

SPRAY DRYING OF ATI-ATI PLANT BOILING EXTRACT

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ABSTRACT

In this work, optimization and scale up of the spray drying *ati-ati* plant boiling extract was studied. The variables that influence the antioxidant activity of *ati-ati* plant are studied and determined. In this research spray drying has been adopted to produce the dried extract from the liquid form of the boiling extract of *ati-ati* plant. Analysis on the dried extracts has been conducted using an antioxidant assay are based on measurement of the loss DPPH color at 517 nm after reaction with test compound and the reaction is monitored by UV-VIS spectrophotometer. The 2^2 factorial experiment method is used to design the experiment around a centre point of temperature and concentration of maltodextrin which gave the optimum antioxidant activity at small scale. Among the experimental points, the highest yield of antioxidant activity is produced in condition of 150 °C and 10% concentration of maltodextrin. This experiment was followed by mathematical analysis using Yates' method and linear regression in order to study the main effect and interactive effect of changing the level of experimental variables. The calculation proved that concentration of maltodextrin has the biggest main effect on antioxidant activity while the interactive effect of the combination of temperature and concentration of maltodextrin is also significance. The 2^2 factorial experiments were complemented with more experiments to make a composite design in order to determine the maximum point. The maximum predicted yields according to the optimized levels of variables are 57.3427% of antioxidant activity at temperature of 165.26°C and 19.35% concentration of maltodextrin.

ABSTRAK

Dalam kajian ini, pengoptimum dan skala besar dari pengeringan semburan ekstrak didihan tanaman ati-ati dipelajari. Pembolehubah yang mempengaruhi aktiviti antioksidan tanaman ati-ati dipelajari dan ditetapkan. Dalam kajian pengeringan semburan telah digunakan untuk menghasilkan ekstrak kering dari bentuk cair dari ekstrak didihan tumbuhan ati-ati. Analisis ekstrak kering dilakukan menggunakan ujian antioksidan berdasarkan pada pengukuran kehilangan warna DPPH pada 517 nm selepas reaksi dengan sebatian uji dan reaksi dipantau oleh spektrofotometer UV-VIS. Kaedah percubaan faktorial 2^2 digunakan untuk merancang percubaan sekitar titik pusat suhu dan kepekatan maltodekstrin yang memberikan aktiviti antioksidan yang optimum pada skala kecil. Di antara eksperimental pada titik pusat, hasil tertinggi aktiviti antioksidan dihasilkan adalah dalam keadaan $150\text{ }^{\circ}\text{C}$ dan konsentrasi 10% dari maltodekstrin. Percubaan ini diikuti dengan analisis matematik menggunakan kaedah Yates dan regresi linear untuk mempelajari kesan utama dan kesan interaktif menukar tahap pembolehubah eksperimen. Perhitungan membuktikan bahawa kepekatan maltodekstrin mempunyai kesan utama yang terbesar kepada aktiviti antioksidan sedangkan kesan interaktif dari kombinasi suhu dan kepekatan maltodekstrin juga signifikan. Percubaan 2^2 faktorial yang dilengkapi dengan percubaan lebih banyak untuk membuat desain komposit dalam rangka untuk menentukan titik maksimum. Hasil yang dijangka dengan peningkatan optimum pembolehubah adalah 57,3427% aktiviti antioksidan iaitu pada suhu dan kepekatan $165.26\text{ }^{\circ}\text{C}$ dan 19,35% dari kepekatan maltodekstrin.

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LIST OF SYMBOLS

ml	-	Mililiter
min	-	Minute
t	-	Time
T	-	Temperature
X	-	Concentration
°C	-	Degree Celsius
DPPH	-	1,1-Diphenyl-2-picrylhydrazyl
g	-	Gram
A	-	Absorbance
Max	-	Maximum

CHAPTER 1

INTRODUCTION

In this chapter it gives the ideas about the rationale of the research formulation. This first chapter discovers the subtopic of background of study, problem statement, definition of terms, scope of study and significance conducting the research.

1.1 Background of Study

Ati-ati plant is well-known as one of indigenous plant or herb in traditional practitioner. Scientific name for *ati-ati* plant is *Coleus Blumei* (family Labiate or mostly known as Lamiaceae). Malay traditional practitioners have adopted boiling method to extract useful component from plants. In past, human use this plant as medical treatment and as food supplement. By drinking extraction water from the plant it was believed can cure or treat an illness. *Ati-ati* plant was believed can treat and cure influenza, cold, *athsma*, critical bronchitis and removed toxic from seafood.

It becomes crucial for citizens to concern about the health. In this bygone era, people are exposed to many types of pollution such as air, water and soil pollution. The pollution exposed human to high levels of free radicals. Free radicals are capable of attacking the healthy cells of the body. This will cause them to lose their structure and function. Cell damage caused by free radicals appears to be a major contributor to aging and to generative diseases of aging for instance cancer, cardiovascular diseases, cataracts, immune system decline and brain dysfunction. This damage can be controlled by practicing healthy lifestyle such as exercising and good habitual diet. Food is one of and

main contributor of our health. Fruits and vegetables are good sources of micronutrients provide health benefit to human diet and inversely related to cancer risk. Fruits and vegetables have protective effects against cellular damage caused by exposure to high levels of free radicals. All plants produce antioxidants. Even meat, dairy products, and eggs contain some antioxidants, which mainly come from the nutrient-rich plants the animals fed on. Plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases.

Nowadays, there has been growing interest in the research subject to develop new technologies in finding standardized dried plant and food extracts. In this research, spray drying has been adopted to produce the dried extract from the liquid form of the boiling extract. Lower storage costs, higher concentration, and stability of active substances are the advantages of the dried extract over conventional liquid forms. In powders manufacturing, spray drying has been adopted due to its ability to create a product with precise quality specifications in continuous operation. According to Mouere *et. al.* (2001), the temperature during extraction and drying, affects the activity and stability of compounds due to chemical and enzymatic degradation and losses by volatility or thermal decomposition. Thus, the aim of this research was to optimize the spray drying of the liquid extract of the selected *Lamiaceae* plant obtained by boiling.

1.2 Problem Statement

Nowadays, due to adverse side effects of modern drugs, people have resorted to natural products instead of modern drugs. Fundamentally, activities of dangerous free radicals from the pollution can be lowered by using an antioxidant. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are examples of the most commonly used antioxidant. However research revealed that these are suspected of being responsible for liver damage and carcinogenesis in laboratory animals (William *et. al.*, 1999). Hence, more effective antioxidant of natural originate desired to be develop and utilise. In higher education institution, scientists have worked with new plant foods that have potential to be effective natural remedies and as well as food supplement. As a result

they come up with new products in form of drinks, juice, herbal tea, dietary supplements or pills. In Malaysia, many plant of foods that have potential to be effective natural remedies have been abandoned due to lack of information and research about their nutritional values. In tropical countries such as Indonesia and India *Lamiaceae* is widely used as home remedies to treat and relieve fever, headache cough and asthma. In Malaysia, the use of *Lamiaceae* plants remedies is unknown and they used instead of ornamentals. It is surprise to realize that people grow them without knowing they are rich with nutrient.

This research is therefore aimed to study and determine the effect of levels of the operational variables that optimizes a product yield from extraction of *Lamiaceae* plant. The main problem here is how to conduct the drying without damage the antioxidant activity. Thus, research is a further research on finding the suitable parameters that can produce higher yield without loosing desired properties of the plant. By making and converting extraction of *Lamiaceae* plant into a tablet or capsule it will give many benefit to human and saving too. Lower storage costs, higher concentration, and stability of active substances are the advantages of the dried extract over conventional liquid forms. By this research hopefully can optimize the production of spray drying of the liquid extract of the selected *Lamiaceae* plant obtained by boiling and maintains the quality of the product.

1.3 Research objectives

This research is aim to investigate the antioxidant contain in selected of *Lamiaceae* plant, *Coleus Blumei*. Thus, this research aims to achieve the following objectives:

- (i) To measure the antioxidant activity in *Lamiaceae* plant.
- (ii) To study effect of levels of the operational variables that affect drying of *Coleus Blumei*.
- (iii) To optimize the spray drying of the liquid extract of *Lamiaceae* plant obtained by boiling.

1.4 Scope of Research

The main scopes of the research are:

1. To study the effects of temperature of spray dryer to *Coleus Blumei*.
2. To study the effects of % concentration of maltodextrin to *Coleus Blumei*.
3. To study and determine of levels of operational variables that optimizes product yield from extraction of *Coleus Blumei*.

1.5 Rational and Significance of the Research

People become more health-conscious. People are also become more aware of significance of good nutrition. Natural food provides best nutrition as human body is adapted for natural food intake. Regrettably, some of vegetables have no extraordinary reputation even though many of these foods have been traditionally known as health diet. As a result, nowadays they have been largely abandoned and ignored. It is expected from this study and knowledge obtained will affirm good habitual practitioner and habitual traditional diet among population in developing countries.

CHAPTER 2

LITERATURE REVIEW

2.1 Properties of *Coleus Blumei* (*Lamiaceae*)

Coleus Blumei is part of *Lamiaceae*. *Lamiaceae* or *Labiatae* also known as the mint family which is a family of plants. The common name for *Coleus Blumei* are *Coleus x hybridus* and most recently as *Solenostemon scutellarioides*. In Asia *Coleus Blumei* is known as *ati-ati*. *Coleus* is from the Greek “*koleos*”, meaning sheath. The male portions of *coleus* flowers, the stamens, are fused into a tube or sheath while *blumei* is named for Karl Ludwig Blume (1796-1862), a Dutch writer who wrote about plants from Java.

Coleus Blumei Benth. (*Lamiaceae*) is an ornamental plant, growing all over the world in an enormous number of different cultivars that vary in colour and shape of the leaves. It is used in India, Indonesia and Mexico as a medical plant. One of the most prominent secondary compounds in *C. blumei* is rosmarinic acid (RA), which is believed to be a part of the plant’s defense system against fungal and bacterial infections and predators (Bauer *et al.*, 2002)

The plants grouped in *Lamiaceae* are important and many are of great economic importance. They are widely used in traditional systems of medicine and horticulture. The *Lamiaceae* plants are herbs shrubs, or trees comprising about 258 genera and 6,970 species, commonly with aromatic, herbage, quadrangular stems, and verticillate inflorescences. The leaves are usually opposite or whorled, and are simple or occasionally pinnately compound; stipules are absent (Zakaria *et al.*, 2008)



Figure 2.1: *Coleus blumei*

Phytochemicals are extensively found at different levels in many medical plants. Numerous plants used in traditional medicine are effective in treating various ailments caused by oxidative stress, bacterial and/or viral infections. Research has shown that medical plants exhibit antioxidant activity. Because of their antibacterial and antioxidant properties, herbs are used as natural food and cosmetics preservatives and are considered in formulation of new functional food products (Samec *et al.*, 2010).

2.2 Antioxidant of *Coleus Blumei*

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.

Free radicals are potentially important in a number of ailments states that can have severe effects on the cardiovascular system, either through lipid peroxidation or vasoconstriction (Lachance *et al.*, 2001). Although the antioxidant defense systems includes both endogenously and exogenously derived compounds, dietary plants based antioxidant have recently received a great attention (Bravo L, 1998).

Free radicals other reactive oxygen species are generated by exogenous chemicals or endogenous chemicals or endogenous chemicals or endogenous metabolic process in food systems or the human body (Georgetti et al., 2007). The radicals may cause oxidative damage by oxidizing biomolecules and results in cell death tissue damage However, ingestion of antioxidative supplements, or foods containing antioxidants, may reduce the oxidative damage on the human body (Wang *et al.*, 2006).

Chemical constituent with antioxidant activity found in high concentration in plants determine their considerable role in prevention of various degenerative diseases (Challa, Ahmad & Mukhtar, 1997; Diplock *et al.*, 1998; Hu & Willett, 2002). Besides the fruits and vegetables that are recommended at present as optimal sources of such components, the supplementation of human diet with herbs, containing especially high amounts of compounds capable of deactivating free radical, may have beneficial effects (Capecka *et al.*, 2005).

Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.

There are two types of antioxidants: exogenous and endogenous. Exogenous antioxidants are antioxidants we get from our diet and endogenous antioxidants are made by our bodies. Endogenous antioxidants repair free radical damage on the inside by initiating cell regeneration; exogenous antioxidants repair some free radical damage from the outside on in by stimulating cell regeneration. Endogenous antioxidants more potent than exogenous antioxidants, unfortunately, the body's production of endogenous antioxidants declines with age and certain diseases. This decrease in endogenous antioxidants is found to be a strong factor in contributing to premature aging and degenerative diseases. The body is incapable of producing enough endogenous antioxidants to combat free radicals on their own. So it is essential to get additional antioxidants thru diet. The body makes five types of endogenous antioxidants which are superoxide dismutase (SOD), alpha lipoic acid (ALA), coenzyme Q10 (CoQ10), catalase and glutathione peroxidase. Catalase and glutathione peroxidase are important because the body can produce more of them when certain free radicals are present.

2.3 Significance of Antioxidant

Antioxidant is discovered for the first time in 1920. It is defined as compounds that fight metal corrosion and all processes involving oxidation (Norman, 2008). There exist various kinds of antioxidants. Examples are vitamins, plant substances and trace minerals. Antioxidants stop the chain reactions of free radical, and inhibit other oxidation reactions. An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Antioxidants have extra electrons, so they can give one or more of their extra electrons to the free radicals. When antioxidants donate an electron to a free radical, they neutralize that free radical and stop the oxidative stress in our cells.

Oxidant itself is defined as atoms or groups of atoms with one or more unpaired electrons. This property makes them very unstable and highly reactive, trying to seek out and capture the needed electrons from other compounds to gain stability. When the 'attacked' molecule loses its electron, it becomes a free radical itself (Surh and Packer, 2004).

In other words, free radicals are incomplete molecules and unstable because lacking of an electron. When these molecules lose their electron resulting from chemical reaction or external factor, it will become free radical which is unstable and reactive. The following figure will show how the antioxidant plays its role in fighting free radicals.

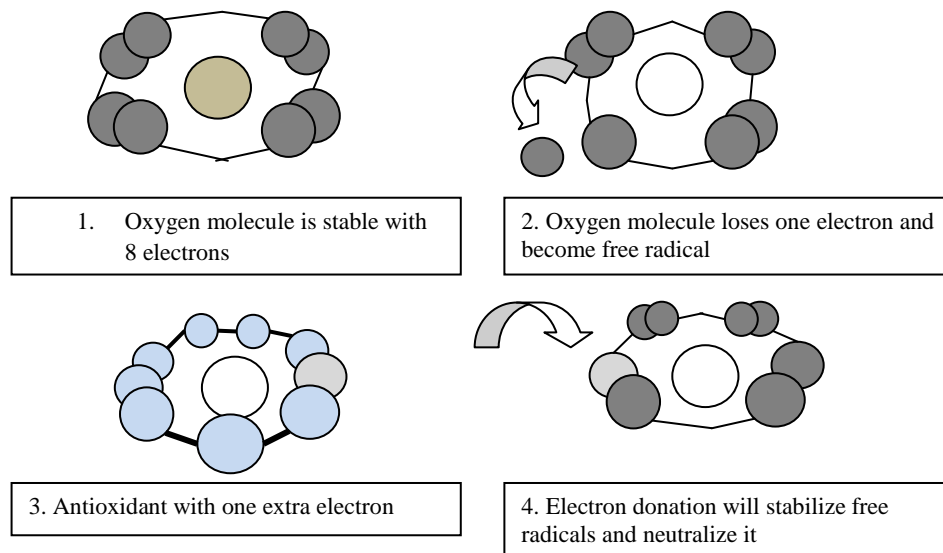


Figure 2.2: Antioxidant fights free radicals by releasing extra electron to free radical in order to stabilize its molecule

Source: Norman (2008)

The free radical trigger chain reaction in the imbalanced cells which eventually will damage the cells. Free radical which ‘rage’ in the cell will finally destroy genetic material (DNA), fat molecule and protein. Effect from this damage will contribute to aging, chronic degenerative disease and even cancer (Norman, 2008).

It is impossible to avoid from free radicals attack. Thus, human beings need antioxidant protection. Plants have their own protection to defend themselves from the effect of free radicals produced by radiation from sunlight.

God has created human with special creation. Humans have evolved a highly sophisticated and complex antioxidant protection system to protect the cells and organ systems of the body against reactive oxygen species (Percival, 1998). Antioxidant can act in different ways: by metal chelation (preventing free radical formation), scavenging free radicals, acting as a chain-breakers (stopping propagation of the free radicals), being part of the redox antioxidant network, and/or regulating gene expression (Surh and Packer, 2004).

Antioxidant protection system involves a variety of components, both endogenous and exogenous in origin. They function interactively and synergistically to neutralize free radicals. Antioxidant can be categorized into four categories. Firstly, the natural antioxidant which are the antioxidant enzymes produced within the human body include superoxide dismutase, glutathione peroxidase, coenzyme Q10, alpha lipoic acid, as well as cytochrome P450 (Rimbach *et al.*, 2005). The second category is vitamins. Most of the vitamins like vitamin A, vitamin C and vitamin E have properties as antioxidant. Thirdly, the minor elements; in a very low doses, it functions to help producing natural antioxidant, such as selenium, zinc, copper and manganese. The fourth category is phytonutrient. The phytonutrient is a material produced by plants which helps antioxidant network like flavonoids, carotenoids, proanthocyanidin, resveratrol, tannin, curcumin and catechin (Norman, 2008).

Antioxidant compounds in food play a significant role as a health-protecting factor. With increased exposure to free radicals, the need for antioxidants in the human body becomes even more vital. Pollution, cigarette smoke, drugs, illness, stress and even exercise can increase free radical exposure (Percival, 1998). Whole grains, fruits and vegetables are the primary sources of naturally occurring antioxidants. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk (Miller, 2000). Many naturally occurring antioxidants—from plant sources—have been identified as free radical- or active oxygen-scavengers (Zheng and Wang, 2001).

Synthetic material is always comparable with the original or natural ones. Lately, restriction of using synthetic antioxidants due to their adverse side effects such as carcinogenicity has considerably sparked an interest to replace them with naturally occurring antioxidants in food or medicinal flora (Ito *et al.*, 1983). Natural antioxidants can defend the human body from free radicals and impede the progress of many chronic diseases as well as lipid oxidative rancidity in food, cosmetics and pharmaceutical materials (Kinsella *et al.*, 1993; Lai *et al.*, 2001). In the past few decades, antioxidant activity and radical scavenging in some medicinal plants and fruits have been widely studied in the search for plants as a source of natural antioxidants (Singh *et al.*, 2002; Velioglu *et al.*, 1998). Among the various natural antioxidants, phenolic compounds are reported to be active, quenching oxygen-derived free radicals by donating a hydrogen atom or an electron to the free radical (Wanasundara and Shahidi, 1996; Yuting *et al.*, 1990). Also, phenolic compounds from plant materials have been shown to neutralise free radicals in various in vitro model systems (Ruch *et al.*, 1989; Zhang *et al.*, 1996).

2.4 Antioxidant Activity

The antioxidant activities of the extracts and fractions, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, were determined by the method described by Brand-Williams *et al.* (1995). The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourisation is stoichiometric with respect to number of electrons captured (Prakash, n.d.). This widely used decolouration assay was reported by Brand-Williams and co-workers (William *et al.*, 1995).

2.5 Free Radical

A free radical is any atom or molecule that is missing an electron from its outer shell, making it unstable. Free radicals float around until they stabilize. They stabilize by attacking another molecule and stealing an electron. There are a wide variety of molecules from which they can steal an electron. The molecule that has been attacked by a free radical is now missing an electron and has become another free radical. This creates a chain reaction.

Oxidation reactions are necessary part of life, unfortunately they can also be damaging. Our body produces free radicals almost constantly. Free radicals are by-products of a basic metabolic process called oxidation. Immune cells will also release free radicals in order to fight invading viruses and bacteria in, making them an important part of the body's defenses.

The second source of free radicals is the environment. Free radicals from your environment can be ingested, inhaled or absorbed into the body. Air pollution, tobacco smoke, herbicides, and pesticides all form free radicals. UV rays, from sun exposure are also a cause of free radicals.

Damage caused by free radicals includes aging, destruction of DNA and clogging of arteries. It is also believed that free radicals may play a role in cancer, strokes, and heart disease. Oxidative stress may also damage or kill cells. Free radicals cause damage to your skin's structural support and decrease its elasticity, resilience, and suppleness.

The free-radical theory of aging (FRTA) states that organisms age because cells accumulate free radical damage over time. There is evidence that reducing oxidative damage can extend life span. Dr. Denham Harmon, M.D., Ph.D., first proposed a theory of

aging in the 1950's, it is now considered a major theory of aging. Dr. Harmon's theory also implies that antioxidants will slow the aging process.

2.6 Extraction via Boiling

Nowadays, the development of new technologies to obtain standardized dried plant and food extracts is an important research subject. The advantages of the dried extract over conventional liquid forms are lower storage costs, higher concentration and stability of active substances (Oliveira, Bott & Souza, 2006).

For a practical application in industry, the antioxidant should be first extracted. The efficiency of the extraction process affects the antioxidant activity of the extract. The Malay traditional practitioners have adopted the boiling method to extract useful components from plants in the production of *jamu*. *Jamu* is a traditional medicine that is prepared from indigenous plants in the form of powder, pills, capsules and drinks. It is traditionally used to treat illness in the Malay Archipelago (Taher *et. al.*, n.d).

2.7 Spray Drying

Spray drying is the most popular drying technology used within the food, chemical and pharmaceutical industries for the production of dry particles from liquids. The modern industries emphasize the production of high-quality products at minimum cost (low investment and running cost). Due to the complexity of the drying, new drying technologies have been developed.

Spray drying is one of the most used methods in microencapsulation, due to the wide availability of the equipment, low processing costs, possibility of using a large variety of carriers, and good final product stability (Favaro-Trindade *et al.*, 2009).

The spray drying has been adopted for manufacture of powders due to its ability to generate a product with precise quality specifications in continuous operation (Souza & Oliveira, 2006). Several drying techniques can be utilized including freeze drying, spray drying and spouted beds with spray dryers being the most commonly used in herbal processing industries. One main reasons why spray drying has been adopted for manufacture of powders is its ability to generate a product with precise quality specifications in continuous operations (Souza & Oliveira, 2006).

2.8 Response Surface Method

Response Surface Methodology is a successive, exploratory approach to establish the relationship between more than one variable with the obtained responses. The analysis develops a model by fitting the experimental data in a generalized smooth curve, from which a specific predicted response could be calculated. In addition, contour plots are also generated which delineates predicted response over a range in the design surface. Thus, a step-by-step approach for response surface analysis establishes a relation between variable and response more efficiently than traditional design (Launen *et. al.*, 1999).

The statistical software package, SPSS was used for regression analysis of the experimental data. Analysis of variance (ANOVA) was used to estimate the statistical parameters. The second order polynomial equation was employed to fit the experimental data. The significance of the model equation and model terms were evaluated by F-test. The quality of fit of the polynomial model equation was expressed by the coefficient of determination (R^2), adjusted R^2 and “adequate precision”. To optimize the level of each factor for maximum response “Point Optimization” process was employed. The combination of different optimized parameters, which gave maximum response, i.e. maximum antioxidant activity yield was tested experimentally to see the validity of the model (Vallapil *et. al.*, 2007)

2.9 Method of Factorial Experiment

There are actually many types of factorial experiments, but we will restrict ourselves to that involving 2 levels, ie the 2^n factorial experiments (Montgomery, 2001). The method of factorial experiments has been designed to allow the effects of a number of experimental variables on the yield to be investigated simultaneously. It gives the “main effects” and the “interactive effects” of changing the experimental variables from a lower level to higher level. The main effect of an experimental variable is defined as the average of the effects of changing its value from the lower level to upper level among all the experiments. It is derived by assuming that the experimental variable is an independent variable and all the variations in its effect are due to experimental errors only. The interactive effects between two or more experimental variables are calculated on the assumption that the experimental variables are not independent but are in fact interacting between them.

The factorial experiments make use of a mathematical method known as the Yates' Method (Yates, 1937) to analyse the main effects and the interactive effects. In giving the main effects and the interactive effects the result of the analysis by Yates' Method (Yates, 1937) also indicate whether the “yield response surface” in the area examined is curve or uncurved, and if it is uncurved, whether it is flat with the respect to the experimental variables or increasing or decreasing with respect to one or more experimental variables and if so, in which direction. The yield response surface itself is not actually a surface in the sense that a surface can only have a maximum of three dimensions whereas in this theoretical response surface the number of dimensions that can be considered is limitless. In this method, each of the experimental variables that is relevant to the yield is given 2 levels equidistant from the centre point, far enough from each other so that the effect of the difference in levels can be detected in the yields.

CHAPTER 3

METHODOLOGY

3.1 Material

Fresh samples of *Coleus Blumei* were collected at Institute Perguruan Tuanku Bainun (IPTB) from Penang during the early month of November 2010.

3.2 Chemical

Maltodextrin was obtained from San Soon Seng Food industries Sdn. Bhd. (Selangor, Malaysia). Methanol (MeOH) was purchased from Merck (Darmstadt, Germany). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich (Steinheim, Germany). All the chemicals used were analytical grade.

3.3 Equipment and Instrumentation

Spray dryer, stirrer and UV-VIS spectrometer

3.4 Preparation of Plant Extract

Extracts were prepared by the technique of decoction. Plant was weighed and washed. About 2 kg plant materials was weighed and washed. Then it was boiled in 20 L distilled water based on ratio 1:100 (2 kg in 20 L). The sample was boiled at 100°C and 150 minutes. The boiled was conducted for four times and the plant extracts were mixed

together. The antioxidant activity of the mixture was determined prior to feeding it into the spray dryer.



Figure 3.1: Raw Material (*ati-ati* Plant)



Figure 3.2: Preparation of Liquid Extraction by Boiling Method



Figure 3.3: Liquid Extraction from the *ati-ati* plant