

**OPTIMIZATION OF EXTRACTION OF ANTIOXIDANTS  
FROM LAMIACEAE *sp.* PLANTS BY BOILING**

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## ABSTRACT

This research is conducted to study the effects of temperature and time of boiling in the extraction of antioxidants from *ati-ati* (Lamiaceae *sp.*) plants. In this research, there are two major objectives which are the study of the effect on antioxidants activity of *ati-ati* (Lamiaceae *sp.*) plants extraction conducted by repeated boiling and the study of scale up and optimization of the antioxidants extraction from *ati-ati* (Lamiaceae *sp.*) plants. In the scale up and optimization study, the experiments were conducted under different levels of two variables of temperature (50, 75 and 100 °C) and time (60, 105 and 150 minutes). In the repeated boiling study, the levels of temperature and time used are at the optimum condition only which are at 100 °C and 150 minutes. The method used to analyze antioxidants levels was 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. Antioxidants activity assay are based on measurement of the loss of DPPH colour at wavelength of 517 nm monitored via UV-VIS spectrophotometers. The percentage of the DPPH remaining is calculated as % antioxidant activity. In order to determine the optimum condition of the antioxidants activity, several mathematical method were used such as the  $2^2$  factorial experiments and using linear regression. In the use of Yates' method, the calculation has proved that temperature has contributed to the biggest main effect on antioxidants activity while the interactive effect of temperature and time on antioxidant yield is also significant. For the linear regression method, the coefficients  $a_1$  to  $a_2$  that are obtained from calculation are small compared to the constant  $a_0$ . The area investigated is a plateau which may contain the maximum yield of antioxidants activity.

## ABSTRAK

Kajian ini dijalankan adalah untuk mengkaji kesan suhu dan masa pemanasan terhadap pengekstrakan antioksidan dari pokok *ati-ati* (Lamiaceae *sp.*). Di dalam kajian ini, terdapat dua objektif utama iaitu mengkaji kesan pada aktiviti antioksidan terhadap pengekstrakan pokok *ati-ati* (Lamiaceae *sp.*) menggunakan kaedah pemanasan berulang dan mengkaji kesan terhadap antioksidan pada ekstrak pokok *ati-ati* (Lamiaceae *sp.*) pada skala besar dan kadar paling optimum. Di dalam kajian skala besar dan kadar paling optimum ini, eksperimen dijalankan berdasarkan dua tingkat pembolehubah yang berbeza iaitu suhu (50, 75 dan 100 °C) dan masa (60, 105 dan 150 minit). Di dalam kajian pemanasan berulang, tingkat suhu dan masa yang digunakan adalah pada keadaan yang optimum sahaja iaitu 100 °C dan 150 minit. Kaedah yang digunakan untuk menganalisa tingkat antioksidan adalah kaedah pencerakinan 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Aktiviti antioksidan diukur berdasarkan kehilangan warna DPPH pada gelombang 517 nm dengan menggunakan spektrofotometer UV-VIS. Peratus DPPH yang tinggal dikira sebagai peratus aktiviti antioksidan. Di dalam menentukan keadaan optimum pada aktiviti antioksidan, beberapa kaedah matematik digunakan seperti kaedah Yates' dan regresi lurus. Pengiraan kaedah Yates' telah membuktikan suhu adalah penyumbang utama kesan terbesar pada aktiviti antioksidan sementara kesan berkait suhu dan masa pada aktiviti antioksidan juga adalah signifikan. Bagi kaedah regresi lurus, pekali  $a_1$  kepada  $a_2$  yang diperolehi adalah kecil berbanding dengan pekali  $a_0$ . Kawasan kajian menunjukkan dataran tinggi dan mengandungi kandungan aktiviti antioksidan yang maksimum.

**TABLE OF CONTENTS**

<b>CHAPTER</b>	<b>TITLE</b>	<b>PAGE</b>
	<b>TITLE PAGE</b>	<b>i</b>
	<b>DECLARATION</b>	<b>ii</b>
	<b>DEDICATION</b>	<b>iii</b>
	<b>ACKNOWLEDGEMENT</b>	<b>iv</b>
	<b>ABSTRACT</b>	<b>v</b>
	<b>ABSTRAK</b>	<b>vi</b>
	<b>TABLE OF CONTENTS</b>	<b>vii</b>
	<b>LIST OF TABLES</b>	<b>x</b>
	<b>LIST OF FIGURES</b>	<b>xi</b>
	<b>LIST OF SYMBOLS/ABBREVIATIONS</b>	<b>xii</b>
	<b>LIST OF APPENDICES</b>	<b>xiii</b>
<b>1.0</b>	<b>INTRODUCTION</b>	
1.1	Background of Study	1
1.2	Problems Statement	3
1.3	Research Objectives	4
1.4	Scopes of Research	5
1.5	Significance of Research	6

<b>2.0</b>	<b>LITERATURE REVIEW</b>	
2.1	Introduction	7
2.2	Herbs	10
2.3	Extraction Process <i>via</i> Boiling	12
2.4	Lamiaceae Plants	13
2.5	Antioxidants Activity	14
2.6	An Overview of the Assays Method Used to Estimate Antioxidant Content	19
2.6.1	DPPH Radical Scavenging Assay	19
<b>3.0</b>	<b>METHODOLOGY</b>	
3.1	Research Methodology	21
3.1.1	Chemicals	21
3.1.2	Equipment and Instrumentation	22
3.1.3	Sample Selection	23
3.1.4	Sample Preparation	25
3.2	Data Collection	26
3.3	Data Analysis	28
<b>4.0</b>	<b>RESULTS AND DISCUSSIONS</b>	
4.1	Effect of Repeated Boiling on Lamiaceae Plants	29
4.2	Scale Up and Optimization	32
4.2.1	The Method of Factorial Experiment	32
4.2.2	The Linear Approximate Equation of the Yield Response	37

4.2.3	Summary	41
<b>5.0</b>	<b>CONCLUSION AND RECOMMENDATION</b>	
5.1	Conclusions	42
5.2	Recommendations	43
	<b>REFERENCES</b>	45
	<b>APPENDICES</b>	51

**LIST OF TABLES**

<b>TABLE</b>	<b>TITLE</b>	<b>PAGE</b>
2.1	Antioxidant Compounds in Foods	9
2.2	Activity of Antioxidant Enzymes	17
4.1	The Results of % Scavenging Activity against the Number of Boiling	31
4.2	Level of Experimental Variables in the 2 <sup>2</sup> Factorial Experiments	34
4.3	The Plan of the 2 <sup>2</sup> Factorial Experiments and the Untreated Results	35
4.4	The Results of the Calculation of Main Effects and Interactive Effects using Yates' method	36
4.5	The Plan of the Replication of the Centre Point and the Results of Experiments	37
4.6	F-distribution	38
4.7	The Results of the F-test on the Main Effect and Interactive Effect	38
4.8	Regression Coefficients of the Linear Equation for the Response Surface	40
4.9	The Evaluation of Linear Regression Equation	41



**LIST OF FIGURES**

<b>FIGURE</b>	<b>TITLE</b>	<b>PAGE</b>
2.1	Lamiaceae <i>sp.</i> Plants	14
2.2	Formation of Free Radicals	15
2.3	Role of Antioxidants in Fighting Free Radicals	16
2.4	Decolourization of DPPH Solution	21
3.1	DPPH Scavenging Radical Assay	23
3.2	UV-VIS Spectrophotometer Model U-1800	24
3.3	Experimental Plot	25
3.4	Lamiaceae <i>sp.</i> Plants Leaves	26
4.1	Graph of % Scavenging Activity against Number of Boiling Lamiaceae Plants	32

**LIST OF SYMBOLS / ABBREVIATIONS**

g	-	Gram
kg	-	Kilogram
L	-	Litre
M	-	Molar
mg	-	Miligram
min	-	Minutes
ml	-	Mililitre
mM	-	Milimolar
T	-	Temperature
t	-	time
°C	-	Degree Celsius
%	-	Percentage

**LIST OF APPENDICES**

<b>APPENDIX</b>	<b>TITLE</b>	<b>PAGE</b>
A	List of Tables	51
B	List of Figures	58
C	Statistical Analysis	63

## CHAPTER 1

### INTRODUCTION

This introduction gives the idea about the rationale and general understanding of the research. This chapter discovers the subtopic of background of study, problem statement, research objectives, scope of research and significance of research.

#### 1.1 BACKGROUND OF STUDY

Plants produce an extensive range of chemicals which may exert beneficial health effects when consumed by man. Many of the plant secondary metabolites act as antioxidants. Antioxidant action is a combination of the several distinct chemical events such as metal chelation; quenching free radicals by hydrogen donation from phenolic groups; oxidation to a non-propagating radical; redox potential, enzyme inhibition (Williamson et al., 1999). When oxidation caused by free radicals and reactive oxygen species emerges in food or in biological systems, antioxidants can prevent or delay this process *via* single or combination of aforementioned mechanisms. Hence, antioxidants may help the body to protect itself from various types of oxidative damage which are linked to diseases (Halliwell, 1991).

Nowadays, antioxidant are widely used as ingredients in dietary supplements in order to maintain the health and preventing diseases such as cancer, cardiovascular

disorders, diabetes and aging. Chemical constituents with antioxidant activity found in high concentrations in plants (Velioglu et al., 1998) determine their considerable role in the prevention of various degenerative diseases (Challa et al., 1997; Diplock et al., 1998; Hu and 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 deactivating free radicals (Madsen and Bertelsen, 1995), may have beneficial effects (Lutomski, 2001). Therefore, the incorporation of seasoning based on herbs into everyday meals may be crucial importance.

Consequently, search for food and drinks with high antioxidant content and enhancement of their antioxidant properties for nutritional purposes are currently of major interest. Food such as common fruits and vegetables are main contributor of our health. Fruits and vegetables that are good sources of micronutrients provide health benefits to human diet and inversely related to cancer risk (Ziegler, 1991). All these fruits and vegetables which are consumed all over the world as well as herbs plants that are consumed by local inhibition are screened for their antioxidant capacity. It has been confirmed that fruits and vegetables have protective effects against cellular damage caused by exposure to high levels of free radicals (Ames et al., 1993). However, scientific information on antioxidant properties of various herbs plants, particularly those that are less widely used due to their endemic nature is still rather scarce. The assessment of antioxidant capacity of such herbs plants remains an interesting and useful task for finding new sources of natural antioxidants.

## 1.2 PROBLEMS STATEMENT

Nowadays, the demanding on supplementation dietary is high and increase time by time due to the concerning and awareness among people about the health and the prevention of various degenerative diseases. Many supplement food were produced and sold in the pharmaceutical market. In this lately period of time, many people have resorted to natural products instead of modern drugs due to adverse side effects of modern drugs. Fundamentally, activities of dangerous free radicals can be lowered by using antioxidants. Some of the examples of the most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). However research revealed that these are suspected of being responsible for liver damage and carcinogenesis in laboratory animals (Williams et al., 1999). Hence, more effective antioxidant of natural origin are desired to be developed and utilized (Oktay et al., 2003).

There are many plant foods outside there that have the potential to be effective natural remedies have been abandoned due to lack of information about their nutritional values. One of these is the Lamiaceae plants. In tropical countries such as India, Vietnam and Indonesia, Lamiaceae *sp.* is widely used as home remedies to treat and relieve fever, headache, cough, asthma and boil (Che Aniha, 2008). In Malaysia, the use of Lamiaceae plants as remedies was previously unknown and they are used instead as ornamentals. It is a surprise to realize that the people that grow them without knowing they are rich in nutrient. Lamiaceae *sp.* is highly beneficial to human health which is commonly used to flavor food, candy, teas, breath fresheners, antiseptic mouth rinses and toothpastes. Some part of the Lamiaceae also used as an herb or for medication purpose. This because it has some sort of antioxidant activity in the Lamiaceae species.

One of the productions techniques is by extraction. Extraction is a method of separating the constituents of a mixture utilizing preferential solubility of one or more components in a second phase. Commonly, this added second phase is liquid while the

mixture to be separated may be either in solid or liquid. However, how processing methods affect the health promoting antioxidant activity have not been systematically studied. For the practical application in industry, the antioxidant activity that has in the Lamiaceae plants should be first extracted. The efficiency of the extraction process affects the antioxidant activity of extract (Hinneburg and Neubert, 2005). Usually, in the Malay culture, traditionally, they have adopted the boiling method as the extract method in order to extract the useful component from plants/herbs in production of medicine or *jamu*. *Jamu* is a traditional medicine that is prepared from indigenous plants or herbs in the form of powder, pills, capsules, drinks and ointments. It is traditionally used to treat illness in the Malay Archipelago (Taher et al., n.d).

In this research, the boiling method is chosen as an extraction technique for the studies in the extraction of antioxidants activity in Lamiaceae plants. Boiling is most widely applied for the extraction as it is most sufficient and conventional technique and can save cost of production. Therefore, in this study, the extraction process on boiling Lamiaceae plants are developing in order to avoid damage on antioxidants activity and minimize percentage (%) loss of antioxidants activity in the plant. Furthermore, this research also investigates the effect of repeated boiling on the antioxidants activity of Lamiaceae plants and the scale up and optimization of this extraction method.

### **1.3 RESEARCH OBJECTIVES**

This study is to achieve the following objectives:

1. To study the effect on antioxidants activity of *ati-ati* (Lamiaceae *sp.*) plants extraction conducted by repeated boiling.
2. To scale up and optimize the effect of antioxidants extraction from *ati-ati* (Lamiaceae *sp.*) plants.

## 1.4 SCOPE OF RESEARCH

In order to achieve the objectives, the scopes were:

1. The plant materials were collected at the research plot near UMP Gambang Campus.
2. The method used to analyze antioxidants was 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. DPPH is preferable because it is generally a more cost efficient method to analyze antioxidant activity. The test is easy and needs only a UV-VIS spectrophotometer to perform. The DPPH assays also contribute the good reproducibility (Prior et al., 2005).
3. The antioxidant activity was chosen to be studied because it has many benefits to human health (Norman, 2008; Packer and Colmon, 1999; Hermani and Rahardjo, n.d).
4. The extraction of Lamiaceae plants was scale up and optimize by boiling in 20 L of distilled water combining with fresh Lamiaceae plants leaves. Hence, the antioxidant capacity is investigated.



## 1.5 SIGNIFICANCE OF RESEARCH

Many species of Lamiaceae plants are currently not threatened. However, habitats in which they occur are threatened by human impact and thus may change their status in future. Many species are endemic to restricted area and are regardless as rare because of their narrow distribution but are not considered as threatened at this stage. On the other hand, green leafy vegetables and other edible wild plants are considered to be “poor food” and have no extraordinary reputation even though many of these foods have been traditionally known to be health-promoting and have formed a component of the habitual traditional diets of populations of developing countries.

The benefits resulting from the use of natural products rich in bioactive substances has promoted the growing interest of pharmaceutical, food and cosmetic industries as well as of individual consumers in the quality of herbal produce. Halvorsen et al. (2002) demonstrated over a 1000 fold difference between total antioxidant in dietary plants and stressed the need for investigations on the role of processing and storage of raw materials in this respect. Among the important constituents participating in the cell defense system against free radicals are phenolic compounds and also ascorbic acid and carotenoids (Diplock et al., 1998; Gao et al., 2000; Szeto et al., 2002). Natural food provides the best nutrition as human bodies are adapted for natural food intake. Hence, it is expected that from this research it will affirm good habitual traditional diet among populations of developing countries.

## CHAPTER 2

### LITERATURE REVIEW

This literature review provides information of background to a particular topic. In this chapter, it explores the subtopic of introduction, herbs, extraction process *via* boiling, Lamiaceae plants, antioxidant activity and an overview of the assay methods used to estimate the antioxidant content.

#### 2.1 INTRODUCTION

Interest in natural antioxidants for both health and improvement of food stabilization has been intensified dramatically over a few years ago. Currently, food and health are synonyms to people especially for those who don't have time for exercise in their daily routine. Health is the precious gift by God to human as it the important aspect in human life. Serving food as a medicine is a current hot trend that is capturing everyone's imagination with images of a new "magic bullet" or "fountain of youth". The correct consumption of food should be practiced every day in order to obtain an optimum health. Consumption of nutritional food will make us have a strongest shield to dangerous diseases that could not easily treated.

Chemical constituents with antioxidants activity found in high concentrations in plants (Velioglu et al., 1998) determine their considerable role in the prevention of

various degenerative diseases (Challa et al., 1997; Diplock et al., 1998; Hu and Willett, 2002). Traditionally, antioxidants have been used to inhibit oxidation in foods as well as it also quench dreaded free radicals and stop oxidation chain in vivo. So, they have become viewed by many as nature's answer to environmental and physiological stress, aging, atherosclerosis and cancer. The fruit and vegetables 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 radicals (Madsen and Bertelsen, 1995), may have a beneficial effects (Lutomski, 2001).

There are varying amount of antioxidant can be found in food such as fruits, vegetables, eggs, grain cereals, nuts and etc. Some of the antioxidants such as ascorbic acid can be destroyed by long-term storage or prolonged cooking. Polyphenolic antioxidants are other example of the stable antioxidant compounds that can get in foods. The effect of cooking and food processing are complex as these processes can also increase bioavailability of antioxidants, such as some carotenoids in vegetables. In general, processed foods contain fewer antioxidants compared to fresh and uncooked foods. This is because the processed foods have been exposed to oxygen.

**Table 2.1:** Antioxidant Compounds in Foods

<b>Antioxidant Compounds</b>	<b>Foods Containing High Levels of Antioxidants</b>
Vitamin C (ascorbic acid)	Fresh fruits and vegetables
Vitamin E (tocopherols, tocotrienols)	Vegetables oils
Polyphenolic Antioxidant (resveratrol, flavonoids)	Tea, coffee, soy, fruit, olive oil, chocolate, cinnamon, oregano
Carotenoids (lycopene, carotenes, lutein)	Fruits, vegetables, eggs

For the food industry, moving to natural antioxidants is a potential goldmine that offers a “green” label for food stabilizers plus intriguing new opportunities for formulating for health and specific medical benefits. Antioxidants included such as vitamin E, C, zinc, selenium and carotenoids (Parker and Colman, 1999) can be found in hundreds of natural substances especially in fruits and vegetables. Regular consumption of fruits and vegetables are good dietary practice because they are rich with vitamins, minerals, fibers, and nutritional which are included in the group of antioxidants. According to Hermani and Rahardjo n.d., the spices also can be used as a protection of body health as well as can be use as medicines.

The nutraceutical trend towards doubling the impact of natural antioxidants that stabilize food and maximize health impact presents distinct challenges in evaluating antioxidant activity of purified individual compounds, mixed extracts, and endogenous food matrices and optimizing applications. Halvorsen et al. (2002) demonstrated over a 1000 fold difference between total antioxidants in dietary plants and stressed the need for investigations on the role of processing and storage of raw materials in this respect.

The important constituents participating in the cell defense system against free radicals are phenolic compounds, ascorbic acid and carotenoids (Diplock et al., 1998; Gao et al., 2000; Szeto et al., 2002). The antioxidative properties of carotenoids seem to be controversial and under certain conditions such as high oxygen pressure in the plant tissue can be converted into pro-oxidant activity (Larson, 1998). Herbs are used as fresh or dried with regardless of the purpose they serve. Enzymatic processes during drying fresh plant tissues may lead to significant changes in the composition of phytochemicals (Jambor and Czosnowska, 2002). The evaluation of antioxidant properties of the raw material allows the determination of its suitability as high quality food beneficial for human health. Therefore, it is considerable as an importance factor to human health.

## **2.2 HERBS**

Malaysia is among the finest country with herbs in the global where is listed as fifth in the world and fourth in Asia in plant diversity. There are about 15, 000 species of plants; each has its own use in the nutrition, medicine or even cosmetics (Che Aniha, 2008). Herbs are plants which have some soft stems, aromatic and used as food in fresh or dried in order to promote general health. There are benefits of using herbs because each herb has their own function to human health. This is based upon the people who have used it throughout the ages and from information obtained from traditional medicine users.

An herb is the plant that is valued for flavor, scent, medicinal and food which are widely use in cooking, traditional medicines and also spiritual purposes. Commonly, herbs are significant to medicinal purposes. Herbs have been long used as the basis of the traditional Chinese herbal medicine. The main contain of the herbs is it own *phytochemicals* that have effects on the body immune system. It is good to use herbs in our daily life but the amount of usage must be controlled. Large amounts of herbs usage may be lead to toxic overload in our body system and might involve complications, which is some of serious nature and should be used in caution.

Traditionally, Malay natives already had scientific knowledge in natural healing and the treatment system of the body. People in the olden days took traditional medicines that were made from herbs, jungle plants and wild plants. They have used various types of plant-based ingredients in treating various types of diseases. The ingredients can be used fresh, or active constituents from the plant essence can be extracted to be mixed with other extracts to produce a final medicine drink (Hermani and Rahardjo, n.d).

Presently, aborigines are still using wild plants that are found in the jungle to heal themselves from diseases or to avoid certain diseases. It is not surprising that dangerous diseases like malaria do not touch aborigines even though they are exposed to mosquitoes in their settlements. Herbs like *Tongkat Ali* (*Eurcyoma longifolia*) and *Kacip Fatimah* (*Labisia pumilia*) are commonly used by them. There may be many more herbs in the jungle that have not revealed by them.

### 2.3 EXTRACTION PROCESS *via* BOILING

In chemical engineering and related fields, unit operation is a basic step in a making a process. Chemical engineering unit operations and chemical engineering unit processing form the main principles of all kind of chemical industries. It also as a foundation of designs of chemical plants, factories and equipment used. Fluid flow processes, heat transfer processes, mass transfer processes, thermodynamic processes and mechanical processes are some of the classification in the unit operation. Extraction is the mass transfer processes.

Extraction is a method of separating the constituents of a mixture utilizing preferential solubility of one or more components in a second phase. Commonly, this added second phase is liquid while the mixture to be separated may be either in solid or liquid. Depending on the nature of the extraction process, the temperature, pH resistance and residence time could have an effect on the yield and selectivity. The extraction efficiency is affected by multiple variables such as extraction temperature, extraction time, and solvent composition (Wettasinghe and Shahidi, 1999). Operating pressure has a negligible effect on extraction performance. Therefore, most extractions take place at atmospheric pressure unless governed by vapor pressure considerations. Temperature can also be used as a variable to alter selectivity. Elevated temperatures are sometimes used in order to keep viscosity low and thereby minimizing mass-transfer resistance. Other parameters to be considered are selectivity, mutual solubility, precipitation of solids and vapor pressure. Furthermore, residence time is an important parameter in reactive extraction processes and in processes involving short-life components.

In this extraction technique, boiling method is chosen in order to get the extraction product. Boiling is the process in which a liquid phase is converted into a vapor phase. The energy for phase change is generally supplied by the surface on which boiling occurs. Boiling differs from evaporation at predetermined vapor/gas-liquid interfaces because it also involves creation of these interfaces at discrete sites on the

heated surface. Boiling is an extremely efficient process for heat removal and is utilized in various energy-conversions, heat-exchange systems and in the cooling of high-energy density components. Boiling bring about a number of changes in physical characteristics and chemical compositions in Lamiaceae. As a result, it will affect the changes in the antioxidants activity in Lamiaceae due to the effect of temperature and residence time.

## **2.4 LAMIACEAE PLANTS**

Lamiaceae or Labiatae are well-known as the mint family. It is classified as family of plants. It is a cosmopolitan family with many well-known members of horticultural and economic importance. The Lamiaceae families were established by De Jussieu in 1789 as the Order Labiatae and were changed to Lamiaceae after the genus *Lamium* by botanists. Lamiaceae is highly beneficial to human health. Lamiaceae plant which is one of the traditional medicines is believed to have originated from Indonesia and Africa as a plant that is commonly used as ornaments in domestic compounds. It is also known to be used as traditional remedies.

Lamiaceae plants are herbaceous perennials in their native habitat, with thick stems and stalks which can reach up to one meter tall. The species with red leaves is very nutritious while species with other colours are usually used as ornamentals (Ong, 2008). It has the unique characteristic of opposite leaves. The shape of its leaves form the shape of a heart with a potential edge and coarsely toothed in the margins. The coloured foliage for which Lamiaceae is so famous include red, pink, purple, green, yellow, orange, brown and all shades in between (Ombrello, 2008).

Commonly, Lamiaceae are used to flavor food, candy, teas, breath fresheners, antiseptic mouth rinses and toothpastes. Some part of the Lamiaceae also used as an herb or for medication purpose. This because it has some sort of antioxidant activity in the Lamiaceae. The evaluation of antioxidant properties of the raw material allows the



determination of its suitability as high quality food beneficial for human health and therefore is of considerable importance.

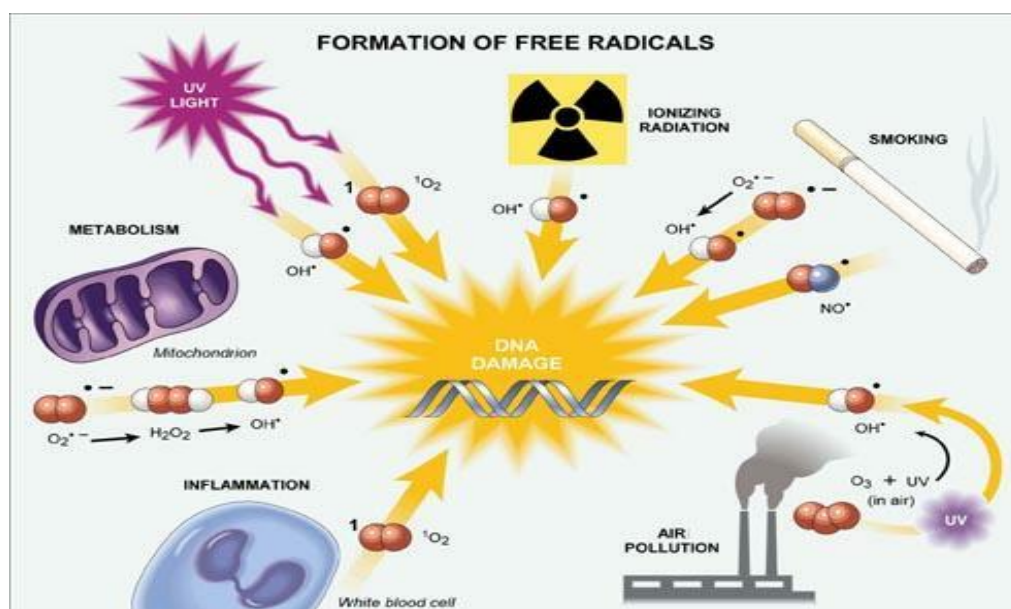


**Figure 2.1:** Lamiaceae *sp.* Plants

## 2.5 ANTIOXIDANTS ACTIVITY

Antioxidant is a molecule capable of preventing the oxidation of other molecules which is the chemical reaction of oxidation will transfer the electrons from a substance to an oxidizing agent. It is defined as compounds that fight metal corrosion and all processes involving oxidation (Norman, 2008). 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 electrons, it becomes a free radical itself (Surh and Packer, 2004).

Oxidation reactions can produce free radicals which start chain reactions that could damage cells. These chain reactions will be terminated by antioxidants with removing free radical intermediates and inhibiting other oxidation reactions by being oxidized themselves. In other words, free radicals are incomplete molecules and unstable because they lack an electron. When these molecules lose their electron resulting from a chemical reaction or external factor, they become free radicals which are unstable and reactive. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.



**Figure 2.2:** Formation of Free Radicals

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 diseases and even cancer (Norman, 2008). Research has shown that medicinal plants exhibit antioxidant (Nam and Kang, 2004; Katalinic et al., 2006; Kiselova et al., 2006) as well as antimicrobial (Chan et al., 2008) activity. Because of their antibacterial and antioxidant properties, herbs are used as natural food and cosmetics preservations (Chan et al., 2007; Özkan et al., 2007) and are considered in formulation of new functional food products (Kiselova et al., 2006).