STUDY ON BIOACTIVE COMPOUND DEGRADATION FROM BELIMBING BULUH *(AVERRHOA BILIMBI)*

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Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
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JULY 2014

ABSTRACT

Averrhoa bilimbi Linn. are type of family Oxalidaceae that had a common name such as bilimbi, belimbing buloh and balimbing. They have an excellent source of bioactive compounds such as flavonoid, phenolic and antioxidant included oxalic acid, vitamin C, tannins also minerals. A. bilimbi, has been widely used in traditional medicine since gives high good impact to humankind for cured disease such as cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough and hypertension. However, this active compound will reduce its value during undergo heating and drying process especially when exposed to high temperature. Since bioactive compounds are unstable when it exposed to heat, the product will become low in the nutritional value. Therefore, it very important to understand the thermal degradation of A.bilimbi to maintain the product quality and produce nutritional product that can used for human health, and hence this is the objective of this work. Extraction of A. bilimbi fruit were performed using 50% concentration methanol as a solvent. Then, the heat treatment was performed using a metal tube containing A. bilimbi extracts at various temperature and time exposure. Total flavonoids (TFC) and phenolic (TPC) content were analyses using colorimetric assay. Meanwhile, the free-radical scavenging (antioxidant) activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. All analysis was performed using a calibrated ultraviolet-visible spectroscopy (Hitachi U-1800, Japan). TPC and DPPH were found to decrease relatively as the heating time prolonged and temperature increased were, from 885 to 877 mg GA/100 g *Averrhoa bilimbi* and from 42 to 40 % after 120 minutes heat treatment respectively. However, TFC increased significantly with temperature and heating time were from 852 to 2133 μg/100 g *A.bilimbi*. From this study, it showed that a long period of heat treatment of A. bilimbi at a relatively high temperature destroy the overall antioxidant activity. Therefore, the overall antioxidant activity of A. bilimbi should be enhance to minimize the degradation of natural antioxidant during thermal processing.

ABSTRAK

Averrhoa bilimbi terdiri daripada kumpulan Oxalidaceae dan di kenali sebagai belimbing buluh, bilimbi dan juga balimbing. *A. bilimbi* kaya dengar sumber sebatian bioreaktif seperti flavonoid, phenolic dan antioksidant termasuk asid oksalik, vitamin C, tannin dan juga mineral. Oleh itu, A. *bilimbi* telah digunakan secara meluas dalam perubatan tradisional untuk merawat penyakit seperti batuk, sejuk, gatal-gatal, bisul, sakit sendi, siflis, kencing manis dan darah tinggi. Walaubagaimnapun, sebatian bioreaktif akan berkurangan apabila dipanaskan dan di keringkan terutamanya apabila terdedah kepada suhu yang tinggi. Oleh sebab A. *bilimbi* tidak stabil apabila dikenakan haba, ini akan menyebabkan nutrisi produk mempunyai kualiti yang rendah. Oleh itu, sangat penting untuk memahani kesan degradasi haba terhadap sebatian bioreaktif untuk mengekalkan kualiti nutrisi produk dan ini adalah tujuan kajian ini dilakukan. Pertama sekali, A. *bilimbi* akan diekstrak mengunakan 50 % kepekatan methanol sebagai pelarut. Kemudian, ekstrak A. *bilimbi* akan dimasukkan dalam tiub logam dan dipanaskan terhadap suhu dan masa yang berbeza. Kandungan flavonoids dan phenolic akan di analisis mengunakan cara kalorimetrik. Manakala, antioksidan mengunakan 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Kandungan phenolic dan antioksidan berkurang apabila dipanaskan dalam tempoh yang lama dan suhu yang tinggi iaitu dari 885 ke 877 mg GA/100 g *A. bilimbi* dan dari 42 ke 40 % selepas 120 minit di panaskan. Tetapi kandungan flavonoids bertambah secara mendadak dengan peningkatan suhu dan masa pemanasan iaitu dari 852 to 2133 μg/100 g *A.bilimbi*. Daripada kajian ini, dapat disimpulkan bahawa masa pemanasan yang lama dan suhu yang tinggi akan memusnahkan antioksidan dalam buah A. *bilimbi.* Oleh itu, antioksidant dalam A. *bilimbi* mesti ditingkatkan untuk mengelakkan penurunan antioksidan semulajad semasa proses pemananasan haba

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LIST OF ABBREVIATIONS

A C (o)	Absorbance of the blank
A S (t)	Absorbance of the sample
°C	Celsius

LIST OF ABBREVIATIONS

LC-MS	Liquid Chromatography Mass Spectra
NMR	Nuclear Magnetic Resonance
UV-Vis	Ultraviolet-Visible Spectrophotometry

1 INTRODUCTION

1.1 Backgrounds of study

Averrhoa bilimbi Linn. or commonly known as a pickle fruit that growth in well-drained soil also can live in sand and limestone. Generally, this fruit tree has a small sized which is growing up from 5 to 10 m tall and 3 to 6 cm long in leaf. It also had sour taste. They are an excellent source such as oxalic acid, vitamin C, tannins and minerals as well as fifty three (53) volatile components included aliphatic acids, hexadecanoic acid, 9-octadecanoic acid, esters and butyl nicotinate also hexyl nicotinate. Besides that, extract fruit of *Averrhoa bilimbi* had many useful bioactive compounds that exhibit excellent antibacterial. These *Averrhoa bilimbi* easily found in most country like in Indonesia, Malaysia, Brazil, Cuba, Philippines and Sri Lanka as well as in Bangladesh and Myanmar (Burma). It used for treatment of children cough, stomach ache and as a cooling drink. On the other hand, *A. bilimbi*, has been widely used in traditional medicine for treatment of cough, rheumatism, itches, boils, diabetes, syphilis, whooping cough, cold and hypertension (Goh et al., 1995).

Figure 1-1: Ripe and unripe fruits of *A. bilimbi*

Previous scientific studies revealed that phytochemicals that presence in vegetables and fruits consists of phenolics, alkaloids and carotenoids, also various nitrogenous compounds have excellent variety of bioactivities such as antioxidant, anti-fungal, antiproliferation and antiviral activities as well as have antibacterial (Liu, 2003). Thus, most of studies take the opportunities to deep studied about medicinal plant *like Pithecellobium confertum, Averrhoa bilimbi, Portulaca oleracea, Solanum torvum, Solanum nigrum, Persicaria tenella, Cosmos caudatus, Pandanus amaryllifolius, Curcuma mangga, Ocimum basilicum, Anacardium occidentale* and *Melicope ptelefolia,* (Abas et al., 2006) since give beneficial effect on the human health. As a result, they found that, the *Averrhoa bilimbi* gives high good impact to humankind since the plant produce antimicrobial to against microorganism. This protective effect also associated with high level of antioxidant properties and bioactive contents in *Averrhoa bilimbi* that can help to avoid the human body from damage by reactive oxygen and nitrogen species. This fact also supported by Beecher (1999) which are frequently taking of fruits in diet, leading to reduce the risk of critical diseases such as stroke and cancer.

The bioactive compound that identified from fruit *Averrhoa bilimbi* is flavonoid content, phenolic content and antioxidant activity. Flavonoid has a low toxicity other than bioactive compound it also rich in antiviral, anti-allergic and anti-tumor. However, among the various bioactive compounds, phenolic compounds which are plant secondary metabolites that act as reducing agents for redox potential also inhibit the formation of free radical scavenges. Moreover, recent studied indicate that, most of fruits provided lots source of natural antioxidants that can decrease the risk of critical disease like diabetes, cancer, Alzheimer's and cardiovascular diseases since antioxidant responsible to lowering the incidence of oxidative damage to human body (Liu, 2003). Other than that, vitamin C is natural antioxidants that can act as a most powerful anti-oxidant present in food and beverages.

In order to perform analyses, the fruit of *Averrhoa bilimbi* must be extracted out. These extraction processes involve raw material and methanol as a solvent. However, the focus is now to studies the thermal degradation from the extract fruit of *Averrhoa bilimbi*. After the desired products are yielded, an assay will be performed in order to measure the bioactive content in *Averrhoa bilimbi* by using total phenolics content (TPC), total flavonoid content (TFC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay to determine the thermal degradation of each compound. This is because, to maintain the nutritional quality inside the fruits to ensure human health.

1.2 Motivation and Problem Statements

Research on medicinal plants is important because of the plant like *Averrhoa bilimbi* produces bioactive components with different medically useful functional properties. The *Averrhoa bilimbi* is excellently rich in bioactive compounds that could potentially give benefits to human being and health applications. Moreover, *Averrhoa bilimbi* easier to found because it is not a seasonal fruit and cheaper than other fruit. Bioactive compound that contain inside these *Averrhoa bilimbi* are flavonoid, phenolic and antioxidant activity. Unfortunately, it is well understood that heat can destroy the quality of food attributes, such as color, texture, nutrients and substances beneficial to health.

This natural antioxidants compound will reduce its value during undergo heating and drying process especially when exposed to high temperature maybe due to loss in moisture content. Recent studies believed that, most of the bioactive compounds are relatively unstable when exposed to heat (Roy et al., 2007) and caused the product has low nutritional value. Moreover, the previous scientific studies emphasized that, increased the extraction time leading to degradation effects (Garcia-Sales et al., 2010). Therefore, it very important to understand the thermal degradation of *Averrhoa bilimbi* to maintain the product quality and produce nutritional product that can used for human health.

1.3 Objectives

The following are the objectives of this research:

- To elucidate the thermal degradation of bioactive compounds from *Averrheo bilimbi*

1.4 Scope of this research

The following are the scope of this research:

i) Extraction of Averrheo bilimbi using 50 % concentration of methanol as solvent

ii) Heating Treatment at various temperature and time exposure for *Averrheo bilimbi* sample

iii) Perform analysis for TPC, TFC and DPPH assays using UV-VIS spectrophotometer

1.5 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 gives review of *A. bilimbi* fruit, bioactive compounds and thermal degradation of bioactive compounds. . A summary of the previous experimental work on bioactive compounds is also presented.

Chapter 3 explained more details on methodology and procedures of this study. In addition, in this chapter also explained the material used in this experiment and the method use to analysis the data.

Chapter 4 will be covered on the results and discussion of the .All the experimental result and data will be discussed in details which are effect of thermal degradation bioactive compounds contain in *A. bilimbi.* The detailed report on the product quality analysis was evaluated.

Chapter 5 will be discussed on the conclusion can be made for the study and some recommendations can be taken.

2 LITERATURE REVIEW

2.1 Description of Averrheo Bilimbi

Averrhoa bilimbi Linn.are type of family Oxalidaceae that has different common names in different countries for example, in Malaysia it is popular with *belimbing buloh, b'ling,* or *billing-billing* and *belimbing asam,* while in Indonesia, it is known as *belimbing besu, balimbing, blimbing,* or *blimbing wuluh.* Other than that, in Filipino it is famous with *kamias,* while English names it is called are *cucumber tree* and *tree sorrel*; in Creole it is *bumbling plum* or *blimblin* and in French normally called it *carambolier bilimbi, blinblin* or *blimblim* (Ali et al., 2013). In Thailand, it is famous with *talingpling,* or *kalingpring.* Wherease, in Haiti, it is known as *blimblin.* While, in Jamaica it is called *bimbling plum* and famous with *grosella china* in Cuba. In addition, El Salvador and Nicaragua named it as a *mimbro.* The other common names for *A.bilimbi* included Costa Rica as *mimbro* or *tiriguro;* in Venezuela famous with *vinagrillo;* in Surinam and Guyana known as *birambi;* in Argentina called as *pepino de Indias* (Morton, 1987).

Figure 2-1: Tree and leaf of *A. bilimbi*

This *Averrhoa bilimbi* can be categorized as an attractive tree, long-lived tropical tree that can reach 16 to 33 ft (5-10 m) in height; has a short stem and a lot of thickest branch. Most of the leaves mainly clustered at the branch also have a 5-petalled flower. While, the fruit have a shape like ellipsoid and obovoid or just about cylindrical, faintly 5-sided and can reach from 4 to 10 cm in long. Besides that, capped by a thin, star-shaped calyx at the stem-end and tipped with 5 hair-like floral remnants at the apex. The fruit is crusty when unripe, change from bright-green to yellowish-green and lastly to white color when ripe and falls to the ground. The outer skin is more bright, very small, soft and tender, and the flesh green, jelly-like, juicy and highly acid (Morton, 1987). Besides that, the *Carombola* (one of the family member of Oxalidaceae) is more resistance to cold than *Averrhoa bilimbi*.

The *Averrhoa bilimbi*s are essentially originated from Moluccas, Indonesia. It also grown semi-wild everywhere in the Brazil; much planted in Cuba, Sri Lanka and Bangladesh. Besides that, it is very familiar in Philippines, Malaysia and Burma (Ali et al., 2013). Besides that, the previous study revealed that, both of leaves and fruits of *Averrhoa bilimbi* had an antibacterial activity against bacteria but the fruit it's more excellent than the leaf (Das et al., 2011). However this fruit contain of moderate antioxidant activity and nitric oxide inhibition activity than *O. Basilicium* (Abas et al., 2006).

In Philippines, most of the leaves used as an adhesive on itches, rheumatism, swellings of mumps and on skin eruptions. In another place, they are applied on stings from poisonous creatures (Das et al., 2011) and Malaysians used the fresh leaves or fermented as a remedy for venereal disease *(*Morton, 1987). A leaf infusion is a therapy for coughs and treatment after childbirth as a tonic. Also the flower infusion is believed can be applied for healing coughs, cold and thrush (Das et al., 2011). Moreover, it is used to cure itches, cough, syphilis, boils and rheumatism (paste of leaves); and as a cooling drink (juice of preserved fruits); to relieved children's cough (syrup of flowers) and stomach ache (fruits) (Ali et al., 2013). Besides that, *Averrhoa bilimbi* has been extensively used in traditional medicine for cure disease like cough, whooping cough, itches, diabetes, rheumatism, boils, syphilis, cold, and hypertension (Abas et al., 2006). In Indonesia, *Averrhoa bilimbi* used as treatment for diabetes mellitus (Chin, 1992). Furthermore *Averrhoa bilimbi* also can be used for established anti-cancer activity (Ali et al.,

2013) and can be applied in production of vinegar, pickles, wine, jams, jellies and as a treatment after birth (Chauhan and Kapfo 2013).

2.2 Bioactive Compounds

Recent studies indicated that, fruit and vegetable play important role to prevent the chronic disease included cancer, cardiovascular and diabetes as well as neurological disease due to lot of sources of vitamin C and phenolic antioxidant (Caro et al., 2004). Some studied also revealed that, the fruit and vegetables had a beneficial nutritional that rich in carbohydrate, protein, mineral and vitamin C (Ismail, 2000). Since the vegetables excellent in medicinal properties it can be used as blood cleansing, induction of uterine contraction also reduce risk some disease like high blood pressure, arthritis, cardiovascular disease, diabetes, fever and coughs (Abas et al., 2006). Besides that, each fruit have a different antioxidant activity and identified based on their flavonoids, polyphenol content, carotenoids and vitamin C, E (Saura-Calixto & Goni, 2006).

Bioactive compounds that determined from *Averrheo bilimbi* are flavonoid, phenolic and antioxidant activity. Polyphenol compounds such as flavonoid, tannin and phenolic compounds are the major group of antioxidant inside the plant. Polyphenols have received most attention because of their preventive potential that gives benefits to human health. There is strong evidence from the studies which is fruit that containing dietary fiber and polyphenols can enhance the lipid metabolism and avoid the oxidation of low density lipoprotein cholesterol (LDL-C) as well as can inhibit the growth of atherosclerosis (Gorinstein et al., 1998).

Phenolic categorized as simple phenol and the most natural compounds in plant, it also found that, about 2% of all the carbon from plants during photosynthesis process was converted into the flavonoids (Vijayakumar et al., 2008). Among the variety of polyphenol compounds, phenolic are classified as a secondary metabolites plant that act as reducing agents for redox potential also inhibit the formation of free radical scavengers (Hanasaki et al., 1994). The free radicals released during respiration can damage DNA, which results in mutation and consequently leads to cancer. Other than that, the phenolic can controlled the nitric oxide, reduce the leukocyte

immobilization, and inhibit cell proliferation and angiogenesis also exhibit the phytoestrogenic activity (Pellati, et al., 2004). Phenolic compounds consist of aromatic ring that contained one or more hydroxyl group and range from simple phenolic compounds to highly polymerized compounds.

Flavonoids are polyphenols with have characteristic diphenylpropane ($C_6C_3C_6$) chemical structure as shown in Figure 2-2. Flavonoids are extensively distributed in the plant kingdom with large variety of structures (Iwashina, 2000). They are ubiquitous among the plant and categorized according to four major subgroups which are isoflavones, 4-oxoflavonoids (flavones,flavonols), anthocyanins, and flavan-3-ol derivatives (catechin and tannins) (Rhodes & Price, 1996). More than 4000 flavonoids have been identified from the fruits and vegetables. This flavonoid has antiviral, anti-inflammatory, anti-allergic, antitumor, antiplatelet, and antioxidant activities. There is strong evidence from studies that fruits and vegetables containing antioxidant in the diet are associated with a lower incidence of age related disease and various cancers (Anlasik, et al., 2005). Because of this, the idea has come out that it is the antioxidants in these foods that are the effective preventive agents. Besides that, Flavonoids are the most important sources in plant since can provided flower pigments to attract pollinator and help to avoid UV radiation. They also give good beneficial to human health since the studies revealed that flavonoids from various botanical sources can react as a strong antioxidants hence can be used as traditional vitamins (Lewis et al., 1999).

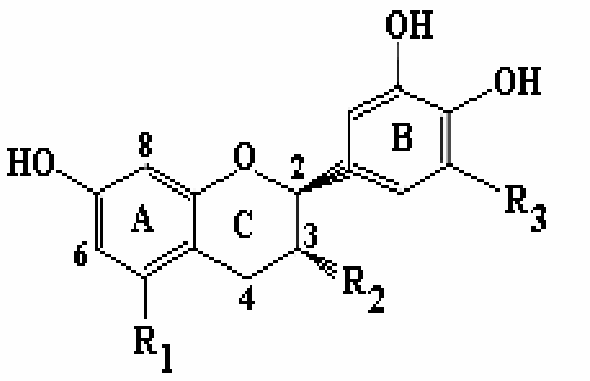

Figure 2-3: Chemicals structure of flavonoids

Antioxidants can be classified to two classes which is as synthetic and natural. Example of synthetic antioxidants that commonly used is butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG). While, a-tocopherol, phenolic compounds and polyphenolics are some example of natural antioxidant (Hall & Cuppett, 1997). In addition, ascorbic acid or vitamin C is one of the natural antioxidant mostly exists in plants and quite important in biochemical processes for example in formation of collagen, in immune responses, in neurotransmission and iron absorption (Martínez, 1998). Unfortunately, adverse effects will occur when high content of ascorbic acid inside human body. Thus, it is very important to determine the antioxidant level in each food. Several studies indicate that, some of synthetic antioxidants could be toxicity, required high manufacturing costs and lower efficiency compared to natural antioxidants (Soong & Barlow, 2004). Therefore, needed to identified the natural antioxidants that more economic, safer and effective that can be applied into foods processing. Meanwhile, certain natural antioxidants have already been isolated from variety of plant parts, like vegetables, oil seeds, leaves, spices, cereal crops, fruits, roots, and herbs (Ramarathnam et al., 1995). However, Fruit seeds have not received most attention as antioxidant sources compare to oil seeds may be due to their less of commercial applications (Soong & Barlow 2004).

There are two types of antioxidants which is primary and secondary antioxidants. Whereas, the primary antioxidants responsible for scavenge free radicals to prevent the chain initiation and break chain propagation. While, the secondary antioxidants responsible for block the formation of radicals and protect against from oxidative damage (Chauhan and Kapfo 2013). Recent studies indicate that free radicals are leading to break the proteins, lipids and nucleic acids in cells (Leong & Shui, 2002) and caused several physiological and pathological abnormalities, such as inflammation, cardiovascular diseases and ageing diseases. According to Abas et al. (2006) antioxidants help to inhibit the growth of free radicals that responsible for many oxidative processes. This fact supported by Tan et al. (2005) those free radicals which release from phagocyte cell that leading to formation of nuclear factor jB (NF-jB) that activate the transcription of inflammatory cytokines and COX-2 can prevent effectively by antioxidant through stabilization of NF-*k*B/I *k*B-∝.

The leaves of *Averrheo Bilimbi* such as AF and BuF contain strong hypoglycemic and hypotriglyceridemic properties in HFD–STZ–diabetic rats that have potential to reducing the blood glucose level in diabetic rats (Tan et al., 2005).

Other study emphasized that, plants which rich in antioxidant properties as well as excellent in antibacterial, anticarcinogenic, antiviral, and antiallergic, estrogenic and immune-stimulating effects (Larson, 1988). Other than that, the polyphenolic compounds that founds in plants have multiple biological effects include antioxidant, also tannins and flavonoids are the strong antioxidants founds in plants (Chowdhury et al., 2012). There is convincing evidence from epidemiological studies that fruits and vegetables containing phytonutrients are preventive potentially to prevent human body from damage by reactive oxygen and nitrogen species (Diplock et al., 1998; Halliwell, 1997). Furthermore, the studies believed that, vegetable have chemopreventive properties, can produce new sources of antioxidants and block production of NO (Abas et al., 2006). Therefore, it is very important to providing adequate contents of antioxidants in human diet. Moreover, there is strong evidence from the studies that fruits containing antioxidant have potential to reducing incidence of stroke and cancer (Bae et al., 2008; Beecher et al., 1999), since can be applied in therapy for anticancer (Ramjan, et al., 2013).

Table 2-1: Previous Work on *Averrheo Bilimbi*

AUTHOR	STUDY	REMARK
Ali et all. (2013)	Cytotoxic activity of different extract of *Averrheo Bilimbi*	• Have cytotoxic potential • Have bioactive compounds that leading for anticancer therapy
Chowdhury et all. (2012)	Antioxidant and cytotoxic activity of *Averrhoa Bilimbi* fruits	• Preliminary phytochemical showed the presence of flavonoids,tannin and reducing sugar and phenolic compounds • Have strong antioxidant properties • Prevent and cure of diseases associated with oxidants or free radicals
Chauhan and Kapfo (2013)	Effect of traditional sun-drying on phenolic antioxidants of *Averrheo Bilimbi*	• Drying treatment and selection solvent compositions during process affect TPC and antioxidant activity. • Degrades the phytochemicals and phenolic decomposed • Fresh fruit extracts have higher antioxidant properties then dried fruit extracts
Das et al. (2011)	Cytotoxic activities and antibacterial of fruits and leaf extracts of *Averrheo Bilimbi* using methanol as solvent.	• Have more antibacterial potential at extract fruits then leaf • Fruits extract have excellent inhibitory activity against pathogen compared with standard antibiotics (kanamycian) • Can be used as an antiproliferative, antitumor, pesticidal and other bioactive agents.

Table 2-1: *Continued*

AUTHOR	STUDY	REMARK
Tan et al. (2005)	Activity of semi–purified fractions of the leaf extract of *Averrhoa bilimbi* (ABe) in HFD–STZ–induced diabetic rats.	• used as antibacterial, antiscorbutic, astringent, inflammation of the rectum and diabetes • Ethanolic extracts of leaves such as AF and BuF have potent hypoglycemic and hypotriglyceridemic properties in HFD–STZ–diabetic rats • Helping in reduced of blood glucose level in diabetic rats
Goh et al. (1995)	Malaysian medicinal plants for the treatment of cardiovascular diseases	• Revealed that have multiple ethnopharmacological properties such as anti-inflammatory, anti-scorbutic, astringent, anti-bacterial, and postpartum protective properties.
Norhana et al. (2009)	Effectiveness of washing shrimps in bilimbi or tamarind juice in reducing the surface population of Listeria monocytogenes Scott A and S. Typhimurium ATCC 14028	• Active compounds in averrheo bilimbi indicate that, strong antibacterial activity since have potential to inhibit the growth of Listeria and Salmonella cells when directly in contact with them.
Abas et al. (2006)	Antioxidant properties and effect on nitric oxide (NO) production of selected Malay traditional vegetables	• Indicated that, averrheo bilimbi have low antioxidant activity and nitric oxide inhibition activity • Antioxidants can prevent the activation of NF-*k*B through the stabilization of NF-*k*B/I *k*B-∝ • Free radical (NO) activate production of inflammatory and COX-2

2.3 Thermal Degradationof Bioactive Compounds

Thermal processing is generally applied to extend shelf life of food products. However, it is well understood that natural antioxidants may be substantially loss during the heat treatment due to the fact that most of the bioactive compounds are relatively unstable when exposed to heat. Therefore, heat processed foods are considered to have a lower health compared to fresh one. There is strong evidence from Rawson et al. (2011) found that most of the bioactive compound in plants for example flavonoid, phenolic, antioxidant, carotenoid and vitamin C will change their chemical and physical structure also loss the bioavailability due to the thermal degradation process when exposed to high temperature. Besides that, most of author revealed that, the bioactive compounds and antioxidant inside the fruit and vegetables slightly reduce its contents because of thermal processing (Busha et al., 2008; Cisse et al., 2009; Ferracane et al.,2008; Jimenez-Montral et al., 2009). Thus, every heating process caused the dropped in flavonoids content (Arancibia-Avilia et al., 2012). While the anthocyanins, flavanols and ascorbic acid are damaged during the drying process occurs as well as drop in antioxidant content (Wojdylo et al., 2009) may be due to oxidation and sensitivity. Besides that, other bioactive compounds like vitamin A, vitamin E also suffer because of thermal degradation process. In addition, vitamins are the most sensitive bioactive compounds will affected by degradation process (Rawson et al., 2011) and might be a reduce the shelf life of these products.

However, recent studies have shown that thermal processing does not necessarily reduce the quality of fruits. In some cases, heat treatments retain or enhance the effect on the antioxidants activity. For instance, it has been found that the total carotenoid content in tamarillo nectar is slightly not affected by thermal degradation while, oxygen dissolved from liquid foods leads to increasing in degradation rate on bioactive compounds (Rawson et al., 2011). Dewanto et al. (2002) found that, heating treatment significantly increases the fruits and vegetables biological activities compared to fresh one due to the various chemical changes during thermal processing. This fact supported by Stahl and Sies (1992) whereas, the tomatoes and carrots have higher content of lycopene and β-carotene after cooked rather than fresh one. Recently, numerous studies revealed that, most of polyphenol and antioxidant activity derived from citrus peel affected by heating

treatment (Jeong et al., 2004; Xu et al., 2007; Garau et al., 2007). For instance, Jeong et al. (2004) illustrated that, the antioxidant activity derived from citrus peel relatively increases when exposed to heat treatment. Garau et al. (2007) also mentioned that, heat treatment at 50 and 60℃ may be interrupt the plant cell wall and enhance the antioxidant capacity of citrus (Citrus aurantium v. Canoneta). Moreover it has been found that, the heat treatment significantly increases the antioxidant activity of blood-orange juice, Shiitake mushrooms and grape seed (Scalzo et al., 2004; Choi et al., 2006; Kim et al., 2006).

2.4 Summary

As a conclusion, since the *Averrhoa bilimbi* had excellent sources of bioactive compounds such as flavonoid, phenolic and antioxidant it can be classified as an important medicinal plant that can cure and prevent the critical disease like diabetes, high blood pressure, cancer, age related disease and etc. However, it is well understood that natural antioxidants compounds might be substantially loss during the heat treatment due to the fact that most of the bioactive compounds are relatively unstable when exposed to heat. Hence, the products from degradation will have a low nutritional value compared to the fresh one. Therefore, it is important to understand the degradation of bioactive compound derived from *A. bilimbi* to avoid any loss of nutrition content to ensure the human health.

3 MATERIALS AND METHODS

3.1 Overview

The flowchart below shows the overview of methodology that will be conducted in order to fulfill the objective of this research.

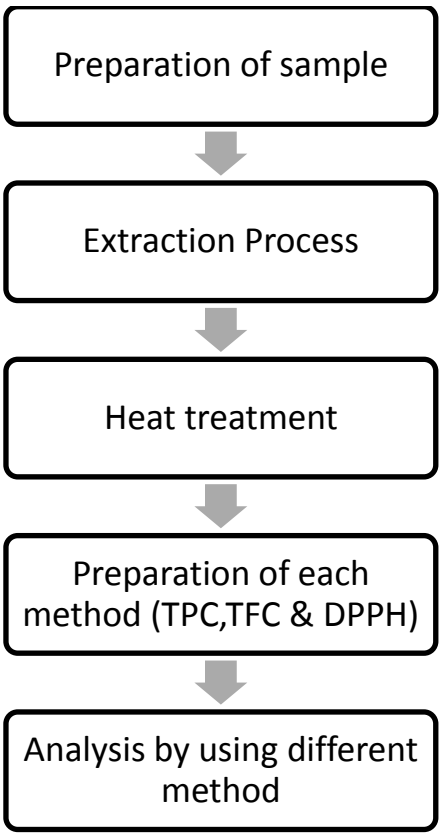

Figure 3-1: An overview flowchart for methodology

3.2 Chemicals

Sodium nitrate, methanol, sodium hydroxide, follin-ciocalteau reagent, sodium bicarbonate and gallic acid were obtained from Merck (Darmstadt, Germany). The other chemical included aluminium chloride (Fluka), quercetin (Acros) and DPPH solution (2,2- diphenyl-1-picrylhydrazyl) were obtained from Sigma Aldrich (St. Louis, MO). Fruits were collected in Gambang, Pahang, Malaysia from a *Averrhoa bilimbi* trees.

3.3 Experiment Procedure

3.3.1 Preparation of sample and extraction

The fresh fruit of *A. bilimbi* was washed under running tap water to remove dirt and other foreign materials and wiped with tissue paper. The edible portion was then cut into small pieces and stored at -80 °C before lyophilization using a bench-top freeze dryer. The lyophilized fruits were ground into powder form, and kept at -20 °C prior to analysis. The extraction process was performed in ultrasonic water bath at 30 °C and 90 minutes using 50 % concentration of methanol. The insoluble matter was then separated by centrifuge and filtered again using filter paper (Whatman No. 1) to obtain a clear solution. After that, the fruit extracts was wrapped with aluminum foil and stored under nitrogen at -20 °C prior to analysis and heat treatment. The extract was used for determination of flavonoid, phenolic and antioxidant content.

3.3.2 Heating Experiment

The heat treatment was performed using *Averrhoa bilimbi* extract and carried out at different temperatures (90 and 120 °C) and various time exposure such as 10, 20, 40, 60, 80,100 and 120 minutes. *Averrhoa bilimbi* extract was enclosed in seal metal tubes (outer diameter = 16 mm; innerdiameter = 13 mm, length = 150 mm) and placed in the water bath for various treatment times, depending on the temperature. The temperature was measured by a thermometer. For all treatment, preheating time was taken into account for the extract to reach the isothermal phase. After treatment, the tubes were immediately cooled in an ice bath to stop further thermal degradation. The sample was analyzed using TFC, TPC, and DPPH assays to determine the degradation of individual compound.

3.3.3 Total Flavonoid Content

TFC of the fruit extracts was determined according to the colorimetric assay was described by Alothman et al. (2009). First, 1 ml of properly diluted fruit extract was mixed with 4 ml of distilled water. Then, at zero time, 0.3 ml of (5% w/v) $NaNO_2$ was added. After 5 min, 0.3 ml of (10% w/v) $AlCl_3$ was added. Then, at 6 min, 2 ml of 1 M solution of NaOH also added. After that, the volume was made up to 10 ml, immediately by the addition of 2.4 ml of distilled water. The mixture was shaken