ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF SOME MALAYSIAN HERBS

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Date : 20 January 2011

To my beloved parents, sister, families and friends…

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بسم الله الرحمن الرحيم
In the name of Allah SWT, most Grateful and most Merciful

Alhamdulillah, all praise goes to Him, Allah Almighty for giving me everything that I have today; families, friends, knowledge, opportunities and inysa-Allah, a bright future. With His blessing, I finally able to finish this research successfully.

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ABSTRACT

Antioxidant, usually found in food has the ability to prevent the oxidative damage to body cell by trapping free radicals, a highly unstable molecule. In this research, the antioxidant activity of extract from three selected Malaysian herbs was investigated. The herbs were *Euodia redlevi* (Tenggek Burung), *Cosmos caudatus* (Ulam Raja) and *Premma cordiflora* (Bebuas). Besides, the Vitamin C content in the herbs was also determined. The herbs were collected from Tanjung Sedili (Kota Tinggi, Johor) and Kuantan (Pahang). The herbs were extracted using sonication extraction method at different interval of times (30, 60, 90 and 120 minutes) and at different concentrations of solvent (50%, 70% and 90% of ethanol). After the extraction, the extracts were analyzed for total phenolic content using Folin-Ciocalteu's reagent and antioxidant activity using DPPH assay. The results show that the three herbs exhibited significant value of ascorbic acid content. The highest amount of ascorbic acid was obtained in *Cosmos caudatus* in 60 minutes of extraction using 70% of ethanol. As for *Premma cordiflora* and *Euodia redlevi*, the highest amount of ascorbic acid were obtained in 90 minutes of extraction using 90% of ethanol. The herbs extracts of the said parameters were analyzed again for their ascorbic acid or vitamin C content using High Performance Liquid Chromatography (HPLC). The results showed that *Cosmos caudatus* has the highest vitamin C content, followed by *Premma cordiflora* and finally *Euodia redlevi*. This research provides the information on the promising source of natural antioxidants in Malaysia.

ABSTRAK

Bahan antioksida, biasanya ditemui dalam makanan mempunyai keupayaan untuk mencegah kerosakan oksidatif kepada sel-sel badan dengan memerangkap radikal bebas, molekul yang sangat tidak stabil. Dalam kajian ini, aktiviti antioksida bagi ekstrak tiga herba terpilih Malaysia telah dikaji iaitu herba *Euodia redlevi* (Tenggek Burung), *Cosmos caudatus* (Ulam Raja) dan *Premma cordiflora* (Bebuas). Selain itu, kandungan Vitamin C di dalam herba-herba ini juga telah dianalisis. Herba-herba ini diperolehi dari Tanjung Sedili (Kota Tinggi, Johor) dan Kuantan (Pahang). Herba-herba ini diekstrak menggunakan kaedah pengekstrakan gegaran ultrasonik pada selang masa yang berbeza (30, 60, 90 dan 120 minit); juga pada kepekatan pelarut untuk proses pengekstrakan yang berbeza (50%, 70% dan 90% etanol). Selepas proses pengekstrakan, ekstrak dianalisis untuk kandungan jumlah fenol menggunakan reagen Folin-Ciocalteu dan aktiviti antioksida dengan menggunakan ujian DPPH. Hasil kajian menunjukkan bahawa ketiga-tiga herba mengandungi asid askorbik. Jumlah tertinggi asid askorbik telah diperolehi dalam ekstrak *Cosmos caudatus* dalam 60 minit proses pengekstrakan menggunakan 70% etanol. Sementara itu, bagi *Premma cordiflora* dan *Euodia redlevi*, jumlah tertinggi asid askorbik telah diperolehi dalam 90 minit proses pengekstrakan menggunakan 90% etanol. Ekstrak herba menggunakan parameter yang telah menghasilkan kandungan tertinggi bagi askorbic asid atau vitamin C dianalisis semula bagi kandungan asid askorbik atau vitamin C menggunakan High Performance Liquid Chromatography (HPLC). Hasil kajian menunjukkan bahawa *Cosmos caudatus* mempunyai kandungan vitamin C yang tertinggi, diikuti oleh *Premma cordiflora* dan akhirnya *Euodia redlevi*. Kajian ini berjaya menyediakan maklumat baru mengenai sumber antioksida semulajadi di Malaysia.

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LIST OF SYMBOL

%	-	Percentage

LIST OF ABBREVIATION

GAE	-	Gallic Acid Equilibrium
TPC	-	Total Phenolic Content
AA	-	Ascorbic Acid
DPPH	-	1,1-Diphenyl-2-picrylhydrazyl
HPLC	-	High Performance Liquid Chromatography
min	-	Minutes
mg	-	Milligrams

CHAPTER 1

INTRODUCTION

1.1 Research Background

Antioxidant is very important in human's life. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease (Strand, 2002). The diseases are mainly cause by free radicals, a by-product from respiration and other metabolism activity that can cause oxidative damage to cell's body. Free radicals may also come from smoking, pollutions, poisons and fried-foods. Other than that, components of antioxidant like Vitamin C and E are not only good for health but also been used in cosmetic industry for years.

Herbs like *Euodia redlevi* (Tenggek Burung), *Cosmos caudatus* (Ulam Raja) and *Premma cordiflora* (Bebuas) have been used for traditional medical purposes and consumed by Malay people for many years. *Euodia redievi* has been used for years as therapy for hypertension and *Premma cordiflora* has been used traditionally as therapy for traditional massage and also been used to enhance the production of mother's milk, especially among mothers who just deliver their baby. *Cosmos caudatus* is believed by Malay people traditionally as dietary supplement that can benefit their health (Rasdi et al., 2010). These herbs can be easily collected as they are abundantly available around the village and also around the rainforest areas.

1.2 Problem Statement

Free radicals, known as oxidative stress are organic molecules responsible for aging, tissue damage and also cause of almost all of chronic generative diseases (Strand,

2002). The examples of these diseases are cancer, heart problems kidney diseases and many others. These molecules are very unstable, therefore they look to bond with other molecules, destroying their vigor and perpetuating the detrimental process. Antioxidants, present in many foods, especially fresh food like vegetables and fruits, specifically in vitamins found in those particular vegetables or fruit like vitamin A, E and beta-carotene, are molecules that prevent free radicals from harming healthy tissue (Tsang, 2005).

In this study, *Euodia redlevi*, *Cosmos caudatus* and *Premma cordiflora* were chosen because there are no scientific evidences on their actual potential in antioxidant activity. The herbs are normally used based on traditional beliefs. For example, *Euodia redlevi* is long use by the locals for its nutritional value that helps to treat hypertension, information or nutrition value that has never proven, not even until now. *Premma cordiflora* is a herb that has been used for such a long time by the locals as one of follow-up therapy for traditional massage and also to enhance the production of mother's milk, especially among mothers who just deliver their baby. The effects may be associated with the antioxidant properties contained in these herbs.

1.3 Research Objectives

The objective of this research is to investigate the antioxidant activity of ethanolic extract of three selected Malaysian herbs which is *Euodia redievi* (Tenggek Burung), *Cosmos caudatus* (Ulam Raja) and *Premma cordiflora* (Bebuas) by using sonication extraction method with different time of extraction (30, 60, 90 and 120 minutes) and ethanol with different concentration (50%, 70% and 90%) as the solvent of extraction.

1.4 Research Scopes

i. Identifying the phenolic content of ethanolic extract of *Euodia redievi*, *Cosmos caudatus* and *Premma cordiflora* by using different intervals of extracting time using sonication method of extraction and also different concentration of solvent for the extraction process.

ii. Identifying the antioxidant activity of ethanolic extract of *Euodia redievi*, *Cosmos caudatus* and *Premma cordiflora* by using different intervals of extracting time using sonication method of extraction and also different concentration of solvent for the extraction process.

iii. Determining the composition of Vitamin C from the ethanolic extracts by using High Performance Liquid Chromatography (HPLC).

1.5 Significance of Research

The finding of this study will provide the promising sources of potential antioxidant in some Malaysian herbs for future studies and also, through this research, the new natural antioxidant therapy could be produced in promoting health and wellness.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In this chapter, subjects that are vital to this research will be elaborated in detail based on numbers of references that are related and useful to this research. Subjects like types of the extraction process and the solvent used in this research, herbs used in this research which are *Euodia redlevi* (Tenggek Burung), *Cosmos caudatus* (Ulam Raja) and *Premma cordiflora* (Bebuas) will be elaborated in detail, covering aspects like the significance and reason behind the decision of choosing these herbs, the traditional usage and previous research regarding to these type of herbs (if any). The other things that are also being covered in this chapter are about antioxidant activity, phenolic content and the relationship between these two matters and also, vitamin C.

2.2 Ethanolic Extract

2.2.1 The Extraction Process

By definition, extraction process is a separation process consisting in the separation of substance from a matrix. There are two general type of extraction, the first one is liquid-liquid extraction and the other one is solid-liquid extraction. Liquid-liquid extraction, also known as solvent extraction and partitioning is a method used to separate compounds based on their relative solubility in two different immiscible liquids. Examples of this type of solvent are water and organic solvents. Generally, it is an extraction of a substance from one liquid phase into another liquid phase. Liquid-liquid extraction is a basic technique in chemical laboratories, where it is performed

using a separator funnel. Solvent extraction can also be referred as the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. In this case, a soluble compound is separated from an insoluble compound or a complex matrix.

Solvent extraction can also be referred as the separation of a substance from a mixture by dissolving that substance in a suitable solvent. In this case, a soluble compound is separated from an insoluble compound or a complex matrix. Solvent extraction is used in many industrial fields such as nuclear and ore processing, the production of fine organic compounds like vitamins, the processing of perfumes, the production of vegetable oils and biodiesel, and many other industries involving chemical type production.

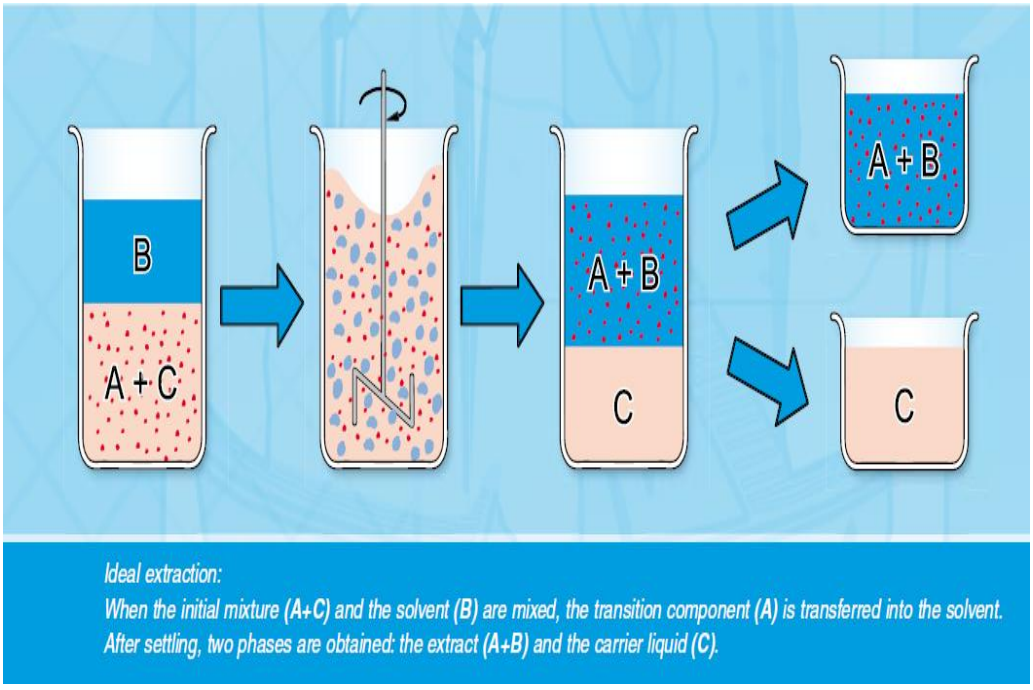

Figure 2.1: Liquid-liquid Extraction (Sarawagi, 2007)

In some special cases of liquid-liquid extraction, liquid-liquid extraction is also possible to be done in non-aqueous systems. For example, in a system consisting of a molten metal in contact with molten salt, metals can be extracted from one phase to the other. This situation is much related to a mercury electrode where a metal can be

reduced, the metal will often then dissolve in the mercury to form an amalgam that modifies its electrochemistry of the metal greatly. For example, it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations are not reduced. Instead, water is reduced to hydrogen. A detergent or fine solid can be used to stabilize an emulsion, or third phase (Sarawagi, 2007).

The other type of extraction is solid-liquid extraction. Basically, solid-liquid extraction allows soluble components to be removed from solids using a solvent. Applications of this unit operation include obtaining oil from oil seeds or leaching of metal salts from ores. Basically, an easy example of solid-liquid extraction is an everyday example coffee preparation. Coffee preparation involves water as solvent and the water (solvent) is used to ‘transfer’ the coffee flavours (transition component), nutritional value and also colour and texture from the coffee powder (extraction material, consisting of solid carrier phase and transition component) to the solvent, in this case, water. Ideally, this results in drinkable coffee (solvent with dissolved flavours), with the completely depleted coffee grounds (solid carrier phase) remaining in the coffee filter. In reality, the solid carrier phase will still contain some transition component after completion of the extraction. In addition, some of the solvent will still be adsorptive bonded to the solid carrier phase.

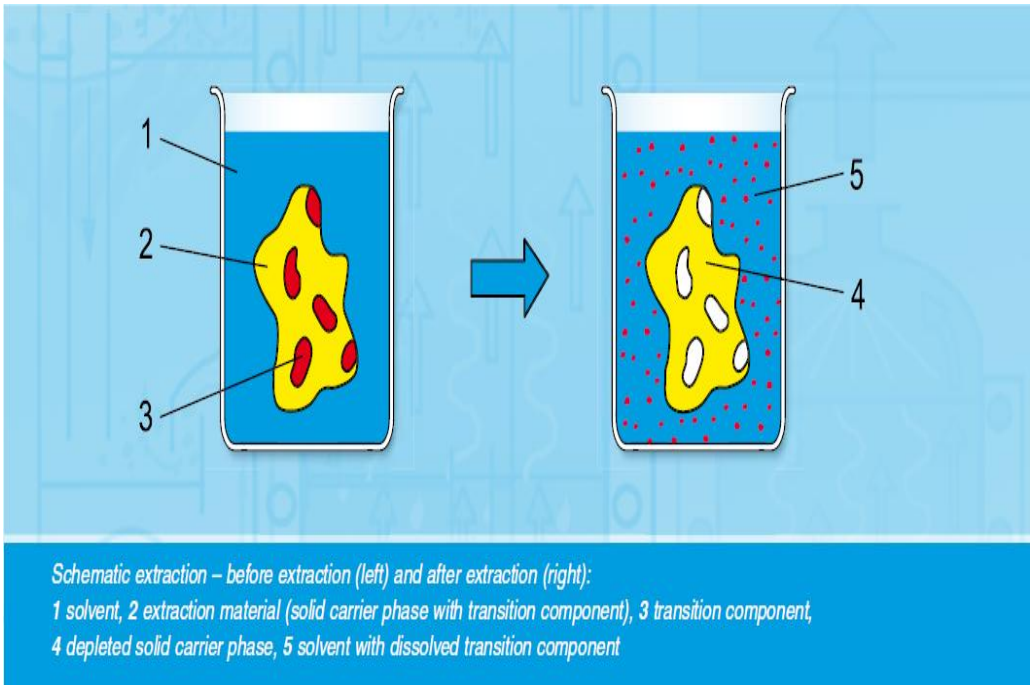

Figure 2.2: Solid-liquid Extraction (Sarawagi, 2007)

To achieve the fastest and most complete solid extraction possible, the solvent must be provided with large exchange surfaces and short diffusion paths. This can be done by pulverizing the solid to be extracted. However, an excessively small grain size can cause agglutination and make it more difficult for the solvent to permeate. In the simplest form of this unit operation, the extraction material and the solvent are mixed well. The solvent and the dissolved transition component are then removed and regenerated. The extraction material can also take the form of a fixed bed with the solvent flowing through it. In a further form of the application, the extraction material is led through the solvent. The solvent is normally regenerated using evaporation/distillation. The solvent is evaporated and a concentrated extract solution is left behind as the product. The solvent is condensed and can then be reused.

Aside from the two general type of extraction, both of it can done using numbers of possible method of extraction. There are many type of extraction method such as supercritical carbon dioxide extraction, ultrasonic extraction, heat reflux extraction or microwave-assisted extraction (Pan *et al.*, 2003).

The usual, most common method of extraction procedures for the isolation of organic compounds of medicinal plants are maceration, percolation, and Soxhlet extraction (Barboza *et al*., 1992). These techniques usually require long extraction times, providing low efficiencies and consume a lot of energy. Moreover, many natural products are thermally unstable and may degrade during conventional processes where the matrix is kept in a boiling solvent, such as Soxhlet extraction. Many reports on the beneficial effects of medicinal plants ultrasound extractions have been published, and the main reported improvements have been found to be enhanced efficiency and shortening of extraction time with ultrasound extraction (Schinor *et al*., 2004).

By ultrasound vibration, the effects of the collapse of cavitations bubbles produced by ultrasound on the cell walls of plants can best be described as follows; First, numbers of plant cells have its own glands filled with essential oil and one characteristic of external glands that makes their skin is very thin and can be easily destroyed by ultrasonic vibration, the vibration thus facilitate the release of essential oil contents into the extraction solvent (Huie, 2002). On the other hand, ultrasound vibration or sonication can also facilitate the swelling of plant materials which may cause the enlargement of the pores of the cell wall, wider than its original size, thus helping the extraction process by easing the release of essential oil containing target compound into the extraction solvent. Better swelling will improve the rate of mass transfer and occasionally, break the cell walls, thus resulting in increased extraction efficiency and/or reduced extraction time. Other advantages of this technique are its high reproducibility, the possibility of using a wide range of sample sizes and the low cost of the whole process (Luque-Garciaa *et al*., 2004).

The optimization of ultrasound vibration or sonication operational parameters such as solvent polarity, sample particle size, and others according to a specific plant matrix like leaves, flowers, stems and roots is also the main importance in order to achieve high extraction efficiency (Toma *et al*., 2001).

2.2.2 Ethanol as Solvent for the Extraction Process

As for the properties first, ethanol (also known as ethyl alcohol or grain alcohol) is a clear, colorless liquid with a characteristic, agreeable odor. In dilute aqueous solution, ethanol has a somewhat sweet flavor, but in more concentrated solutions it has a burning taste. Ethanol, CH_3CH_2OH, is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom. The word alcohol derives from Arabic ‘al-kuhul’, which denotes a fine powder of antimony used as an eye makeup. Alcohol originally referred to any fine powder, but late alchemists in medieval times later applied the term to the refined products of distillation, and this led to the current usage as a beverages and many other usage. The physical properties of ethanol is it melts at –114.1°C, boils at 78.5°C, and has a density of 0.789 g/mL at 20°C. Its low freezing point has made it useful as the fluid in thermometers for temperatures below –40°C, the freezing point of mercury, and for other low-temperature purposes, such as for antifreeze in automobile radiators.

Ethanol also has been long known as its function as one of the best extraction solvent since it has bi-polar properties in nature. This property has made ethanol being proved to be the best solvent for herbs extraction (Kumar *et al*., 2008).

2.3 The Herbs

Within the past decades, researchers around the globe have started to identify the potential value of fruits and vegetables beyond their well-known contribution in helping people preventing themselves from vitamin deficiencies. A significant number of phytochemicals have also been found in fruits, vegetables and other plant food-sources, which is linked to the reduction of risk of disease. As an example, the anti cancer potential obtained from fruits and vegetables have highly been promoted recently, but fruits and vegetables are not only able to decrease the chances of developing cancer, they also able to decrease the risk of developing coronary heart disease and strokes. Other than that, compounds obtained from fruits and vegetables have also an anti-aging compounds.

Malaysia, a well known highly diverse tropical country is among the world's top twelve hotspots in the field of biodiversity. Malaysia, located within the equator intersection is richly endowed with a profusion of diverse Flora which potentially may have immense benefits to people from generations to other generations. Recent scientifically accepted ethno-botanical studies suggest that at least 20% of the estimated 12,000 higher plant species in Malaysia may possess either medicinal or therapeutic properties. With Malaysia's richly abundant natural tropical rainforest, the opportunity exists to identify, catalogue and study the vast number of indigenous flora at our own country and hence uncovering their hidden secrets and true potential which can contribute not only to the people of Malaysia, but also people around the globe and ultimately, benefiting our country in term of economy. The screening, analysis and processing of native Flora has already been successfully carried out on *Eurycoma Lingifolia* (Tongkat Ali), *Orthosiphon Staminues* (Misai Kucing) and *Labisia Pumila* (Kacip Fatimah), just to name a few. Because of this, three herbs have been selected to be analyze in this research. The herbs are; *Euodia redlevi* (Tenggek Burung), *Premma cordiflora* (Bebuas) and *Cosmos caudatus* (Ulam Raja).

Euodia redlevi (Tenggek Burung), *Premma cordiflora* (Bebuas) and *Cosmos caudatus* (Ulam Raja) has been used widely among Malay people not only as food but also for traditional medical purposes. For example, *Euodia redlevi* (Tenggek Burung) has been used for years as therapy for hypertension. Other than that, *Premma cordiflora* (Bebuas) has been used traditionally as one of follow-up therapy for traditional massage and also been used to enhance the production of mother's milk, especially among mothers who just deliver their baby. Finally, *Cosmos caudatus* (Ulam Raja) has been used traditionally as additional food that can remove toxic in blood stream. Among all of these three selected herbs, only *Cosmos caudatus* (Ulam Raja) has been prove scientifically to contain some amount of antioxidant (Shui *et al*., 2005) but in this research, different method of extraction may enhance the obtaining value of antioxidant from the very same herbs.

2.3.1 *Euodia redlevi*

Tenggek Burung, or its scientific name, *Euodia redlevi* has been long used by the Malay natives in Malaysia as a daily consumed food aside from its traditional medical potential in healing the wound caused by delivery for a new mother and also improve the mother's womb condition which the natives also believed. Unfortunately, this potential has never before being prove scientifically. That is why in this research, this herb is chosen to be analysis so that after this there will be at least a reference for future and deeper analysis toward this herb.

This herb has been collected for extraction and other analysis process from a countryside area, specifically at Kampung Teluk Jeri Luar, Tanjung Sedili, Kota Tinggi, Johor where this kind of herbs can be easily obtained since it is abundant here.

2.3.2 *Cosmos caudatus*

Ulam Raja, or its scientific name, *Cosmos caudatus*, has been used traditionally as additional food by the natives around Malaysia especially among Malay people. It is believed among the natives that it has the ability to remove harmful impurities in blood stream. Among all of these three selected herbs, only this herb has been prove scientifically to contain some amount of antioxidant (Shui *et al*., 2005) but in this research, different method of extraction may enhance the obtaining value of antioxidant from the very same herbs.

Unlike the other two herbs chosen to be analyzed in this research, *Cosmos caudatus* has been scientifically prove to contain a significance amount of antioxidant. The reason behind the decision to include this herb in this research is because it can provide a guide to the extraction process and also analysis process of the other two herbs. Aside from that, with most research used the traditional extraction method using Soxhlet extraction method, in this research a bit different extraction method is approached which is ultrasonic extraction.

2.3.3 *Premma cordiflora*

Much like the first herb, *Euodia redlevi* (Tenggek Burung), *Premma cordiflora* (Bebuas) has been collected at the same place where *Euodia redlevi* (Tenggek Burung) is obtained (at Kampung Teluk Jeri Luar, Tanjung Sedili, Kota Tinggi, Johor). The reason behind the decision of choosing this herb for further analysis is because it has lack scientific prove to its actual potential.

This type of herb is also being used as native Malay people as a daily consumed food. It is also being used traditionally for medical purposes. For example, it has been used for generations among Malay people as an additive to a traditional oil massage and also being traditionally extract by boiling it into a jar of water to produce a dark green type of drink to helps the new mother enhance their milk productivity. Unfortunately, much like Tenggek Burung or *Euodia redlevi*, the scientific proves toward its medical potential is very hard to find or can be assumed as not even scientifically prove yet. That is why; the research using this type of herb will add more useful information for future analysis or even can be use as a source of production of antioxidant treatment product like collagen in the future. Table 2.1 summarizes on the traditional use and scientific proofs of selected herbs:

Table 2.1: Summary of traditional usage, scientific proves and its reference of all three herbs (*Euodia redlevi, Premma cordiflora* and *Cosmos caudatus*)

Herbs	Traditional usage / medical purposes	Scientific prove / biological activity	References
***Euodia redlevi* (Tenggek Burung)**	Therapy for hypertension/high blood pressure	Nil	Nil
***Premma cordiflora* (Bebuas)**	An additional ingredients to massage oil for traditional massage among the new-delivered mother and also being extract traditionally using boil water to be drink by the new mother to enhance their milk production	Nil	Nil
***Cosmos caudatus* (Ulam Raja)**	Has the ability to remove impurities (harmful impurities) in the blood stream	Contains significant amount of antioxidant	Shui *et al*., 2005

2.4 Phenolic Compounds

It has been known for such a long time that phenolic compounds are effective antioxidants and because of this, phenolic compounds have been used for decades as industrial antioxidants. Chemically, phenol comprises a benzene ring and an alcoholic hydroxyl group. Phenolic compound was originally called carbolic acid. Phenolic compound has never been used before until in 1865, Joseph Lister used it as an antiseptic in 1865. Although phenol is an alcohol, it does not behave like typical alcohols like ethanol and because of this, phenol has been categorized in its own family.

In contrast with typical alcohols, phenols are more acidic with the pKa value of 9.95. The phenolic compounds are able to eliminate free radicals because of two factors. The first one is the compound's acidity (which make them able to donate protons) and the second one is its delocalized π-electrons (which make them able to transfer electrons while remaining relatively stable) characteristic of benzene rings. The electron delocalization process and ease of ionization are probably responsible for their bright colours.

In general, all plants produce diversity of secondary metabolites. One of the most important groups of these metabolites is phenolic compounds. Phenolic compounds are characterized by having at least one aromatic ring (C6) bearing one or more hydroxyl groups. Basically, the phenolic compounds are synthesized from cinnamic acid (a kind of organic acid), which is formed from phenylalanine by the action of L-phenyloalanine ammonia-lyase PAL (EC 4.3.1.5), which is the branch point enzyme between primary (shikimate pathway) and secondary (phenylopropanoid) metabolism. The significance of this route reaction can be supported as in fact, in normal growth conditions, 20% of carbon is fixed by plants flows through this pathway (Diaz *et al*., 2001).

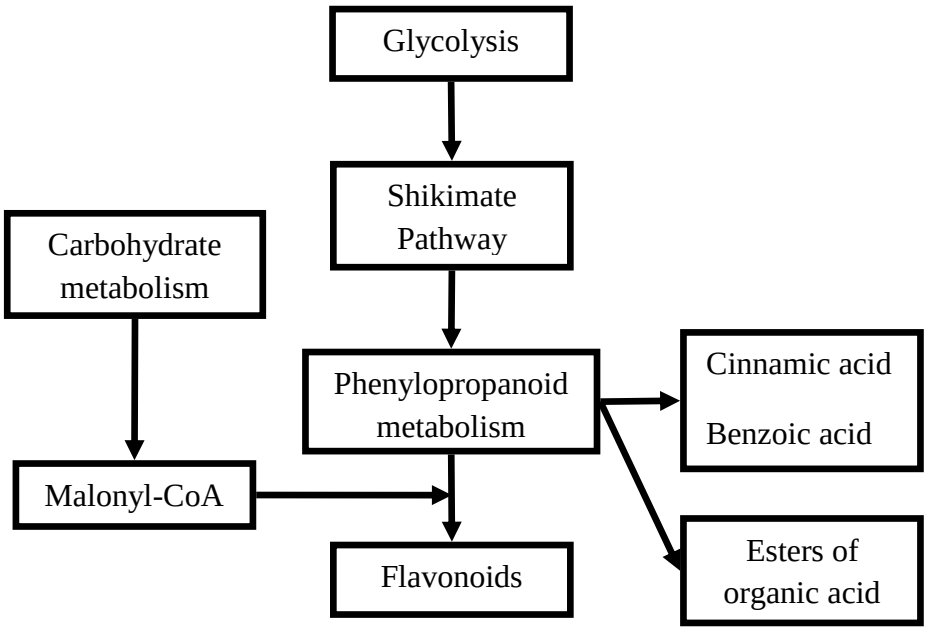

Figure 2.3: Biosynthesis pathways leading to formation of main groups of phenolic compounds (Ryan *et al*., 1999)

2.5 Free Radicals and Antioxidant

Human beings have the ability to utilize the oxygen with benefit of metabolizing fats, proteins and carbohydrates for energy. However, the process did not leave humans without tiny bad consequences. As a fact, oxygen is a highly reactive atom that is capable of becoming free radicals. The reactive oxygen species is an example of this bad consequence.

On the other hand, oxidative stress occurs when there is an imbalance between generation of reactive oxygen species and not enough antioxidant in balance to act as defense systems to avoid cell damage. The consequence of oxidative stress may be oxidative damage of lipids, proteins, and DNA, with subsequent disease development and aging (Finkel *et al*., 2000). The reactive oxygen species production is a result of exogenous factors. The factors are like radiation and drug exposure which might increase the mitochondrial respiration and oxidative enzymes in infections and inflammation.

Free radicals (also share the same term with oxidative stress) on the other hand are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Over the years, cell damage which is cost by free radicals appears to be a major factor of aging and several numbers of degenerative diseases of aging like cataracts, cancer, immune system decline, brain dysfunction and also cardiovascular disease. In general, free radicals have been implicated in the pathogenesis of at least 50 diseases (Strand, 2002). In Malaysia alone, the deaths of people cause by chronic diseases possibly caused by free radicals are increased from day to day.

Disahkan *Medically certified*	%	**Tidak Disahkan** *Not medically certified*	%
1. Ischaemic heart disease	12.8	1. Sakit tua 65+ *Old age 65+*	59.0
2. Cerebrovascular disease	6.8	2. Sakit jantung *Heart disease*	7.1
3. Septicaemia	6.7	3. Barah *Cancer*	7.1
4. Pneumonia	6.0	4. Lelah *Asthma*	6.9
5. Transport accident	5.8	5. Kencing manis *Diabetes*	3.3
6. Chronic lower respiratory disease	2.4	6. Jangkitan kuman *Viral infection*	2.0
7. Malignant neoplasm of trachea, bronchus and lung	2.2	7. Darah tinggi *Hypertension*	1.6
8. Diabetes mellitus	1.8	8. Sakit buah pinggang *Kidney disease*	1.1
9. Certain conditions originating in the perinatal period	1.7	9. Penyakit berjangkit *Infectious disease*	0.9
10. Diseases of the liver	1.3	10. Lumpuh *Paralysis*	0.8
Keseluruhan sebab *All causes* (71,030)		**Keseluruhan sebab** *All causes* (47,137)	

Figure 2.4: Death causes in Malaysia for the year of 2009 (Department of Statistics Malaysia, 2009)

Free radicals are also known for its highly unstable molecules that attempt to achieve more stable state by reacting with other molecules or atom and as a result, damaging the cell (Wu *et al*., 2003). Although the initial capturing electron process causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. This chain reaction is very dangerous because until the subsequent free radicals are deactivated, within only seconds, thousands of other free radicals can be formed.

There are four types of chemical reaction that free radicals tend to undergo. The first one is hydrogen abstraction, a reaction where a radical interacts with other molecules that contain free hydrogen atom or a highly potential of hydrogen donor molecule. The other chemical reaction is addition. This type of reaction involves a free radical, reacting with other originally stable molecule which will produce a new form of unstable free radical originating from the previously stable molecule. The other type of reaction is termination. Termination reaction will cause two free radicals transform into much more stable state by reacting with each other. Lastly, the final reaction is disproportionate in which two identical free radicals; react with each other, forming two new stable molecules by donating an electron from a free radical to another. Fortunately, the problem of free radical formation can be controlled naturally by various beneficial compounds of antioxidants.

Antioxidants are capable of deactivating and also stabilizing the free radicals, produced after the chain reaction before it attacks the cells in human body. In general, antioxidants are absolutely important for maintaining optimal cellular and systemic health. There are several numbers of known antioxidants that can be found from several different kind of food like vitamin A and carotenoids, vitamin C, E and selenium. Some antioxidants have the ability to actually regenerate other antioxidants so they can neutralize more free radicals. For example, in this case, Vitamin C. Vitamin C is water soluble and is therefore the best antioxidant to target free radicals within blood and plasma (Strand, 2002).

2.6 Vitamin C

Vitamin C, also known as ascorbic acid, ascorbate or AA is a water-soluble organic compound involved in many biological processes. Although all the functions of Vitamin C have not been fully understand or even discovered yet, one thing can be sure is it is also involved in maintaining the reduced state of metal cofactors, for example, monooxygenase (Cu^{2+}) and dioxygenase (Fe^{2+}). In cells, the other role of Vitamin C is to reduce hydrogen peroxide (H_2O_2), which preserves cells against oxidative damage cause by free radicals. Primates and several other mammals are not able to synthesis ascorbic acid by itself, therefore the only way humans can obtain ascorbic acid is via food, but, the exact daily requirements of vitamin C for humans are not yet clear (Mitic *et al*., 2011).

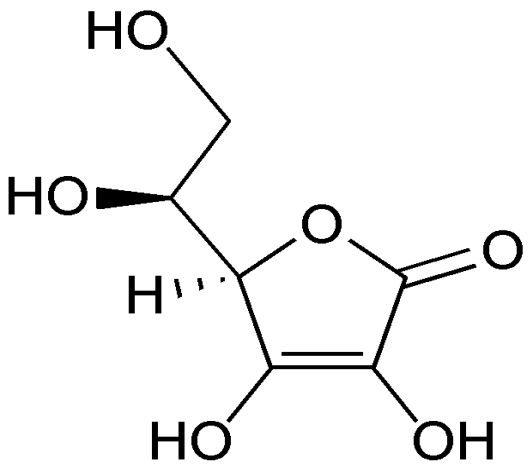

Figure 2.5: The chemical structure of Vitamin C (Mitic *et al*., 2011)

Vitamin C has been studied on many occasions not only on its properties but also in healthcare area. The founding of these research shows that vitamin C is able to act as a preventative or even curative effects in the case of many diseases. Vitamin C is also the most important antioxidant components available in human body. The vitamin C antioxidants neutralize free radicals and slow down the aging process of our bodies. For example, vitamins C can neutralizes the free radicals in aqueous parts in and around the body cells, thus preventing the cells from damage or even avoid the cells to aging rapidly.

As it is proven that free radicals can caused a large numbers of diseases, antioxidant also has been prove to be the counter effect of free radicals and vitamin C, is one of the antioxidant compounds. There are many known function of vitamin C that can help people around the globe improve their health condition. As an example, the eye needs large quantities of vitamin C. If too many free radicals form in the eye area, the eye lens will become cloudy and hence people with this problem will have a disease called cataract. This disease in fact can be treated through regular ingestion of vitamin C. This continuous consumption of vitamin C will make the clouding of the lens be reduced by 40-50%. Other than that, vitamin C can also promote the excretion of heavy metals like mercury and lead. Mercury and lead poisoning in the blood stream is very dangerous and can affect the health of people badly especially for smokers. This is because, aside from dental metals and environmental toxins, all kind of cigarettes is the big sources of mercury and lead. The consumption of vitamin C on daily basis can reduce this kind of poisoning and hence improving the health level and life quality among the people. The other function of vitamin C which also becomes the most important reason to consume vitamin C on daily basis is that vitamin C can work against cancer. Nitrates, which are one of the reasons behind the cancer disease among people to occur, can get into the human bodies easily through drinking water and consuming processed foods. Vitamin C prevents them from being turned into nitrosamines which are carcinogenic that is very harmful to people by causing cancer.

The estimated average requirement and recommended dietary allowance of ascorbic acid are 100 and 120 mg per day, respectively. Many analytical techniques including sensors and biosensors have been suggested for the detection of ascorbic acid in various types of samples. For example, integrated methods, utilizing flow injection analysis, high performance liquid chromatography or capillary electrophoresis and a detector are mostly employed for the determination of vitamin C. However, some of these methods are time-consuming, a bit costly, require special training for operators of the equipment and also suffer from insufficient sensitivity or selectivity.

Vitamin C has been widely employed in pharmaceutical and cosmetic industries. The Vitamin C or ascorbic acid preparations are to protect human against oxidation and to exert physiological/biological activities. In the point of view of the fact that

pharmaceutical dosage forms usually contain a variety of excipients that may appear as interferents, also the likelihood of the presence of degradation products and/or stabilizing antioxidant agents for vitamin C, HPLC method possesses advantages. HPLC is considered a sensitive and selective method and therefore suitable for active substance determination and it is also suitable for the evaluation of stability in formulations in the pharmaceutical and cosmetic industries (Mitic *et al*., 2011).

2.7 Conclusion

In conclusion, the term and actual phenolic compounds, oxidative stress or free radicals, antioxidant and vitamin C have a tight bond together. The three herbs (*Cosmos caudatus*, *Premma cordiflora* and *Euodia redlevi*) that have been chosen to analyze in this research are believed to have significant amount of antioxidant, specifically vitamin C. The herbs, if can be proved to contain antioxidant compounds can be used in the future as one of many sources of antioxidant therapy-based products for medical and cosmetic purposes.

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter covers the extract preparation based on set parameters which are time of extraction and solvent concentration, phenolic content analysis, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay for determining the percentage of scavenging activity and vitamin C or ascorbate analysis using High Performance Liquid Chromatography (HPLC). These methodologies are being used extensively during this research.

3.2 Materials

3.2.1 Plant/Herbs Materials

Three types of herbs have been analyzed in this research. They are *Euodia redlevi* (Tenggek Burung), *Premma cordiflora* (Bebuas) and *Cosmos caudatus* (Ulam Raja).

3.2.2 Chemical Reagents

Chemical reagents used in this research were ethanol for the extraction of plant materials, Folin-cioclateu's reagent, methanol, gallic acid and sodium carbonate for determination of total phenolic compound of the plant extracts and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid for the determination of antioxidant activity of the plant extracts. Ascorbic acid was also used as standard for determination of Vitamin C content in plant extracts using High Performance Liquid Chromatography (HPLC).

3.3 Experimental Procedures

3.3.1 Plant Extraction Preparation

In this research, there only one type of solvent was used (ethanol). Method of extraction in this procedure was ultrasonic-assisted extraction using ultrasonic vibration bath. The temperature of extraction was constant at ambient temperature and time of extraction was the controlling parameters of this research. The set times of extraction are 30, 60, 90 and 120 minutes. Generally, the herbs, *Euodia redlevi* (Tenggek Burung), *Premma cordiflora* (Bebuas) and *Cosmos caudatus* (Ulam Raja) were washed with running water to remove dirt. After that, the clean fresh herbs were dried in a 60℃ oven overnight. The dried herbs were then blended using laboratory grinder to obtain fine powder of the dried herbs. Plant samples were dissolved in ethanol with selected concentration (50%/70%/90%). The plant's powder and ethanol mixture was set to 5mg/mL in concentration. The powder was left soaking in ethanol for about 10 minutes. Then, the mixture was sonicated in sonication bath. Next, the extract was then filtered. The extract was purified using rotary evaporator and stored at low temperature until further analysis (Annegowda *et al*., 2010).

3.3.2 Total Phenolic Content Analysis

Total phenolic content determinations of ethanolic extract of the samples were determined by using Folin-ciocalteu assay using Folic Ciocalteu's reagent as describe by Maisuthisakul *et al*., 2005. Briefly, for each samples, ~0.8 to 0.9g of sample is dilute with 5mL of methanol. 200µL of the solution were then transferred and mixed thoroughly with 1mL of Folin-Ciocalteu reagent in a test tube. After 3 minutes, 0.8mL of 7.5% (w/v) sodium carbonate is added and the mixture is agitated by using a vortex mixture. The mixture will then be leaved in the dark for 30 minutes before centrifugation process of the sample for 5 minutes at 3300g. Using UV-spectrophotometer, absorbance of the sample extracts are measured at 765nm wavelength with gallic acid as blank. The concentration of total phenolic compounds in all plant extracts was expressed as milligrams of gallic acid equivalents (GAE) per gram dry weight of plant.

3.3.3 Ascorbic Acid Content Determination (DPPH Assay)

The determination of antioxidant activity was done using specified and most common, DPPH assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH). In term of procedure, generally, 1,1-diphenyl-2-picryl hydrazyl (DPPH) was weighed and then dissolved in ethanol to produce 0.004% (w/v) solution. 3mL of the DPPH solution was added to 1mL of extract solution (for each of every sample) and leaved at the dark place for 30 minutes in order to complete the reaction. After that, absorbance was measured for every sample at 517nm wavelength using UV-spectrophotometer with ascorbic acid act as blank solution. % of radical scavenging activity was calculated using (Bhuiyan *et al*., 2009):

$$\%\ \boldsymbol{of\ radical\ scavenging\ activity} = \frac{absorbance\ of\ blank - absorbance\ of\ sample}{absorbance\ of\ blank} \times 100$$

3.3.4 Ascorbic Acid Content Determination (HPLC Analysis)

Generally, ascorbic acid was set as standard in this procedure and the peak at certain retention time, in this case about 2.4 minutes, was the standard peak for the three selected herbs extracts (Mitic *et al*., 2011). Ascorbic acid standard curve was made by preparing ascorbic acid blank with different concentration, namely 10, 25, 50, 75 and 100 ppm. The peak produces was in term of peak area with the unit of mAU*s. This became the references to determine the concentration of ascorbic acid in the three selected herbs. The selection was based on the highest amount of ascorbic acid obtained in the previous analysis (DPPH Assay).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Introduction

In this chapter, discussions on the outcomes of this research in detail covering all aspects possible, including the outcomes that are very much related to the objectives and scopes of this research is done. This chapter covers the total phenolic content and antioxidant activity analysis results of all herbs used in this research (*Cosmos caudatus*, *Premma cordiflora* and *Euodia redlevi*) based on all the parameters set which are the time of extraction and ethanol concentration (solvent).

This chapter discuss on the outcome of the HPLC results of determining the Vitamin C content in each of every sample with the highest antioxidant activity.

4.2 Results and Discussions

4.2.1 Standard Curve

4.2.1.1 Gallic Acid Standard Curve (Total Phenolic Content Analysis

Table 4.1: Data for Gallic Acid Standard Curve

Gallic Acid Concentration (mg/L)	Absorbance Unit
50	0.056
100	0.112
150	0.183
250	0.291
500	0.528

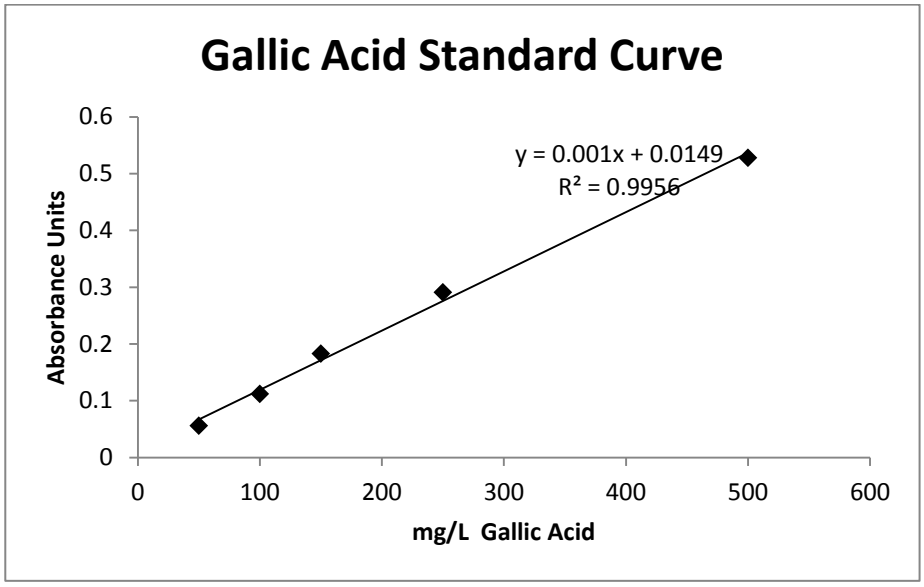

Figure 4.1: Gallic Acid Standard Curve

For this gallic acid standard curve as shown in Figure 4.1, it is clear that the relationship between absorbance unit and concentration of gallic acid is directly proportional. From this relationship, higher absorbance obtained from the plant extract indicates that high phenolic compounds content.

4.2.1.2 Ascorbic Acid Standard Curve (DPPH Assay)

Table 4.2: Data for Ascorbic Acid Standard Curve

Concentration of Ascorbic Acid (mg/L)	Absorbance Unit
1	0.638
5	0.611
10	0.544
50	0.224
100	0.087

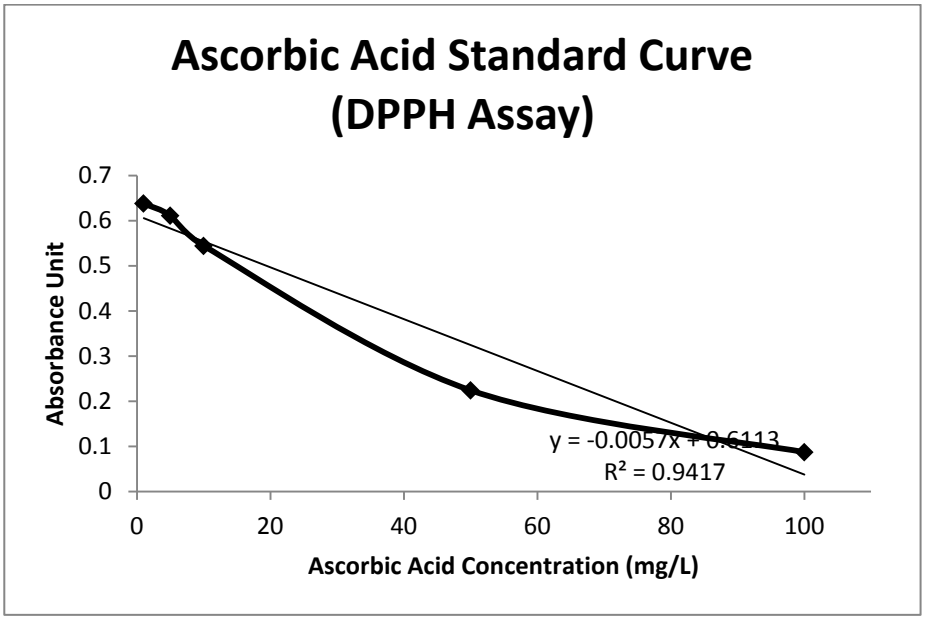

Figure 4.2: Ascorbic Acid Standard Curve (DPPH Assay)

It can be concluded that from Figure 4.2, the relationship between absorbance unit and ascorbic acid concentration is reversely proportional, indicating that herbs with higher absorbance unit contain less amount of ascorbic acid.

4.2.1.3 Ascorbic Acid Standard Curve (HPLC Analysis)

Table 4.3: Data for Ascorbic Acid Standard Curve (HPLC Analysis)

Ascorbic Acid Concentration (ppm)	Area under peak (mAU.s)
10	5.076528
25	40.14957
50	200.0993
75	835.8019
100	1784.809

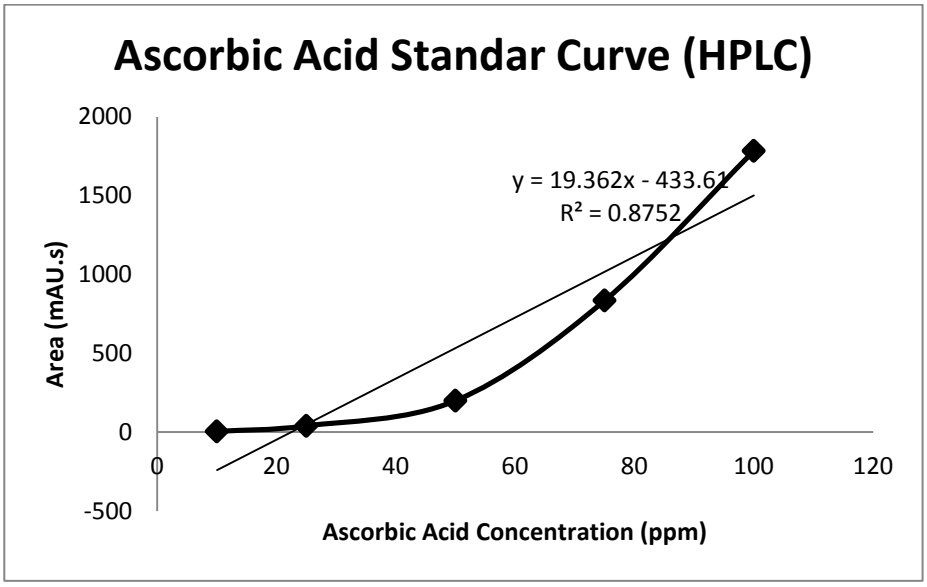

Figure 4.3: Ascorbic Acid Standard Curve (HPLC Analysis)

Figure 4.3 shows the ascorbic acid standard curve by using HPLC. It is clear that the relationship between peak area and concentration of ascorbic acid is directly proportional. From this relationship, higher amount of peak area obtained from the plant extract indicates high ascorbic acid content.

4.2.2 The Effect of Ethanolic Extract of *Cosmos caudatus, Premma cordiflora* and *Euodia redlevi* on Total Phenolic Content

4.2.2.1 Extraction Using 50% Ethanol as Solvent

Table 4.4: Data Analysis of Total Phenolic Content of All Herbs by Using 50% Ethanol as Extraction Solvent

Time of Extraction (min)	Total Phenolic Content (mg GAE/g)								
	Cosmos caudatus			*Premma cordiflora*			*Euodia redlevi*		
	1st Run	2nd Run	Avg.	1st Run	2nd Run	Avg.	1st Run	2nd Run	Avg.
30	43.354	45.42	44.388	74.02	76.22	75.12	177.086	172.554	174.82
60	50.954	50.754	50.854	135.486	133.42	134.454	210.554	208.154	209.354
90	91.686	89.286	90.486	122.02	139.354	130.688	204.554	191.22	197.888
120	60.82	59.22	60.02	122.686	111.886	117.286	190.02	230.42	210.22

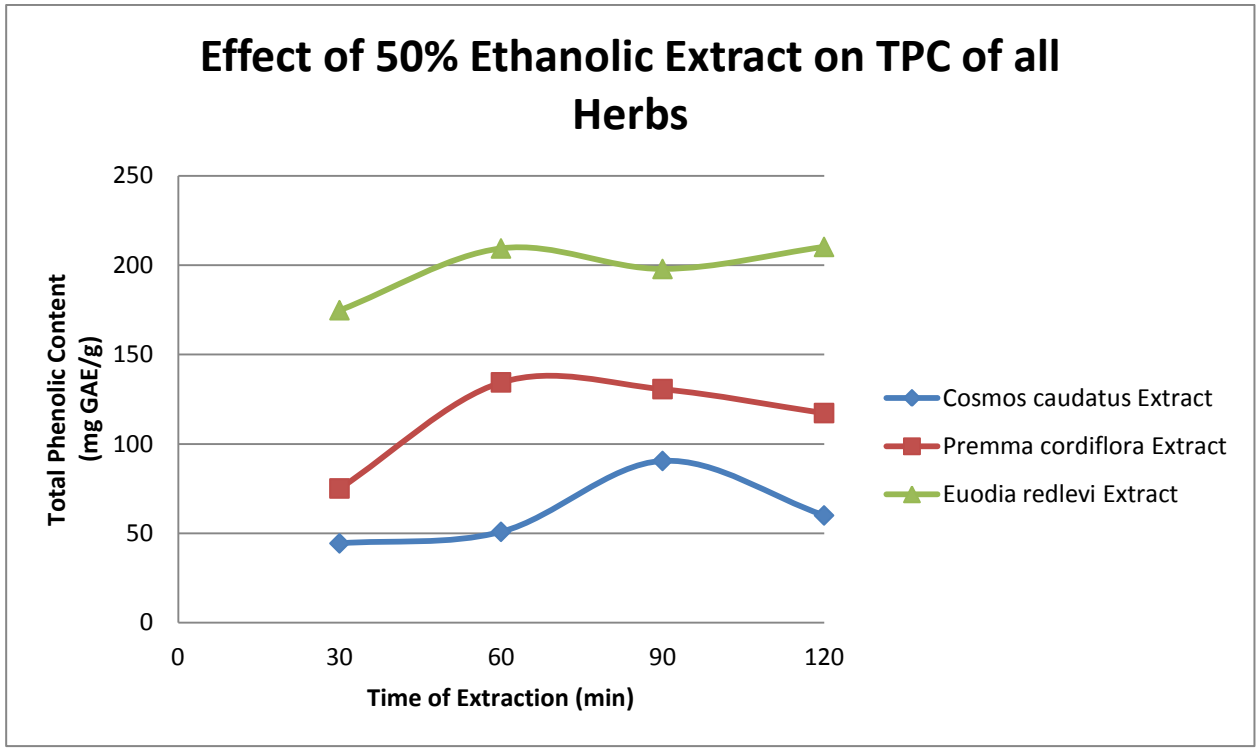

Figure 4.4: Effect of 50% Ethanolic Extract on Total Phenolic Content of the Herbs

Total phenolic content analysis is vital in every single experiment or research related to antioxidant activity of some herbs. Phenolic compound in general is a secondary metabolite which is being synthesize in plants. They contain important biological properties such as antioxidant, anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection and inhibition of angiogenesis and cell proliferation activity (Hodzic *et al*., 2009). In this research, total phenolic compound analysis has been done to analyze the total phenolic compound in an extract of the herb. The total phenolic content in this research was measured in mg GAE/g. In general, considering all of the total phenolic content analysis of all herbs and its extraction parameters, all of them have a significant value of phenolic compounds.

In the extraction process using 50% ethanol, *Cosmos caudatus* extraction sample contains the lowest content of phenolic compound at all extraction time (30, 60, 90 and 120 minutes) compared to the other two herbs as shown in Figure 4.4. From 30 to 90 minutes of extraction, a uniform increase of phenolic compound obtained from the extract can be seen and the phenolic compound value was at the highest at 90 minutes of extraction process in which it contains about 90.486mg GAE/g of phenolic compounds. The yield of phenolic compound decreased when the extraction time was extended to 120 minutes.

In *Premma cordiflora* study, the similar pattern with *Cosmos caudatus* result was observed. A clear pattern of increase in term of value of phenolic compound obtained from the extract, followed by the decreasing of the value over time. In general, *Premma cordiflora* extract produces a slightly higher concentration of phenolic compounds compared to *Cosmos caudatus* extract. Significant increase in phenolic compound content can be seen at 60 minutes of extraction time which contains about 134.454 mg GAE/g of phenolic compounds. Increasing the extraction time from 90 to 120 minutes decreased the value of phenolic content.

Eudioa redlevi extract contains the highest amount of total phenolic compounds compared to the other two herbs. The trend was almost the same with the other two herbs. However, there was an irregularity in the data obtained. For the first 90 minutes of extraction, a trend of increasing and decreasing in the value of phenolic compounds

content was observed. From 30 minutes to 60 minutes of extraction time, a significant increased in TPC values was observed. At 60 minutes of extraction, the content of total phenolic compounds was at the highest. The TPC value was 209.354 mg GAE/g. In 90 minutes of extraction time, the TPC value decreased to 197.888 mg GAE/g. However, the decreasing value did not occur at 120 minutes of extraction time, the situation was believed to be caused by the extraction process itself. The ultrasonic extractor can't be operated continuously for hours. The ultrasonic vibration caused the water bath temperature to increase. Increase in water bath temperature can cause ethanol (the extraction solvent) evaporate at such a small amount hence resulting the herb-solvent mixture became slightly more concentrate compared to the other herb-solvent mixture of the same herb being extracted at different interval of time. This will also cause a slight increase in TPC value per unit of pure extract volume. This situation is believed to be the factor of the slight increase of phenolic compounds content after 90 minutes of extracting time.

4.2.2.2 Extraction Using 70% Ethanol as Solvent

Table 4.5: Data Analysis of Total Phenolic Content of All Herbs by Using 70% Ethanol as Extraction Solvent

Time of Extraction (min)	**Total Phenolic Content (mg GAE/g)**								
	Cosmos caudatus			***Premma cordiflora***			***Euodia redlevi***		
	1st Run	**2nd Run**	**Avg.**	**1st Run**	**2nd Run**	**Avg.**	**1st Run**	**2nd Run**	**Avg.**
30	30.486	31.754	31.12	125.954	132.486	129.22	125.954	71.154	98.554
60	31.486	33.22	32.354	137.82	136.754	137.288	137.82	99.62	118.72
90	40.754	40.354	40.554	137.354	140.354	138.854	137.354	146.554	141.954
120	42.42	44.22	43.32	117.954	135.286	126.62	117.954	166.554	142.254

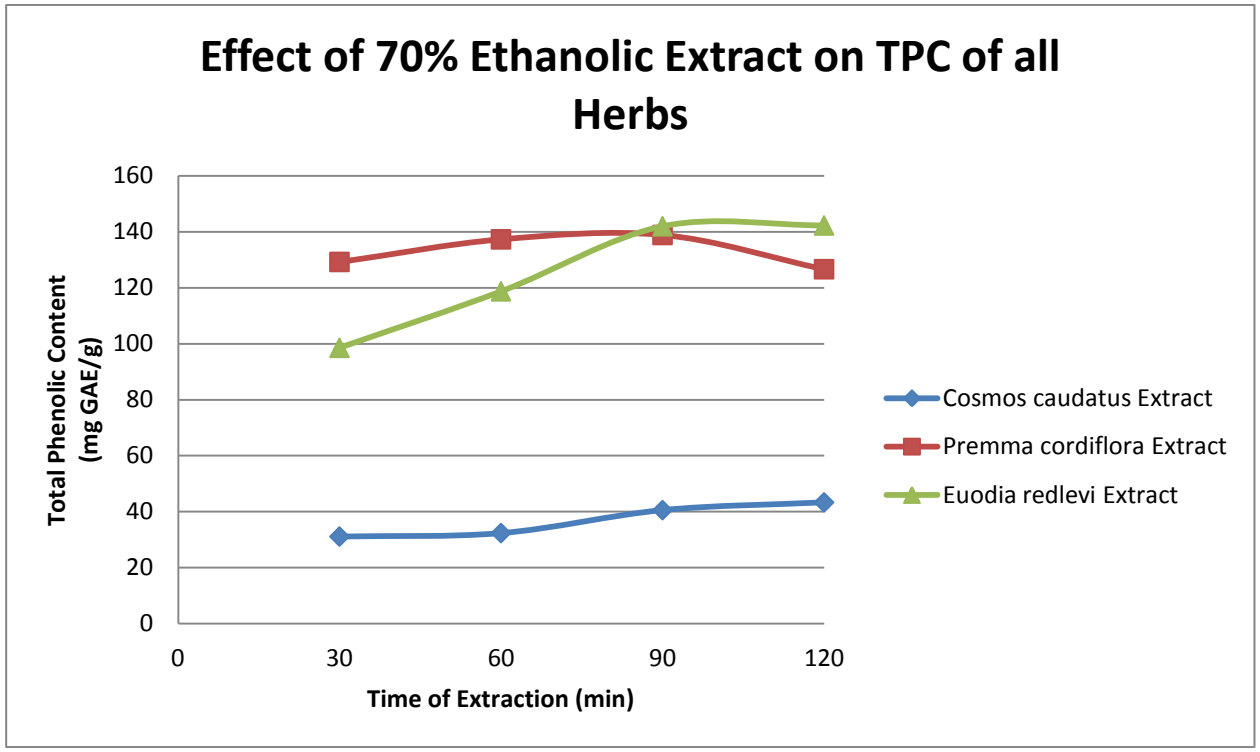

Figure 4.5: Effect of 70% Ethanolic Extract on Total Phenolic Content of the Herbs

Figure 4.5 demonstrates the effect of 70% ethanolic extract of the three herbs on TPC. The results obtained shows the similar trend as the extraction process of the same herbs using 50% ethanol as solvent. The major difference was for *Cosmos caudatus* and *Euodia redlevi* extract samples; it shows a significant decrease at all time of extraction by using 70% ethanol as solvent compared to the same extraction process by using 50% ethanol. However, the different observation was observed in *Premma cordiflora* extract. For *Cosmos caudatus* sample, from 30 to 120 minutes of extraction, an increase of phenolic compound obtained from the extract can be observed. The phenolic compound value was at the highest at 120 minutes of extraction process in which it contains about 43.320 mg GAE/g. To observe the decrease trend of TPC, the extraction time was increased up to 180 minutes. According to Annegowda *et al*., 2010, the extraction time using sonication extraction method should be in a range of 0-180 minutes. The optimum extraction time for 70% of ethanolic extract might be at 120 minutes or more and therefore, extending the time of extraction can be a wise decision to make.

As for *Premma cordiflora*, from 30 to 90 minutes of extraction time, a significant increase was observed. At 90 minutes of extraction, the TPC value was at the highest which is 138,854 mg GAE/g. After that, at 120 minutes of extraction time, the TPC value decrease to 126.620 mg GAE/g. Next is *Euodia redlevi* extract sample. Differ from the other two herbs, *Euodia redlevi* sample shows no decrease pattern with constant increase in term of TPC value was observed starting from 30 minutes of extraction time until 120 minutes of extraction time. At 120 minutes of extraction, this herb's sample contains about 142.254 mg GAE/g of phenolic compound which was the highest.

4.2.2.3 Extraction Using 90% Ethanol as Solvent

Table 4.6: Data Analysis of Total Phenolic Content of All Herbs by Using 90% Ethanol as Extraction Solvent

Time of Extraction (min)	**Total Phenolic Content (mg GAE/g)**								
	Cosmos caudatus			***Premma cordiflora***			***Euodia redlevi***		
	1st Run	**2nd Run**	**Avg.**	**1st Run**	**2nd Run**	**Avg.**	**1st Run**	**2nd Run**	**Avg.**
30	41.754	36.154	38.954	78.82	74.954	76.888	88.886	66.954	77.92
60	39.22	41.886	40.554	102.886	111.954	107.42	131.886	81.086	106.486
90	75.22	72.954	74.088	127.486	108.354	117.92	105.82	111.82	108.82
120	39.22	37.22	38.22	113.686	117.686	115.686	186.62	178.154	182.388

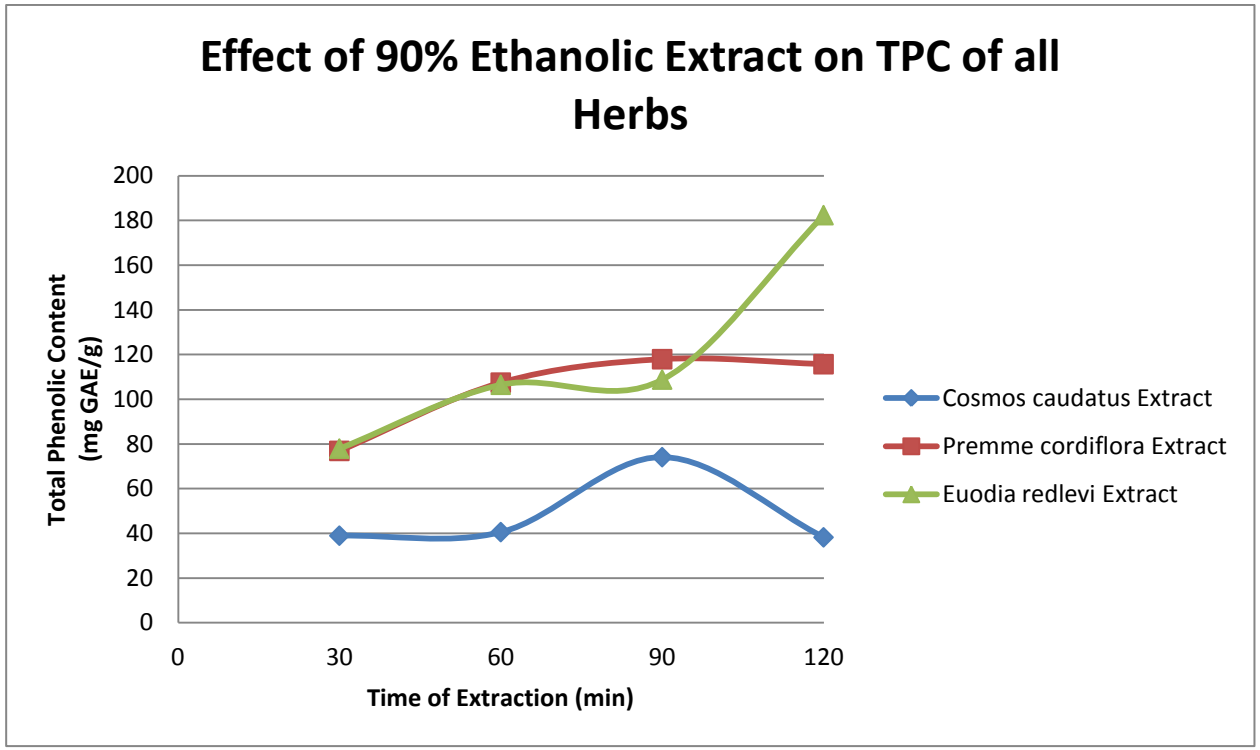

Figure 4.6: Effect of 90% Ethanolic Extract on Total Phenolic Content of the Herbs

Lastly, the total phenolic content analysis of all herbs was continued, this time by using 90% ethanol as solvent.and the results are shown in Figure 4.6. In general, all of the herbs extract shows a significant decrease at all time of extraction by using 90% ethanol as solvent compare to the same extraction process by using both 50% and 70% of ethanol. For both *Cosmos caudatus* and *Premma cordiflora* sample, from 30 to 90 minutes of extraction, a uniform increase of phenolic compound obtained from the extract can be observed. The phenolic compound value was at the highest at 90 minutes of extraction process in which it contains about 74.088 mg GAE/g for *Cosmos caudatus* sample and 117.92 mg GAE/g *for Premma cordiflora* sample. After that, at 120 minutes of extraction, the amount of phenolic compound obtained is decreased. This however does not apply to *Euodia redlevi* samples. There was no significance decrease in yield value noted even until 120 minutes of extraction time. At this time, *Euodia redlevi* sample contains the highest phenolic compound which was at 182.388 mg GAE/g. The cause of this situation was believed to be just the same with the same sample which has been extract by using 70% of ethanol.

4.2.3 The Effect of Ethanolic Extract on Antioxidant Activity using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay

4.2.3.1 Extraction Using 50% Ethanol as Solvent

Table 4.7: Data Analysis of % of Scavenging Activity and Ascorbic Acid Content of All Herbs by Using 50% Ethanol as Extraction Solvent

Time of Extraction (min)	**% of Scavenging Activity**			**Ascorbic Acid Content (mg AA/g)**		
	Cosmos caudatus	***Premma cordiflora***	***Euodia redlevi***	***Cosmos caudatus***	***Premma cordiflora***	***Euodia redlevi***
30	30.8	42.2	49.6	0.0	4.2	8.2
60	25.1	43.5	47.2	0.0	4.6	7.4
90	33.6	34.2	49.1	1.6	1.8	8.1
120	8.0	31.7	42.0	0.0	1.1	6.6

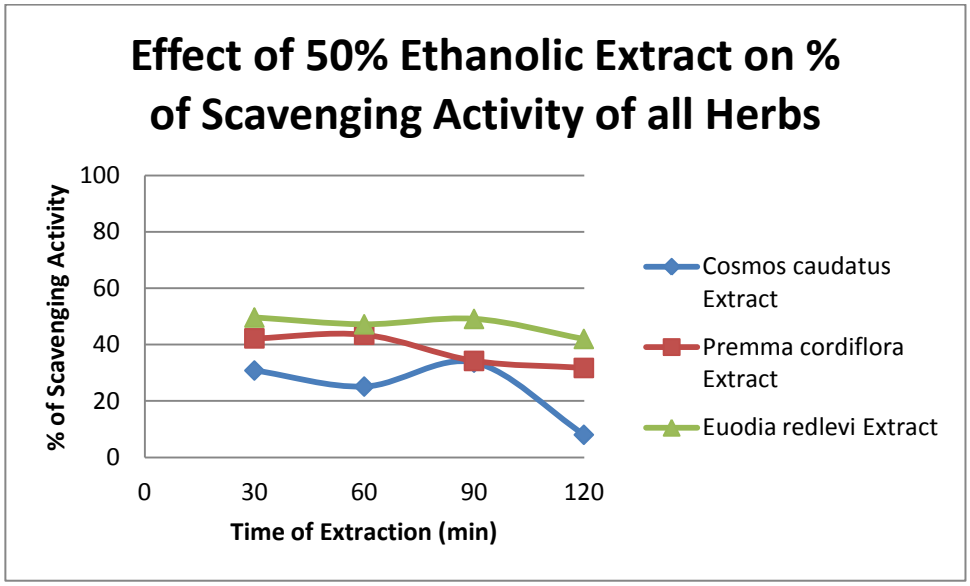

Figure 4.7.1: Effect of 50% Ethanolic Extract on % of Scavenging Activity of the Herbs

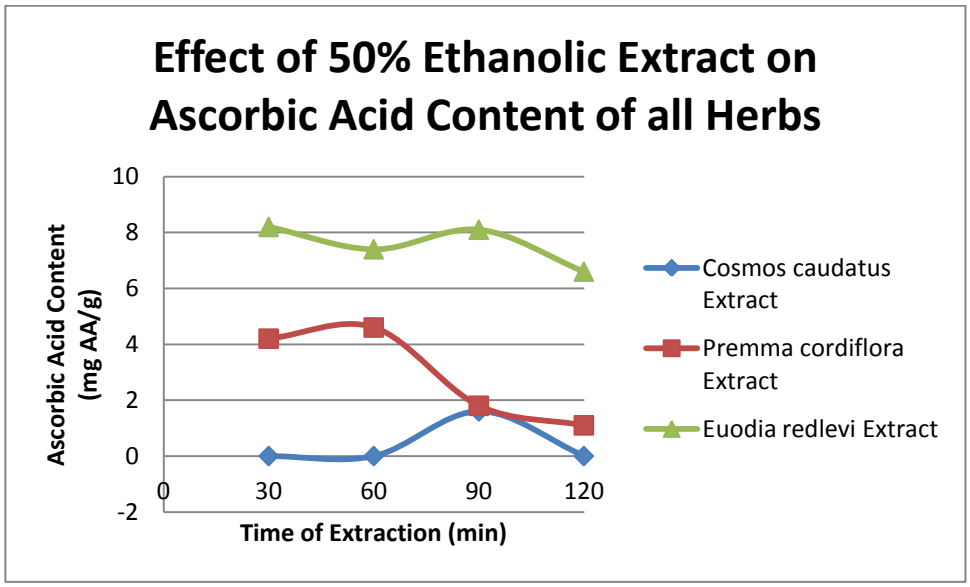

Figure 4.7.2: Effect of 50% Ethanolic Extract on Ascorbic Acid Content of the Herbs

Aside from total phenolic compound analysis, DPPH Assay has also being done in this research. The purpose of this assay was to determine the selected antioxidant compounds in the herbs extracts. There are numbers of known antioxidant; one of them is ascorbic acid, also known as ascorbate or vitamin C (Mitic *et al.*, 2011).

For the next analysis, to determine the effect of ethanolic extract on % of Scavenging Activity and ascorbic acid content of the herbs, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay was used. Taking the extraction process using 50% ethanol as solvent first, *Cosmos caudatus* extraction sample contains the lowest concentration of ascorbic acid at all extraction time (30, 60, 90 and 120 minutes) compare to the other two herbs. For the first 60 minutes, no ascorbic acid content ever recorded. After that, at 90 minutes of extraction time, the amount of ascorbic acid increase, up to 1.63 mg AA/g. At 120 minutes of extraction, there was no AA obtained from the extract. On the other hand, the next herb, *Premma cordiflora*, the trend was just like the previous herb (*Cosmos caudatus*). A clear pattern of increase in term of value of ascorbic acid concentration obtained from the extract, followed by the decreasing of the value over time. In general, *Premma cordiflora* produces a slightly higher concentration of ascorbic acid compare to *Cosmos caudatus*. Significance increase in term of concentration of ascorbic acid concentration can be seen from 30 minutes of extraction time until it reach the highest value of ascorbic acid content at 60 minutes of extraction time which it contains about 4.578 mg AA/g of ascorbic acid. Starting from 90 to 120 minutes of extraction time, the pattern shows a significant decrease in value. The same situation goes to *Euodia redlevi* where it shows increasing in ascorbic acid content value of ascorbic acid, where it was at the highest point of ascorbic acid content at 90 minutes of extraction time with 8.148 mg AA/g of ascorbic acid is obtained from the pure extract.

4.2.3.2 Extraction Using 70% Ethanol as Solvent

Table 4.8: Data Analysis of % of Scavenging Activity and Ascorbic Acid Content of All Herbs by Using 70% Ethanol as Extraction Solvent

Time of Extraction (min)	**% of Scavenging Activity**			**Ascorbic Acid Content (mg AA/g)**		
	Cosmos caudatus	***Premma cordiflora***	***Euodia redlevi***	***Cosmos caudatus***	***Premma cordiflora***	***Euodia redlevi***
30	51.8	38.4	58.4	5.6	2.0	8.7
60	59.9	55.0	56.7	8.2	7.3	8.6
90	42.5	51.6	55.4	2.5	6.2	8.6
120	37.6	44.2	60.9	1.2	3.9	10.3

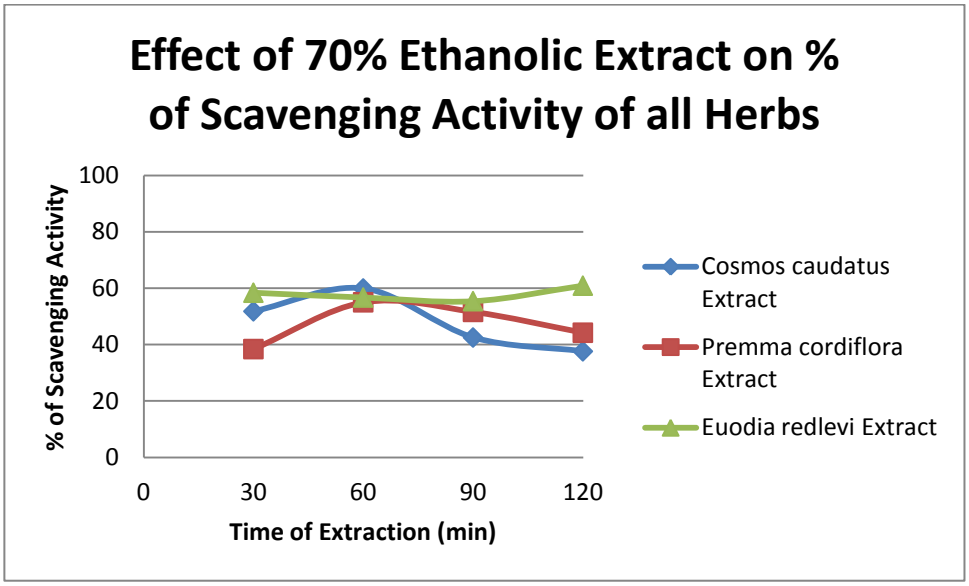

Figure 4.8.1: Effect of 70% Ethanolic Extract on % of Scavenging Activity of the Herbs

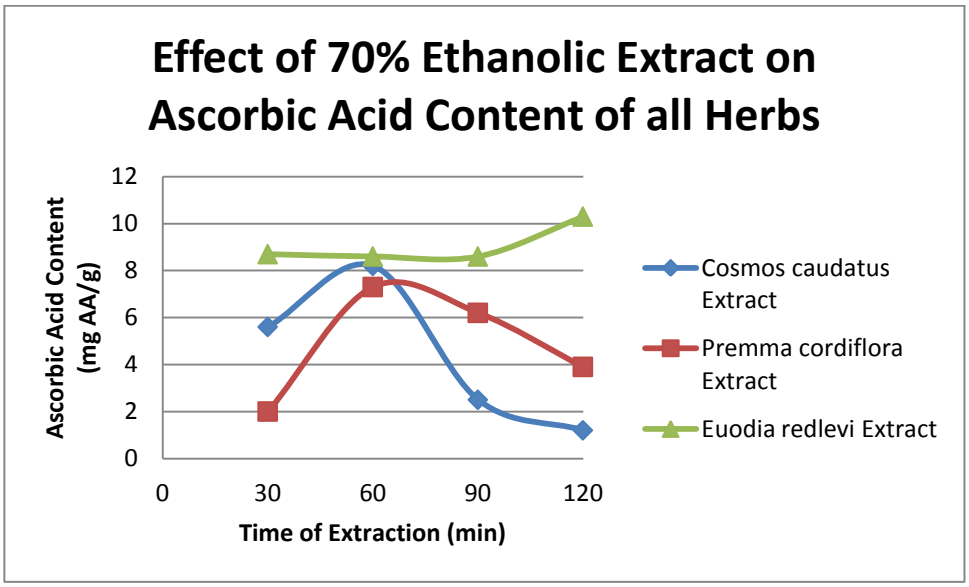

Figure 4.8.2: Effect of 70% Ethanolic Extract on Ascorbic Acid Content of the Herbs

As for the same analysis, by using 70% ethanol as an extraction solvent, in general, the yield of ascorbic acid from the herbs by using 70% ethanol was higher compare the same herbs extract by using 50% ethanol. For both *Cosmos caudatus* and *Premma cordiflora* sample, the pattern is very much the same. Both of the samples shows such a significant increase of the AA content which were at the highest at 60 minutes of time extraction; producing 8.198 mg AA/g and 7.338mg AA/g of ascorbic acid respectively. On the other hand, for *Euodia redlevi* sample, over time, the yield of ascorbic acid was decreased, until 90 minutes of extraction time. At 120 minutes of extraction time, it contains the highest concentration of ascorbic acid; 10,256. This data irregularity may caused by the same factor that affect this very same herbs for total phenolic content analysis, which is the evaporation of sample due to exposing the extract solution to the hot water bath.

4.2.3.3 Extraction Using 90% of Ethanol as Solvent

Table 4.9: Data Analysis of % of Scavenging Activity and Ascorbic Acid Content of All Herbs by Using 90% Ethanol as Extraction Solvent

Time of Extraction (min)	**% of Scavenging Activity**			**Ascorbic Acid Content (mg AA/g)**		
	Cosmos caudatus	***Premma cordiflora***	***Euodia redlevi***	***Cosmos caudatus***	***Premma cordiflora***	***Euodia redlevi***
30	40.0	64.5	62.3	0.5	9.9	10.2
60	47.9	60.8	53.6	3.2	8.8	8.0
90	42.1	66.8	72.8	1.2	10.8	13.4
120	44.7	65.5	65.7	2.1	10.3	11.5

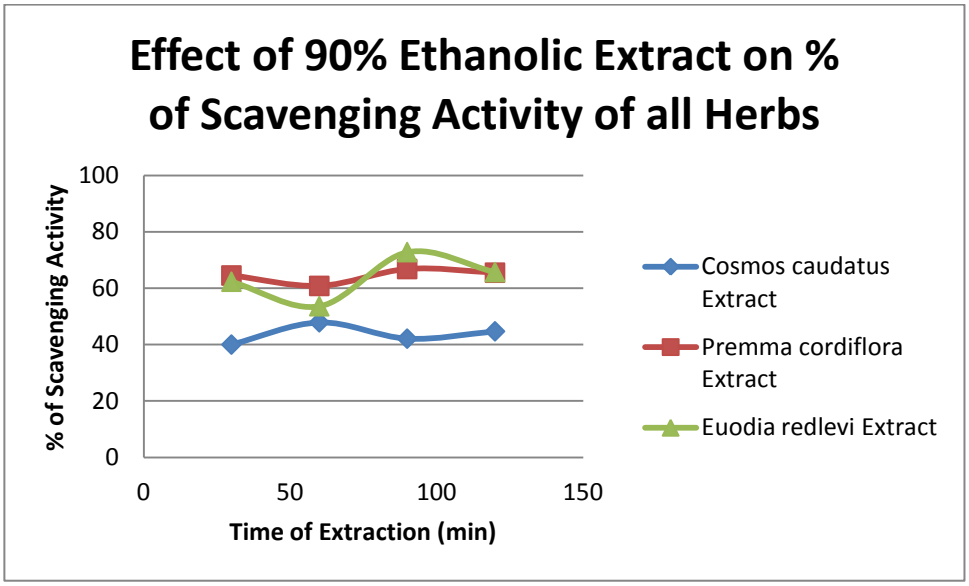

Figure 4.9.1: Effect of 90% Ethanolic Extract on % of Scavenging Activity of the Herbs

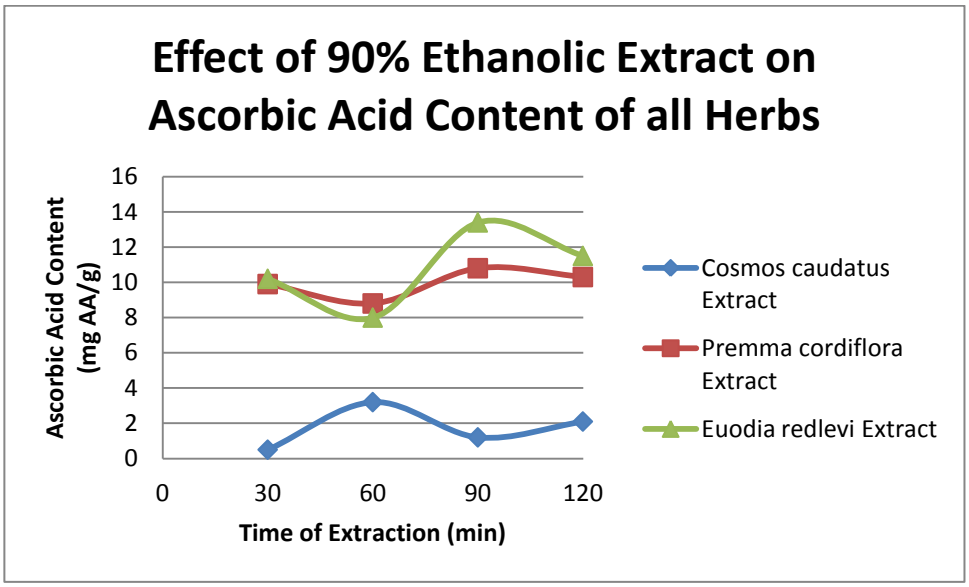

Figure 4.9.2: Effect of 90% Ethanolic Extract on Ascorbic Acid Content of the Herbs

Lastly, DPPH Assay was being done by using the herbs extracts, extracted using 90% of ethanol. In general, except for *Cosmos caudatus* sample, both *Premma cordiflora* and *Euodia redlevi* produced the highest content of ascorbic acid compare to the usage of 70% and 50% of ethanol. Both *Premma cordiflora* and *Euodia redlevi* shows the same pattern. The AA content obtained from both of the samples increases until it reach the highest level at 60 minutes of extraction time. On the other hand, although the pattern of *Cosmos caudatus* sample was very much the same with the other two herbs, it didn't yield the best value of ascorbic acid hence proving that for ultrasonic extraction, the best solvent to be used for *Cosmos caudatus* is 70% ethanol for it yields the highest amount of ascorbic acid.

In conclusion, the highest amount of ascorbic acid yield from all three samples and its extraction parameters are shown in Table 4.10:

Table 4.10: Highest Content of Ascorbic Acid from All of The Herbs and It's Extraction Parameters

Herbs Sample	Time of Extraction (min)	Ethanol Concentration (%)	Ascorbic Acid Conc. (mg AA/g)
Cosmos caudatus	60	70	8.2
Premma cordiflora	90	90	10.8
Euodia redlevi	90	90	13.4

4.2.4 HPLC Analysis

Based on the outcomes as shown in Figure 4.10, the same samples and its set parameters were then analyzed using High Performance Liquid Chromatography (HPLC). The analysis was for determination of ascorbic acid content in the extracts.

Table 4.11: Ascorbic Acid Content in Herbs using High Performance Liquid Chromatography (HPLC) Analysis

Herbs Sample	Area Under Peak (mAU.s)	Ascorbic Acid Conc. (mg AA/g)
Cosmos caudatus	317.09	0.6462
Premma cordiflora	304.66	0.6355
Euodia redlevi	11.10	0.3828

Based on the results obtained, there are some irregularities that can be detected. The concentration of ascorbic acid in *Cosmos caudatus* is the highest, followed by *Premma cordiflora* sample and finally *Euodia redlevi* sample. The results are very much contradicting with the DPPH Assay result which produced result as follow; *Euodia redlevi*, followed by *Premma cordiflora* and finally *Cosmos caudatus* (in term of ascorbic acid concentration). According to Butler, 2004, a high selectivity separation process is needed to distinguish the target product with others but somehow sharing almost similar properties. High Performance Liquid Chromatography has been long known for its sensitivity and accuracy of determining the target product, different with UV-spectrophotometer used in this research for DPPH Assay and Total Phenolic Content Analysis which has its limitation, especially in distinguishing different type of components with almost similar properties.

4.3 Conclusion

In conclusion, all three herbs (*Cosmos caudatus*, *Premma cordiflora* and *Euodia redlevi*) used in this research have their own level of antioxidant properties. Based on all of the outcomes, it can be concluded time of extraction (30, 60, 90 and 120 minutes) using sonication extraction method and solvent concentration (50%, 70% and 90% of ethanol) affect the total phenolic compounds, antioxidant activity and ascorbic acid content in all herbs (*Cosmos caudatus*, *Premma cordiflora* and *Euodia redlevi*).

For total phenolic content analysis, it can be concluded that 90% ethanol is the best solvent to use since all three herbs (*Cosmos caudatus*, *Premma cordiflora* and

Euodia redlevi) have the highest amount of phenolic content using 90% ethanol as extraction solvent. For *Cosmos caudatus*, the highest amount of TPC is 72.088mg GAE/g (90 minutes of extraction time). For *Premma cordiflora*, TPC amount was at the highest at 90 minutes of extraction time (117.92mg GAE/g) and for *Euodia redlevi*, highest amount of TPC was 182.388mg GAE/g (120 minutes of extraction time. 90% ethanol was the best solvent because according to Seeram *et al*., 2006, phenolic compounds, as well as many other antioxidant compounds like carotenoids, vitamin E and also vitamin C are not water soluble and therefore, solvent with less amount of water is the best to choose for the extraction process, which in this case, 90% ethanol. 90% ethanol has only 10% of water, which was the least amount of water in the solvent used in this research, differ from 50% ethanol which contains 50% of water and 70% ethanol which contains 30% of water.

As for 1,1-Diphenyl-2-picrylhydrazyl (DPPH) Assay, where the analysis was about determining the % of scavenging activity and ascorbic acid content of the herbs, the results shows that 90% ethanol is the best solvent for *Premma cordiflora* and *Euodia redlevi* where they both have highest % of scavenging activity and ascorbic acid content which are 66.8% of scavenging activity and 10.8mg AA/g for *Premma cordiflora* and 72.8% of scavenging activity and 13.4mg AA/g for *Euodia redlevi*. On the other hand, for *Cosmos caudatus*, 70% ethanol is the best solvent where the highest point of % of scavenging activity and ascorbic acid content (59.9% of scavenging activity and 8.2mg AA/g) is obtained.

The High Performance Liquid Chromatography (HPLC) Analysis is used in this research to determine the exact amount of ascorbic acid content in the herbs extracts. The result obtained; (*Cosmos caudatus* - 0.6462 mg AA/g, *Premma cordiflora* - 0.6355 mg AA/g and *Euodia redlevi* - 0.3828 mg AA/g) shows differences compared to DPPH Assay and total phenolic content analysis. This is due to the fact that HPLC is very much reliable on determining compounds as it has high selectivity features, unlike UV-spectrophotometer used in DPPH Assay and total phenolic content analysis. DPPH Assay by using UV-spectrophotometer used physical absorbance of certain wavelength to measure the reaction between the reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) with antioxidant compounds in the extract. The absorbance was not 100% reliable on

detecting target compounds, in this case, vitamin C or ascorbic acid. The low selectivity features in the UV-spectrophotometer device limiting its ability to detect the exact amount of target compounds. That is why HPLC analysis result was different from the DPPH Assay analysis.

Based on the results obtained from TPC analysis using Folin-Ciocalteu's reagent and DPPH Assay using 1,1-Diphenyl-2-picrylhydrazyl reagent, although it shows in general that 90% ethanol is the best for extracting higher amount of TPC and ascorbic acid, it did not shows connectivity between the two analysis. For example, highest TPC content for *Cosmos caudatus*, *Premma cordiflora* and *Euodia redlevi* were 90.486mg GAE/g, 137.354mg GAE/g and 210.22mg GAE/g respectively. For *Cosmos caudatus*, highest amount of TPC was obtained from 90 minutes of extraction time using 50% ethanol, a clear opposite results obtained from the DPPH Assay analysis where *Cosmos caudatus* has the highest amount of ascorbic acid at 60 minutes of extraction time using 70% ethanol. The same situation goes to *Premma cordiflora* and *Euodia redlevi*. For *Premma cordiflora*, TPC was at the highest when extracted using 70% ethanol for 90 minutes and for *Euodia redlevi*, the highest TPC amount was obtained at 120 minutes of extraction time using 50% ethanol. According to Javanmardi *et al*., 2003 and Maizura *et al*., 2011, TPC and antioxidant components has linear relationship, which means, high amount of TPC obtained from plant extract indicates high antioxidant components content like vitamin C can be obtained from the same extract. Unfortunately, this research failed to prove the relationship even though all herbs did have significant amount of antioxidant properties. This situation was caused by the extraction process which was unstable for its temperature control features, evaporation of solvent and different antioxidant potential of different batch of herbs used for the TPC analysis and DPPH Assay in this research. The situation was also caused by unreliable results obtained from the usage of UV-spectrophotometer as the working principle of the device is based on physical detection of absorbance of certain wavelength.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, this research is about finding new sources of antioxidant from previously unknown herbs. The objective of this research is considered to be very much successful since all of the herbs (*Cosmos caudatus*, *Premma cordiflora* and *Euodia redlevi*) show a significant amount of antioxidant properties which in this research being represented by ascorbic acid, also known as ascorbate or vitamin C. *Cosmos caudatus* is proved to have the best content of antioxidant with 0.6462mg AA/g based on extraction using 70% of ethanol for 60 minutes, followed by *Premma cordiflora* with 0.6355mg AA/g from the extraction using 90% of ethanol for 90 minutes and finally *Euodia redlevi* with 0.3828mg AA/g based on the extraction process using 90% ethanol for 90 minutes.

In industrial point of view, these herbs have their own potential to become a source of antioxidant based therapy in the future. According to Engredea News & Analysis, 2010, vitamin C (one of major antioxidant components) world demand will increase up to 36.2% by 2012. Vitamin C, as well as other antioxidant components such as vitamin E, has a very high demand, not only in pharmaceutical industry, but also cosmaceutical industry. Beauty products with ‘anti-aging’ description nowadays contain high amount antioxidant components. With China, as of 2006 is the largest exporter and producer of vitamin C decide to reduce its production due to cost and lack of raw materials, these herbs (*Cosmos caudatus*, *Premma cordiflora* and *Euodia redlevi*) with its antioxidant properties represent an opportunity for Malaysia to be a major producer of antioxidant components and antioxidant-based therapy products in the near future.

5.2 Recommendations

In order to obtain such a good results in the near future, the extraction process using ultrasonic vibration should have an extraction temperature control to avoid any irregularities in the data obtained. Unstable temperature reading (due to heating caused by the ultrasonic vibrations) may cause the solvent to evaporate and hence affecting the results. Aside from that, in order to understand more on the effect of solvent concentration and type on total phenolic content and antioxidant activity, it is wise to include one negative control (extraction using 100% pure water) as a point of reference. On the other side, since this research only focused on ultrasonic extraction method, justification can't be made on the decision of choosing this type of extraction method. No comparison can also be made with other type of extraction method like Soxhlet and microwave-assisted extraction to determine which one of these extraction methods is the best in term of time and solvent consumption, energy usage and antioxidant components content in the extract. In the future, it is encourage for the researchers to include these types of extraction methods as a point of reference.

On industrial point of view, if these herbs are about to be extracted for their components in industrial scale, it is wise to use such conventional method of extraction for economic reason, for example Soxhlet extraction. This is because by using the ultrasonic extraction method like the method use in this research, the method consumes a lot of energy which is not practical.

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APPENDICES

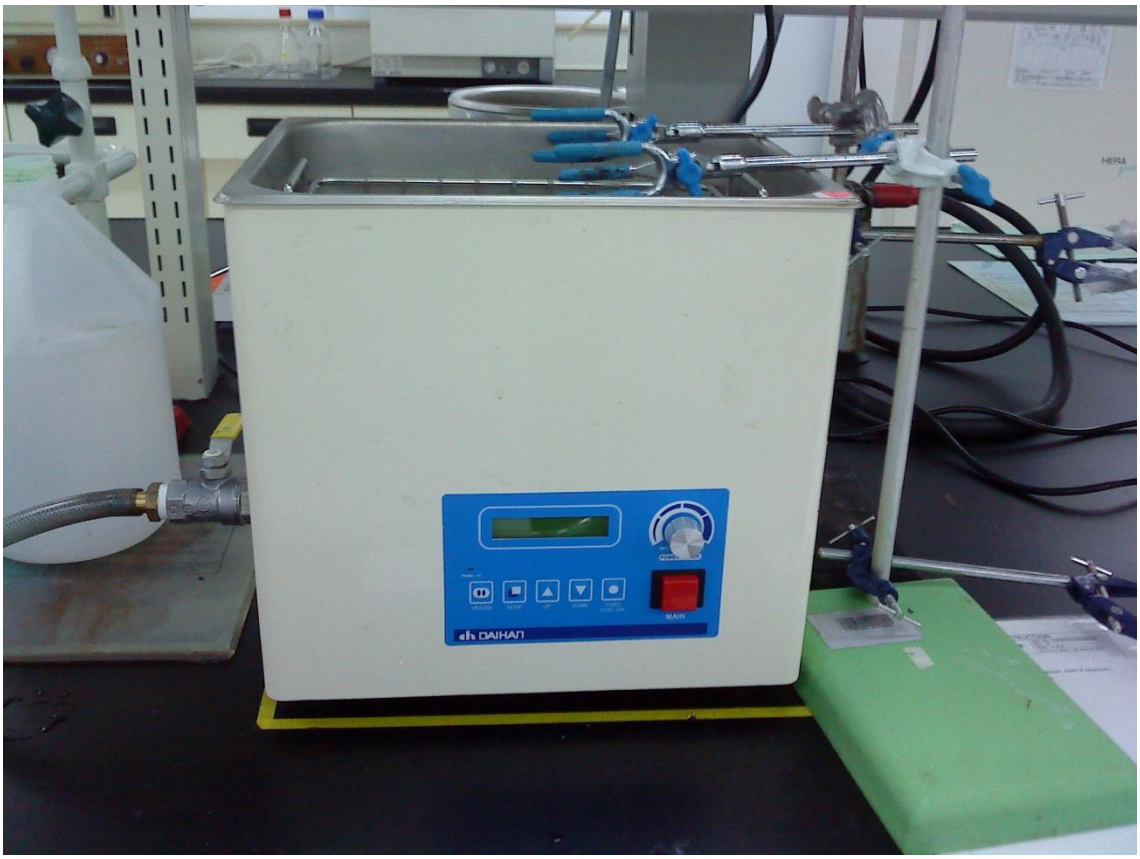

Figure A.1: Ultrasonic Extractor

Figure A.2: Rotary Evaporator

Figure A.3: High Performance Liquid Chromatography (HPLC) Device

Figure A.4: Extraction Process

Figure A.5: Examples of Plant Extract

Experiment Raw Data

1. Total Phenolic Content Analysis

1.1 Extraction using 50% ethanol as solvent

1.1.1 *Cosmos caudatus* – first run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.233	0.237	0.225	0.232	0.005	216.77
60	0.271	0.273	0.265	0.270	0.003	254.77
90	0.427	0.480	0.513	0.473	0.035	458.43
120	0.315	0.320	0.322	0.319	0.003	304.10

1.1.2 *Premma cordiflora* – first run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.385	0.384	0.386	0.385	0.0008	370.10
60	0.702	0.679	0.696	0.692	0.010	677.43
90	0.675	0.661	0.539	0.625	0.061	610.10
120	0.615	0.616	0.617	0.616	0.0008	601.10

1.1.3 *Euodia redlevi* – first run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.903	0.905	0.893	0.900	0.005	885.43
60	1.071	1.073	1.059	1.068	0.006	1052.77
90	0.982	1.074	1.057	1.038	0.040	1022.77
120	0.961	0.963	0.971	0.965	0.004	950.10

1.1.4 *Cosmos caudatus* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.246	0.240	0.240	0.242	0.003	227.10
60	0.265	0.27	0.271	0.269	0.003	253.77
90	0.466	0.458	0.46	0.461	0.003	446.43
120	0.311	0.307	0.315	0.311	0.003	296.10

1.1.5 *Premma cordiflora* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.394	0.393	0.401	0.396	0.004	381.10
60	0.685	0.683	0.678	0.682	0.003	667.10
90	0.712	0.710	0.713	0.712	0.001	696.77
120	0.578	0.572	0.573	0.574	0.003	559.43

1.1.6 *Euodia redlevi* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.887	0.870	0.876	0.878	0.007	862.77
60	1.054	1.061	1.052	1.056	0.004	1040.77
90	0.975	0.973	0.965	0.971	0.004	956.10
120	1.137	1.136	1.228	1.167	0.043	1152.10

1.2 Extraction using 70% ethanol as solvent

1.2.1 *Cosmos caudatus* – first run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.168	0.168	0.166	0.167	0.0009	152.43
60	0.172	0.171	0.174	0.172	0.001	157.43
90	0.219	0.220	0.217	0.219	0.001	203.77
120	0.227	0.228	0.226	0.227	0.0008	212.10

1.2.2 *Premma cordiflora* – first run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.644	0.645	0.645	0.645	0.0005	629.77
60	0.705	0.704	0.703	0.704	0.0008	689.10
90	0.701	0.702	0.702	0.702	0.0005	686.77
120	0.605	0.604	0.605	0.605	0.0005	589.77

1.2.3 *Euodia redlevi* – first run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.644	0.645	0.645	0.645	0.0005	629.77
60	0.705	0.704	0.703	0.704	0.0008	689.10
90	0.701	0.702	0.702	0.702	0.0005	686.77
120	0.605	0.604	0.605	0.605	0.0005	589.77

1.2.4 *Cosmos caudatus* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.174	0.173	0.174	0.174	0.0005	158.77
60	0.183	0.180	0.180	0.181	0.001	166.10
90	0.215	0.218	0.217	0.217	0.001	201.77
120	0.238	0.234	0.236	0.236	0.002	221.10

1.2.5 *Premma cordiflora* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.678	0.676	0.678	0.677	0.0009	662.43
60	0.700	0.697	0.699	0.699	0.001	683.77
90	0.719	0.715	0.716	0.717	0.002	701.77
120	0.692	0.69	0.692	0.691	0.0009	676.43

1.2.6 *Euodia redlevi* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.373	0.368	0.371	0.371	0.002	355.77
60	0.517	0.511	0.511	0.513	0.003	498.10
90	0.744	0.749	0.750	0.748	0.003	732.77
120	0.851	0.847	0.845	0.848	0.002	832.77

1.3 Extraction using 90% ethanol as solvent

1.3.1 *Cosmos caudatus* – first run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.221	0.225	0.225	0.224	0.002	208.77
60	0.210	0.212	0.211	0.211	0.0008	196.10
90	0.394	0.398	0.381	0.391	0.007	376.10
120	0.211	0.210	0.212	0.211	0.0008	196.10

1.3.2 *Premma cordiflora* – first run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.408	0.41	0.409	0.409	0.0008	394.10
60	0.529	0.528	0.531	0.529	0.001	514.43
90	0.669	0.64	0.648	0.652	0.012	637.43
120	0.583	0.584	0.583	0.583	0.0005	568.43

1.3.3 *Euodia redlevi* – first run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.466	0.452	0.46	0.459	0.006	444.43
60	0.677	0.68	0.666	0.674	0.006	659.43
90	0.49	0.572	0.57	0.544	0.038	529.10
120	0.947	0.946	0.951	0.948	0.002	933.10

1.3.4 *Cosmos caudatus* – second run of experiment

Time (min)	**Absorbance**				**Standard deviation**	**Total Phenolic Content (mg GAE/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.196	0.195	0.196	0.196	0.0005	180.77
60	0.226	0.223	0.224	0.224	0.001	209.43
90	0.381	0.380	0.378	0.380	0.001	364.77
120	0.199	0.202	0.202	0.201	0.001	186.10

1.3.5 *Premma cordiflora* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.391	0.390	0.388	0.390	0.001	374.77
60	0.578	0.574	0.572	0.575	0.002	559.77
90	0.557	0.554	0.559	0.557	0.002	541.77
120	0.604	0.604	0.602	0.603	0.0009	588.43

1.3.6 *Euodia redlevi* – second run of experiment

Time (min)	Absorbance				Standard deviation	Total Phenolic Content (mg GAE/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.347	0.354	0.348	0.350	0.003	334.77
60	0.423	0.417	0.421	0.420	0.002	405.43
90	0.574	0.575	0.573	0.574	0.0008	559.10
120	0.899	0.912	0.906	0.906	0.005	890.77

2. DPPH Assay (Ascorbic Acid Content Analysis)

2.1 Extraction using 50% ethanol as solvent

2.1.1 *Cosmos caudatus* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.512	0.506	0.507	0.508	40.27	18.06
60	0.633	0.639	0.64	0.637	25.11	0
90	0.622	0.593	0.585	0.600	29.49	1.98
120	0.832	0.828	0.83	0.830	2.47	0

2.1.2 *Premma cordiflora* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.381	0.379	0.38	0.380	55.35	40.58
60	0.479	0.479	0.479	0.479	43.71	23.21
90	0.542	0.540	0.533	0.538	36.74	12.80
120	0.560	0.556	0.555	0.557	34.55	9.53

2.1.3 *Euodia redlevi* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.429	0.424	0.425	0.426	0.002	49.94
60	0.421	0.422	0.426	0.423	0.002	50.29
90	0.461	0.462	0.459	0.461	0.001	45.87
120	0.646	0.614	0.675	0.645	0.025	24.21

2.1.4 *Cosmos caudatus* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.646	0.645	0.640	0.644	24.36	0
60	0.633	0.639	0.640	0.637	25.11	0
90	0.537	0.528	0.524	0.530	37.76	14.32
120	0.733	0.74	0.737	0.737	13.44	0

2.1.5 *Premma cordiflora* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.609	0.592	0.612	0.604	28.99	1.22
60	0.496	0.473	0.479	0.483	43.28	22.57
90	0.580	0.586	0.577	0.581	31.73	5.32
120	0.613	0.606	0.598	0.606	28.83	0.99

2.1.6 *Euodia redlevi* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.434	0.426	0.433	0.431	49.35	31.63
60	0.476	0.476	0.472	0.475	44.22	23.97
90	0.399	0.415	0.405	0.406	52.25	35.96
120	0.379	0.371	0.378	0.376	55.82	41.28

2.2 Extraction using 70% ethanol as solvent

2.2.1 *Cosmos caudatus* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.434	0.431	0.426	0.430	54.27	31.75
60	0.37	0.442	0.364	0.392	58.34	38.47
90	0.567	0.569	0.563	0.566	39.82	7.89
120	0.682	0.6	0.61	0.631	32.98	0

2.2.2 *Premma cordiflora* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.503	0.506	0.502	0.504	40.81	18.88
60	0.397	0.399	0.394	0.397	53.39	37.65
90	0.416	0.419	0.418	0.418	50.92	33.97
120	0.501	0.502	0.500	0.501	41.13	19.35

2.2.3 *Euodia redlevi* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.25	0.248	0.249	0.249	0.0008	73.54
60	0.437	0.447	0.489	0.458	0.023	51.36
90	0.368	0.368	0.37	0.369	0.0009	60.82
120	0.36	0.345	0.347	0.351	0.007	62.73

2.2.4 *Cosmos caudatus* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.472	0.476	0.480	0.476	49.42	23.74
60	0.364	0.369	0.357	0.363	61.39	43.50
90	0.517	0.514	0.516	0.516	45.20	16.78
120	0.539	0.545	0.546	0.543	42.26	11.92

2.2.5 *Premma cordiflora* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.606	0.599	0.602	0.602	35.99	1.57
60	0.405	0.409	0.409	0.408	56.68	35.73
90	0.447	0.451	0.45	0.449	52.25	28.42
120	0.498	0.496	0.497	0.497	47.18	20.05

2.2.6 *Euodia redlevi* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.536	0.534	0.534	0.535	43.18	13.44
60	0.414	0.416	0.409	0.413	56.11	34.79
90	0.469	0.472	0.471	0.471	49.98	24.67
120	0.385	0.386	0.382	0.384	59.16	39.82

2.3 Extraction using 90% ethanol as solvent

2.3.1 *Cosmos caudatus* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.584	0.585	0.579	0.583	41.68	5.02
60	0.517	0.511	0.505	0.511	48.85	17.60
90	0.588	0.584	0.581	0.584	41.51	4.73
120	0.562	0.557	0.558	0.559	44.04	9.18

2.3.2 *Premma cordiflora* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.304	0.298	0.298	0.300	64.75	54.61
60	0.348	0.346	0.346	0.35	59.26	46.43
90	0.308	0.309	0.309	0.309	63.73	53.09
120	0.314	0.313	0.313	0.313	63.18	52.27

2.3.3 *Euodia redlevi* – first run of experiment

Time (min)	**Absorbance**				**% of scavenge activity**	**Ascorbic Acid Content (mg AA/g)**
	1st rdg	**2nd rdg**	**3rd rdg**	**Avrg rdg**		
30	0.347	0.372	0.373	0.364	0.012	63.56
60	0.472	0.469	0.472	0.471	0.001	52.85
90	0.301	0.3	0.302	0.301	0.0008	69.87
120	0.374	0.372	0.371	0.372	0.001	62.73

2.3.4 *Cosmos caudatus* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.614	0.611	0.619	0.615	38.47	0
60	0.533	0.53	0.529	0.531	46.88	14.15
90	0.573	0.572	0.570	0.572	42.78	6.95
120	0.548	0.547	0.544	0.546	45.31	11.40

2.3.5 *Premma cordiflora* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.355	0.357	0.359	0.357	64.26	44.61
60	0.376	0.38	0.375	0.377	62.26	41.11
90	0.299	0.300	0.303	0.301	69.90	54.50
120	0.319	0.322	0.321	0.321	67.90	50.99

2.3.6 *Euodia redlevi* – second run of experiment

Time (min)	Absorbance				% of scavenge activity	Ascorbic Acid Content (mg AA/g)
	1st rdg	2nd rdg	3rd rdg	Avrg rdg		
30	0.391	0.39	0.388	0.390	60.99	38.88
60	0.454	0.457	0.46	0.457	54.25	27.07
90	0.245	0.244	0.241	0.243	75.64	64.56
120	0.329	0.3	0.312	0.314	68.60	52.22