EFFECT OF DIFFERENT TYPES OF SOLVENT ON EXTRACTION OF PHENOLIC COMPOUNDS FROM *Cosmos caudatus*

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I hereby declare that I have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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I declare that this thesis entitled "*Effect of Different Type of Solvent on Extraction of Phenolic Compound from Cosmos caudatus*" is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is concurrently submitted in candidature of any other degree

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Special Dedication of This Grateful Feeling to My,

Beloved father and mother;
Mr. Sukri bin Mohamad and Mrs. Rohani bin Mohd

Loving brothers and sisters;

Mohd Syairani, Nor Syazwani, Nor Rohaya, Nur Syakira, Nur Fathen, Nurul Fatihah and Muhammad Sukiman

You are everything for me and you my entire all

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ABSTRACT

The preliminary screening indicated that *Cosmos caudatus* had extremely high antioxidant capacity. The antioxidant activity of most of the plant produced is mainly due to the presence of phenolic compounds. Among the phytochemicals, phenolic compounds are the main contributor of antioxidant activity in plant extracts due to their higher value in total phenolic content. The purpose of this study is to investigate the effect of different types of solvent with 50%, 70% and 100% concentration of each solvent on extraction of phenolic compounds from *Cosmos caudatus.* The soxhlet extractor was used in this study. Total phenols in the extract was determine using Folin-Ciocalteu (FC) assay. From the results, 100% ethanolic extract showed the highest of total phenolic content with 15.61 mgGAE/g. However the antioxidant activity was only 14.15%. Meanwhile, 70% acetone extract exhibited the highest inhibition of DPPH. The value obtained was 7.77 mgAAE/g with the antioxidant activity of 84.78%. The polarity of the solvent affects the efficiency of the extraction, total phenolic content and antioxidant activity of the obtained extracts. Total phenolic content is not the only contributor to its antioxidant activity. The existence of other components in fresh extract such as enzymes and vitamin may directly react with free radicals in addition to polyphenolic compound. Further research is warranted to explore the individual or major polyphenolic groups and other bioactive compounds in the *Cosmos caudatus.*

ABSTRAK

Dalam pemeriksaan awal menunjukkan bahawa *Cosmos caudatus* mempunyai kapasiti antioksidan . Aktiviti antioksidan yang banyak dihasilkan dalam tumbuhan ini terutamanya disebabkan oleh kehadiran sebatian fenolik. Antara fitokimia, sebatian fenolik ialah penyumbang utama aktiviti antioksidan dalam ekstrak tumbuhan disebabkan nilai yang tinggi dalam jumlah kandungan fenolik. Tujuan kajian ini untuk menyiasat kesan bagi jenis pelarut yang berlainan dengan kepekatan 50%, 70% dan 100% bagi setiap pelarut untuk pengekstrakan sebatian fenolik daripada *Cosmos caudatus*. Kaedah pengekstrakan daripada soxhlet telah digunakan dalam eksperimen ini. Bagi menentukan jumlah fenol dalam ekstrak perlu menggunakan Folin Ciocalteu. Berdasarkan keputusan, 100% ekstrak ethanol menunjukkan kandungan fenolik yang paling tinggi sebanyak 15.61 mg GAE/ g. Walaubagaimanapun, aktiviti antioksida hanyalah 14.15% Manakala, 70% aseton ekstrak menunjukkan kandungan antioksidan yang tinggi. Nilai yang diperolehi ialah 7.77 mg AAE/g dengan kandungan antioksidan sebanyak 84.78%. Kekutuban pelarut mempengaruhi keberkesanan pengekstrakan, jumlah kandungan fenol dan aktiviti antioksidan untuk ekstrak yang diperolehi. Jumlah fenol bukan hanya penyumbang kepada aktivit antioksida. Kewujudan komponen lain di dalam ekstrak seperti enzim dan vitamin boleh terus bertindak dengan radikal bebas dalam penambahan kumpulan polifenolik. Kajian lanjut adalah wajar untuk meneroka kumpulan polifenolik yang utama atau individu sebatian bioaktif yang lain dalam *Cosmos caudatus*.

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LIST OF ABBREVIATIONS

UV- Vis	Ultra- violet visible spectroscopy
HPLC	High Performance Liquid Chromatography
DPPH	1,1- diphenyl-2- picrylhydrazyl
TPC	Total Phenolic Content
FRAP	Ferric Reducing Antioxidant Power
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
TFC	Total Flavonoid Content
FC	Folin Ciocalteu
GAE	Galic acid equivalent

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Plants are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species in order to survive (Lu and Foo, 1995). For the consumption of raw vegetables they are considered as traditional healthy diet (Ong *et al.*,2004). Consistent intake of raw vegetables is believed can prevent degenerative diseases such as cancer, diabetes, hypertension and cardiovascular. In fact, it can decrease the sign of aging, and improving physical fitness (Mohamed *et al.*, 2005). This diet is a rich source of antioxidant contents like phytochemicals, vitamins and enzymes and also the other minerals and fibers beneficial to health.

Antioxidant is a molecule that capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that can damage cells. In fact antioxidants can terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols (Sies *et al.*, 1997).

Plant phenolics are commonly found in both edible and non-edible plants and also have been reported to have multiple biological effects including antioxidant activity. Mainly the antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agents, hydrogen donators, and singlet oxygen quenchers. The importance of natural phenolic compounds from plants materials is raising interest among scientists, food manufacturers, and consumers due to functional food with specific health effects (Loliger *et a*l., 1991).

Herbs can be eaten fresh as a vegetable like salad and ulam. These herbs are believed to be associated with antioxidant activities and have many beneficial effects and one of them is ulam raja and in scientific name is *Cosmos caudatus*.as shown in Figure 1.1 It is known as an aromatic herb come from tropical Central America and now widespread in almost all tropical regions. Its young leaves are often eaten raw with sambal belacan or coconut paste and normally are used as kerabu. Due to their unique taste and aroma,it is also used as an appetizer and food flavouring. Several antimutagen and antifungal compounds from ulam raja for example lutein (Ragasa *et al.*,2005).

Figure 1.1: *Cosmos caudatus*

In this study, other factors were standardized except for extraction solvent. Thus, no specific or appropriate extraction solvent is recommended for optimal recovery of total phenolic content from fresh sample matrix to the chemical structures phenolics from simple and free to conjugated and polymerized forms that might consequently affect their solubility behavior (Prior *et al*., 2005). For the selection of solvent systems for this study was made on the basis of their reported efficiency in extracting phenols and other antioxidant compounds from fresh sample matrix (Luthria *et al*., 2006; Sun *et al*., 2007; Alothman *et al*., 2009).

Natural antioxidants can obtain from the extraction of fruits or vegetables. For those who need the antioxidant in their bodies, they can get it through the fresh fruit when they eat. But in the food and pharmaceutical industries, the extract of antioxidants from fruits and vegetables is needed for their manufacturing process. The natural antioxidants can be extracted through the fruits, vegetables, and herbs. Extraction is used when to separate substances and the process of extracting the antioxidant can be done by different method of extraction. For example, it can be done by using solvent which is a desired substance dissolves in the extraction and the undesired substance does not dissolve. There are several ways to do in the extraction process such as soxhlet extraction, hydrodistillation, ultrasonic extraction and many more. Soxhlet extraction is one of the oldest method and most widely used approaches for conventional extraction of solid samples (Dingler's *et al*., 1879).

Nowadays, there are numerous techniques such as 1,1- diphenyl-2- picrylhydrazy (DPPH) scavenging activity and ferric reducing antioxidant power (FRAP) assays that are available to evaluate plant phenolics and antioxidant activities (Anatolovich *et al*.,2002). This study is conducted to evaluate the phenolic compounds from *Cosmos caudatus* extract by using different types of solvents and also to investigate the relationship between the antioxidative activity and total phenolic content of the ulam raja extract.

1.2 PROBLEM STATEMENT

Extraction yield of total phenolic compounds and recovery of antioxidant compounds from plant materials are typically depending on different extraction method. Besides, the difference in polarities of extracting solvents might influence the solubility of chemical constituents in a sample and its extraction yield. Therefore, the selection of an appropriate solvent system is one of the most relevant steps to determine of total phenolic content and other antioxidant compound from a sample.

1.3 RESEARCH OBJECTIVES

The main objective of this research is to investigate the effect of different type of solvents on extraction of phenolic compounds from *Cosmos caudatus.*

1.4 SCOPE OF RESEARCH

The scopes of the research are:-

a) Investigating the effects of different types of solvents on the extractability of total phenolic content from *Cosmos caudatus.*The solvent used are acetone , ethanol , and distilled water.
b) Identifying the antioxidant activity of *Cosmos caudatus.*extract.
c) Demonstrating the relationship between phenolic compound and antioxidant activity of *Cosmos caudatus* extract.

1.5 RATIONALE AND SIGNIFICANCE

The rationale and significance in this study is high total phenolic compounds contain in *Cosmos caudatus* act as natural antioxidant and have many medical benefits. The most applicable and healthy way to improve the antioxidant level in the body is by consuming different naturally food resources that available and act as a natural supplement. The best solvent to extract phenolic compounds of *Cosmos caudatus* can promise high potential to be used in nutraceuticals or in food industry as natural preservatives as well.

CHAPTER 2

LITERATURE REVIEW

2.1 PLANT MATERIAL

Ulam raja or *Cosmos caudatus* is an aromatic herb. It came from tropical Central America and now spread in almost all tropical regions. Its young leaves are often eaten raw with chilli or coconut paste and are used in dishes. Due to their unique taste and aroma they are used as an appetiser and food flavouring. There are several bioactive components in ulam raja and according to Ragasa (2005) *Cosmos caudatus* has antimutagen and antifungal compounds. Protein and amino acid are compositions of ulam raja (Zanariah *et al*., 2005). *Cosmos caudatus* is recommended in the traditional medicine system especially for improving blood circulation.

2.2 POLARITY OF SOLVENTS OF *Cosmos caudatus*

Plants are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species in order to survive (Lu and Foo, 1995). The recovery, yield and type of phenolics in an extract are influenced by the type and polarity of extracting solvents, time and temperature of extractions as well as physical characteristic of the samples (Naczk and Shahidi, 2006).

Solvent extraction is most frequently used technique for isolation of plant antioxidant compounds. However, the extract yields and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent due to the presence of different antioxidant compounds of different chemical characteristics and polarities that may or may not be soluble in a particular solvent. Polar solvents are frequently for the recovery of phenols from a plant matrix. The most suitable of these solvents are hot or cold aqueous mixtures containing ethanol, methanol, acetone, and ethyl acetate (Bonoli *et al*., 2009).

In addition, methanol and ethanol have been extensively used to extract antioxidant compounds from various plants and plant-based foods like fruits, vegetables and so on. According to Bonoli *et al* (2009) the maximum phenolic compounds were obtained from the mixtures of ethanol and acetone. In fact, it is important to evaluate and quantify effective antioxidant principles of medicinally or economically viable plant materials.

The polarity of the solvent for the different antioxidant compounds affects the efficiency of the extraction and the activity of the obtained extracts. For example water, methanol, ethanol, acetone, aqueous solutions solvents and ethyl acetate are commonly used as extraction solvents (Shui and Leong, 2006). For the certain complications arise when recovering phytochemical compounds from plant by products due to their high enzyme activity. However, drying the plant by- product before extraction, will immediately immersing the by- product in methanol (Arts and Hollman, 1998).

2.3 ANTIOXIDANT

An antioxidant in food is really important as it can protect human body from free radicals activity. It is also has capable of slowing or preventing the oxidation of other molecules. When electrons are transferred form a substance to an oxidizing agent, it called as oxidation reaction. Free radicals can be produced during the oxidation reactions, where the start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or polyphenols.

Epidemiological studies have strongly suggested that diet plays an important role in the prevention of chronic diseases (Bauman *et al.,* 2004; Willet, 1995). Polyphenolics, thiols, carotenoids, tocopherols, and glucosinolates commonly found in fruits, vegetables and grains, provide chemoprotective effects to combat oxidative stress in the body and maintain balance between oxidants and antioxidants to improve human health (Adom and Liu, 2002; Dragsted *et al.,* 1993; Jia *et al.,* 1999; Wolfe *et al.,* 2003). An imbalance caused by excess oxidants leads to oxidative stress, resulting in damage to DNA and protein and increased risk of degenerative diseases such as cancer (Farombi *et al.,* 2004).

Consumption of fresh fruits and vegetables has been associated with reduced risk of coronary heart disease (CHD) (Bazzano *et al.,* 2003; Joshipura *et al*., 2001; Srinath Reddy and Katan, 2004), stroke (Gillman *et al*., 1995; Voko *et al.,* 2003), symptoms of chronic obstructive pulmonary disease (Fabricius and Lange, 2003; Liu *et al.,* 2004) and different types of cancer including breast and ovarian cancer (Duncan *et al.,* 2004) and colon cancer (Frydoonfar *et al.,* 2003). Polyphenolic compounds, widely distributed in higher plants, have been found to have potential health benefits that are believed to arise mainly from their antioxidant activity (Liu *et al*., 2003). There is considerable scientific and public interest in the important role than antioxidants may play in health care, such as by acting as cancer chemo preventive and anti-inflammatory agents and by reducing risk of cardiovascular mortality (Cos *et al*., 2004).

2.4 TOTAL PHENOLIC CONTENT

In organic chemistry phenols, sometimes called phenolics, are a class of chemical compounds consisting of a hydroxyl group (-OH) attached to an aromatic hydrocarbon group. The simplest of the class is phenol (C_6H_5OH) as shown as Figure 2.2.

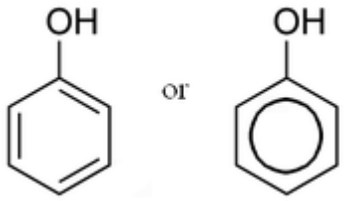

Figure 2.1: Structure of phenol

2.4.1 Phenolic Compound and Phenolic Acids

Phenolic compounds are essential for the growth and reproduction of plants and are produced as a response for defending injured plants against pathogens. The importance of antioxidant activities of phenolic compounds and their possible usage in processed foods as a natural antioxidant have reached a new high in recent years.

Besides that, plant phenolic compounds are diverse in structure but are characterized by hydroxylated aromatic rings for example flavan-3-ols. They are categorized as secondary metabolites, and their function in plants is often poorly understood. Many plant phenolic compounds are polymerized into larger molecules such as the proanthocyanidins and lignins. Furthermore, phenolic acids may occur in food plants as esters or glycosides conjugated with other natural compounds such as flavonoids, alcohols, hydroxyfatty acids, sterols, and glucosides.

Phenolic acids are plant metabolites widely spread throughout the plant kingdom. Recent interest in phenolic acids stems from their potential as protective role through ingestion of fruits and vegetables, against oxidative damage diseases such as coronary heart disease, stroke, and cancers. Its compounds seem to be universally distributed in plants. They have been the subject of a great number of chemical, biological, agricultural, and medical studies. Phenolic acids form a diverse group that includes the widely distributed hydroxybenzoic and hydroxycinnamic acids.

2.5 THE CORRELATION BETWEEN PHENOLIC COMPOUND AND ANTIOXIDANT ACTIVITY

Phenolic compounds in plants are known to act as free radical scavengers. The antioxidant activity of most of the plant produce is mainly due to the presence of phenolic compounds (Skerget *et al*., 2005). Basically antioxidant mechanism of polyphenolic compounds is based on their hydrogen donating and metal ion chelating abilities. Among the phytochemicals, phenolic compounds are the main contributor of antioxidant activity in plant extracts due to their higher value in total phenolic content (Hodzic *et al*., 2009). In vegetables, phenolic compounds were reported to be dominated by glycocidic flavonols and hydroxycinnamic acids (Han *et al*., 2007).

Antioxidant treatments are thought to offset radical damage to biomolecules so slowing or the diseases by preventing oxidative stress. Phenolic compounds as major natural antioxidants of many fruits and vegetables are the focus of nutritional and therapeutic interest. Characterisation of the antioxidant activity of vegetables may also yield more insight into their functionality. Dietary antioxidants are necessary to cope with reactive oxidant species that could damage DNA, RNA and modify proteins. Antioxidants may inhibit the initiation or propagation of oxidation (Velioglu *et al*., 1998). Vegetable extracts with high antioxidant activity may also be useful for food preservation.

In fact, antioxidants are substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species, which include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and non- radicals such as hydrogen peroxide. They scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi *et al.,* 2001). The most abundant antioxidants in fruits are polyphenols and Vitamin C, Vitamins A, B and E and carotenoids are present to a lesser extent in some fruits. These polyphenols mostly contain flavonoids are present mainly in ester and glycoside forms (Fleuriet and Macheix, 2003).

The correlation between phenolic compounds and antioxidant activity has been conducted in several studies. The antioxidative properties of some vegetables and fruits are partly due to the low molecular weight phenolic compounds, which are known to be potent as antioxidants (Wang *et al*., 1999). The effectiveness of phenolics and flavonoids as antioxidants is not only of their composition or relative amount but also by the degree of polymerization, concentration and interaction of their diverse chemical structures to the colorimetric assays. Thus, the higher levels of TPC and TFC do not necessarily correspond to the higher antioxidant responses (Parejo *et al*., 2002).

2.6 SOLVENT SYSTEM

Generally, for the extraction of polyphenols or other bioactive compounds from plant materials, water and organic solvents (ethanol, methanol, acetone, and diethyl ether) are used. Additionally, during the extraction process, the percent recovery depends mainly on the type of solvent and the extraction methods being adapted (Sun and Ho, 2005; Turkmen *et al*., 2006; Hayouni *et al*., 2007).

Solvents with low viscosity have low density and high diffusivity that allows them to easily diffuse into the pores of the plant materials to leach out the bioactive constituents (Naczk and Shahidi, 2006). For the change of solvent polarity, vapour pressure and viscosity of antioxidant compound that are being dissolved in the solvent also varies. As a result of this, the antioxidant activity of the extract observed also varies (Zhou and Yu, 2004; Turkmen *et al.,* 2006; Alothman *et al*., 2009).

For optimal recovery of total phenolic content from fresh sample matrix to the diverse chemical structures of polyphenolics ranging from simple and free to conjugated and polymerized forms (lipophilic) might consequently affect their solubility behavior and there are no specific or appropriate extraction solvent is recommended (Prior *et al*., 2005). The selection of solvent systems was made on the basis of the efficiency in extracting polyphenols and other antioxidant compounds from fresh sample matrix (Luthria *et al*., 2006; Sun *et al*., 2007; Alothman *et al*., 2009).

2.7 SOXHLET EXTRACTOR

2.7.1 The Usage of Soxhlet Extractor

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet (Dingler's *et al*., 1879). The method described by Franz von Soxhlet in is the most commonly used example of a semi-continuous method applied to extraction of lipids from foods. According to the Soxhlet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent usually hexane or petroleum ether under reflux in a special glassware. Four different extraction methods are possible without making any changes to the unit of soxhlet standard, soxhlet warm, hot extraction and continuous extraction. The system has an inert gas supply to avoid oxidation during extraction and to accelerate the evaporation and drying process even with high boiling point solvents (up to 150°C).

Besides that, soxhlet extractor is not limited to the extraction of lipids. Usually soxhlet extraction is only required where the desired compound has a limited solubility in a solvent and also the impurity is insoluble in that solvent. If the desired compound has a significant solubility in a solvent so a simple filtration can be used to separate the compound from the insoluble substance.

2.7.2 The Principle of Soxhlet Extractor

Solid material containing some of the desired compound is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent and then equipped with a condenser. The solvent is heated to reflux. The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools and drips back down into the chamber housing the solid material.

The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will dissolve in the warm solvent. When the soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm with the solvent running back down to the distillation flask. This cycle may be allowed to repeat many times, over hours or days. During each cycle a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. Figure 2.2 shows example of a Soxhlet extractor.

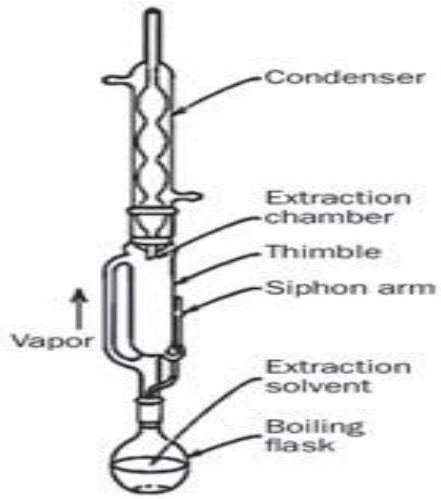

Figure 2.2: Soxhlet Extractor

2.7.3 The Advantages and Disadvantages of Soxhlet Extraction

2.7.3.1 Advantages of Soxhlet Extraction

The advantage of this system is that instead of many portions of warm solvent being passed through the sample just one batch of solvent is recycled. In fact the sample phase is always in contact with fresh solvent so enhancing the displacement of target compound from the matrix and the compound are not discomposed due to moderate extraction condition (Lee *et al*.,2000). After extraction the solvent is removed by a rotary evaporator, yielding the extracted compound. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded. Soxhlet extraction is one of the oldest method and most widely used approaches for conventional extraction of solid samples. It is the most conventional of all methods and consists of a simple distillation process repeated a number of times. Soxhlet extraction is straightforward and inexpensive (Luque de Castro *et.,al* 2004). In fact, it can maintain a relatively high extraction temperature with heat from the distillation flask and no filtration of the extract is required.

2.7.3.2 Disadvantages of Soxhlet Extraction

The disadvantage of this procedure is poor extraction of polar lipids. Agitation is not possible in the Soxhlet device. Besides that, a long time needed for the extraction process. The possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time. It also involved large volumes of solvents and exposed to the hazards of boiling solvents.

2.8 REVIEW RELATED RESEARCH FOR DIFFERENT HERBS USED *Cosmos caudatus*

The data of total phenolic compound with different herbs sample and condition are presented in Table 2.2 and Table 2.3.

Table 2.1: Total phenolic compounds for *Cosmos caudatus* extract

Sample	Condition			Yield of TPC (mg GAE/ 100 g)	Reference
	T (º C)	t (min)	Solvents		
Ulam raja (*Cosmos caudatus)*	80	45	Water Ethanol (50%) Acetone (50%)	844.8 ± 19.7 1144.6 ± 56. 1274.3 ± 98.3	G.Shui *et al* (2005)
Ulam raja (*Cosmos caudatus*)	50	60	Ethanol (95%)	1.52 ± 0.11	N. Andarwulan *et al* (2010)
Ulam raja (*Cosmos caudatus)*	27	60	Water Methanol (70%) Ethanol (70%) Acetone (70%)	0.3±0.1 1.7±0.8 48.8±2.0 17.2±1.0	Sulaiman *et al* (2010)

Table 2.2 : Total phenolic compound for different herbs extract

Herbs	**Solvent**	**Yield of TPC (mg GAE/ 100 g)**	**Reference**
Bunga kantan (*Etlingera elatior. Jack*)	-acetone (50%, 90%100%.) -methanol (50%, 90,100%) -distilled water	Acetone 100% = 142.8 ±14.5 90% = 502.8 ± 15.9 50% = 687.0± 43. 5 Methanol 100% = 361.2±17.1 90% = 431.4±25.8 50% = 615.0± 14.6 Water =90.7± 1.7	Wijekoon *et al* (2010)
Pegaga (*Centella asiatica*)	Methanol	7.79	Huda- Faujan *et al* (2007)
Kesum (*Polygonum minus*)	Methanol	16.73	Huda- Faujan *et al* (2007)
Curry leaf (*Murraya koenigii*)	Methanol	38.60	Huda- Faujan *et al* (2007)
Selom(*Oenanthe javanica*)	Methanol	7.41	Huda- Faujan *et al* (2007)

Table 2.2 : Total Phenolic Compound for different herb(continued)

Herbs	**Solvent**	**Yield of TPC (mg GAE/ 100 g)**	**Reference**
Pegaga (*Centella asiatica*)	Distilled water	3.72	N. Norihan *et al* (2007)
Kesum (*Polygonum minus*)	Distilled water	44.53	N. Norihan *et al* (2007)
Curry leaf (*Murraya koenigii*)	Distilled water	24.62	N. Norihan *et al* (2007)
Selom (*Oenanthe javanica*)	Distilled water	19.96	N. Norihan *et al* (2007)
Petai	Distilled water	33.7	S.P. Wong *et al* (2006)

Table 2.3: Antioxidant Activity from DPPH assays with different raw vegetables extracted using different types of solvent

Herbs	Types of Solvent	DPPH (mg GAE/g dw basis)	Reference
Ardisia crenata	Acetone (70%)	16.7 ±0.9	Sulaiman *et al* (2010)
	Ethanol (70%)	19.6 ± 0.6	
	Methanol (70%)	38.8 ± 0.4	
	Distilled water	45.2 ± 1.6	
Centella asiatica	Acetone (70%)	4.0±1.3	Sulaiman *et al* (2010)
	Ethanol (70%)	5.1±2.1	
	Methanol (70%)	8.1±0.2	
	Distilled water	0.2±0.0	
Cosmos caudatus	Acetone (70%)	19.4±1.3	Sulaiman *et al* (2010)
	Ethanol (70%)	19.6±1.8	
	Methanol (70%)	23.2±1.0	
	Distilled water	0.9±0.2	
Hydrocotyle umbellata	Acetone (70%)	30.2± 0.7	Sulaiman *et al* (2010)
	Ethanol (70%)	25.1± 1.4	
	Methanol (70%)	27.8 ±4.4	
	Distilled water	2.2± 0.2	
Murraya koenigii	Acetone (70%)	9.2± 1.2	Sulaiman *et al* (2010)
	Ethanol (70%)	10.2± 0.6	
	Methanol (70%)	7.0± 0.3	
	Distilled water	9.8± 1.1	
Persicaria minor	Acetone (70%)	8.9 ±0.2	Sulaiman *et al* (2010)
	Ethanol (70%)	12.1± 1.4	
	Methanol (70%)	15.0± 0.6	
	Distilled water	0.4± 0.0	

In Table 2.1 shows the total phenolic compound for *Cosmos caudatus*. The parameters used are different of temperature, time extraction and types of solvent. At temperature of 80°C with 45 minutes time extraction, 50% acetone extract indicated the highest yield of Total Phenolic compound and water extract give the lowest yield of Total Phenolic Compound (G.Shui *et al*., 2005). According to Sulaiman (2010), 70% ethanol extract give the highest value of total phenolic compound. The time of extraction used was 60 minutes and 27°C. Table 2.2 describes total phenolic compound for different herbs. In the extraction of Bunga Kantan, 50% acetone extract showed the highest yield of total phenolic compound. Acetone was found as the best solvent for phenol extraction and water the least effective solvent (Wijekoon *et al*., 2010). Curry leaf exhibited the highest level of Total Phenolic Compound with methanol extract while distilled water extract, Kesum had highest of TPC. From table 2.3, *Ardisia crenata* showed the higher of antioxidant activity for methanolic extract and same as for distilled water extract. Each type of herbs used in extraction indicated different values of antioxidant activity. The lower or higher value of antioxidant activities were detected from different plant part and not restricted toward certain part (Sulaiman *et al*., 2010).

CHAPTER 3

METHODOLOGY

3.1 MATERIALS

3.1.1 Chemical and reagent

All chemicals and reagents used in this study were ethanol, acetone, sodium carbonate annhydrous and from analytical grade of Fisher Scientific(M) Sdn Bhd. Folin-Ciocalteu phenol reagent, and 1,1- diphenyl-2-picryhydrazyl radical (DPPH) were obtained from Sigma-Aldrich Chemicals. The physical properties and polarity index of solvent used are attached as Appendix A1.

3.1.2 Plant materials

Ulam raja or known as *Cosmos caudatus* was used in this study. The leaves were purchased from the local market. After the collection, the samples were brought to the laboratory and keep in the freezer for further experiment.

3.2 EQUIPMENT

There were five equipments used in this research. The equipments are drying oven, Soxhlet extractor, Rotary Evaporator and Ultraviolet- Visible Spectrophotometer (Uv-Vis) and High Performance Liquid Chromatography.

3.2.1 Drying Oven

The drying oven was used to dry the leaves and grinding into powder form. The working principle of the drying oven is intensive, multiple contact of the product with the drying air. Thus, results in fast drying and cross linking of the product inside. The drying process is accelerated by lowering the partial vapour pressure. The drying air is intensively recirculated and partly refreshed. This oven has an adjuster in 0-6 setting for slightly increasing fresh air and a door latch that locks into place with a push on the knob, thus keeping the heat sealed inside. Figure 3.1 shows the drying oven used in this research and model from MEMMERT.

Figure 3.1: Drying Oven

3.2.2 Soxhlet Extractor

A Soxhlet extractor is a piece of laboratory apparatus invented in 1879 by Franz von Soxhlet (Dingler's *et al*., 1879). It was originally designed for the extraction of a lipid from a solid material. Soxhlet extractor is not limited to the extraction of lipids. Basically, Soxhlet extraction is required where the desired compound has only limited solubility in a solvent, and the impurity is insoluble in that solvent. Figure 3.2 shows example of Soxhlet Extractor in laboratory.

Figure 3.2 : Soxhlet Extractor

3.2.3 Rotary Evaporator

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvent from samples by evaporation. Typically, rotary evaporators are used in molecular cooking for the preparation of distillates and extracts. Rotary evaporation is actually applied to separate low boiling solvents such a n-hexane or ethyl acetate from compounds which are solid at room temperature and pressure. However, careful application allows removal of a solvent from a sample containing a liquid compound if there is minimal co-evaporation which is azeotropic behavior and a sufficient difference in boiling points at the chosen temperature and reduced pressure. The applications used in rotary evaporator are reflux distillation, recycling and concentration of solutions, crystallization, synthesis and purification, powder and granules drying. Figure 3.3 shows example of rotary evaporator type Buchi Rotavapor R-200.

Figure 3.3: Rotary Evaporator

3.2.4 Ultraviolet-Visible Spectrophotometer

Uv-Vis Spectrophotometer is an analytical instrument used to gather information about a chemical sample. It exposes a chemical solution to the ultraviolet and visible region of the electromagnetic spectrum. Depend on the type of chemical, certain amount of the light gets absorbed by the chemical which causes electrons to be promoted from one energy level to another. The amount of light that reaches the detector is then recorded as a spectrum. A spectrum is a graphical representation of the amount of light absorbed or transmitted by matter as a function of the wavelength. A UV- visible spectrophotometer measures absorbance or transmittance from the UV range to which the human eye is not sensitive to the visible wavelength range to which the human eye is sensitive. Figure 3.4 shows the example of Uv-Vis Spectrophotometer from Hitachi and model is U-1800.

Figure 3.4: Uv-Vis Spectrophotometer

3.2.5 High Performance Liquid Chromatography

Basically in biochemistry and analytical chemistry, High Performance Liquid Chromatography (HPLC) is used to separate, identify and quantify the compounds. HPLC utilizes a column that holds chromatographic packing material which is stationary phase, a pump that moves the mobile phases through the column while a detector shows the retention times of the molecules. The interactions between stationary phase, the molecules being analyzed and the solvents used will produce different retention time. Figure 3.5 shows the HPLC used in this research from Agilent Technologies 1200 series and type of model is G1322A.

Figure 3.5: High Performance Liquid Chromatography

3.3 EXPERIMENTAL PROCEDURE

3.3.1 Sample Preparation

Cosmos caudatus were cleaned using distilled water to remove any dust particles. After that, the leaves were cut into small pieces and dry in the oven at 60°C for 48 hours. Then, grinded the dried sample into powder form using dry blender. The dried powder form was transferred into a bottle and wrapped with aluminum foil to prevent direct light exposure. The samples were stored in dry and refrigerated places.

3.3.2 Extract Preparation

About 4g of sample was used in this experiment. Three types of solvent used were acetone, ethanol and distilled water. For the extraction, distilled water and different concentrations of acetone and ethanol (50%, 70% and 100% v/v) were used. Each of the extraction was carried out in duplicate (n= 2). In a porous cellulose thimble 4g of sample was placed and then put the thimble in an extraction chamber. In a conical flask, 300 ml of solvent was placed. The extraction process took for 6 hours period at boiling point of the solvent. The yields of extract was collected and evaporated by using rotary evaporator at different boiling point of each solvent until get the concentrated extract. Then the purified of yield was ready for futher analysis. The same methods were repeated for ethanol and distilled water.

3.4 METHOD ANALYSIS

3.4.1 Standard Solution Preparation

The standard gallic acid stock solution for total phenols was prepared by dissolving 0.5 g of dry gallic acid in 10 ml of ethanol and dilute to volume with water in 100 ml volumetric flask. For the calibration curve 0,1, 2, 3, 5, and 10 ml of the gallic acid stock solution into 100 ml volumetric flasks and then dilute to volume with water. There were five concentration values used for standard solution which were 50 mg/L, 100 mg/L, 150 mg/L, 250 mg/ and 500 mg/L. The solution was stored up to 2 weeks at 4 °C. Then, the standard ascorbic acid solution for antioxidant activity was prepared by dissolving ascorbic acid into distilled water (Singleton and Rossi, 1965).

3.4.2 Determination Total Phenolic Compound

Determination of total phenols in the extract was done using Folin – Ciocalteu (FC) assay. Follin Ciocalteu reagent was diluted with an equal volume of distilled water. It was transferred in a brown bottle and was stored in a refrigerator at 4°C. Extract solution of 1ml was added to 60 ml of distilled water. After that, 5 ml of Folin reagent was added. Then after 1 to 5 minutes 4 ml of sodium carbonate was added and made up to 100 ml distilled water. The mixture was stand for 30 minutes at room temperature. The absorbance was measured at 760 nm using UV- Vis Spectrophotometer.(Singleton and Rossi,1965). Total phenolic content was expressed as mg gallic acid equivalent (GAE) as has been carried out by Pitchaon Maisuthisakul (2007).

3.4.3 Determination of Antioxidant activity (DPPH free radical scavenging activity)

Antioxidant activity of *Cosmos caudatus* extracts against stable DPPH (2, 2-diphenyl-2-picrylhydrazyl) was determined spectrophotometrically. When react with antioxidant compound that donate hydrogen DPPH will reduce. The color was changed from deep violet to light yellow and measured at 517 nm by using UV-vis spectrophotometer.

Extract solution of *Cosmos caudatus* was prepared by diluting the extract with concentration of each solvent with 50%, 70% and 100% of ethanol and acetone respectively. For the solution of DPPH was prepared daily by dissolving 2 mg DPPH in methanol and volume was made up to 100 ml with methanol. Then the DPPH solution was stored in dark placed in 30 minutes. About 1 ml of extract was added to 4 ml of methanolic solution DPPH radical (Sanchez-Moreno *et al*., 1998).

The mixture was shaken vigorously and left in the dark for 30 minutes until the changes in color occurred. The absorbance of the mixture was read at 517 nm against a blank of absolute methanol without DPPH.

$$\% \text{ inhibition of DPPH} = [(A_{control} - A_{sample}) / A_{control}] \times 100 \quad (3.1)$$

$A_{control}$ = Absorbance of DPPH solution without extract

A_{sample} = Absorbance of sample with DPPH solution

3.4.4 Determination of Vitamin C content from *Cosmos caudatus* extract

HPLC was used to detect the content of vitamin C in the *Cosmos caudatus* extract. HPLC was very sensitive with concentration, so the samples need to be diluted before run the analysis. There were five concentration values used for standard solution which were 10 ppm, 25 ppm, 50 ppm, 75 ppm and 100 ppm. The standard solution of ascorbic acid were filtered using 0.45 µm membrane filter before injected to HPLC to assure the purity of solutions. The retention time of Vitamin C was 3.260 minutes. The mobile phase used was 25 mM potassium phosphate and dibasic (pH 3.5 with phosphoric acid) .The flow rate was 1.0 m L/min and injection of sample was 10 µL.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 RESULTS ANALYSIS

4.2 GALLIC ACID STANDARD CALIBRATION CURVE

Figure 4.1 shows the Gallic acid Standard Calibration curve with five different concentrations versus absorbance unit.

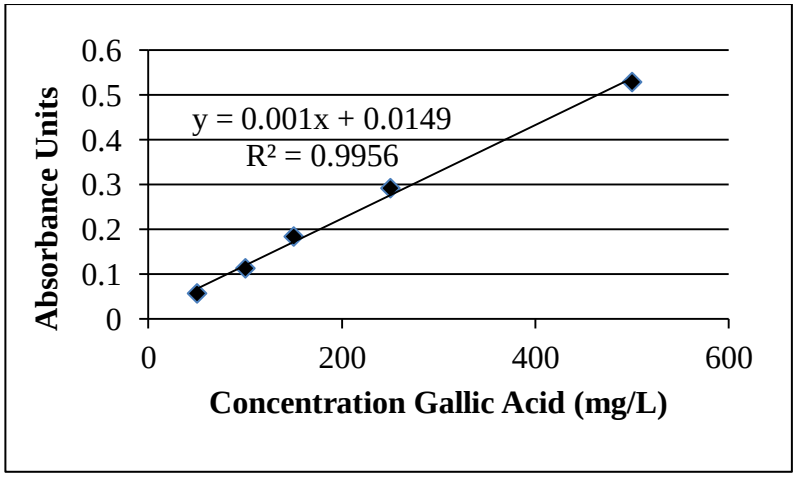

Figure 4.1 : Gallic Acid Calibration curve

Based on Figure 4.1 the concentration of 500 mg/L demonstrate the highest absorbance unit with 0.528 followed by the concentration of 250 mg/L ,150 mg/L , 100 mg/L with the values of absorbance units were 0.291, 0.183, and 0.112 respectively. For the lowest absorbance unit at concentration 50 mg/L the value indicated 0.056 absorbance unit. The absorbance units obtained was directly proportional with the concentration of gallic acid. The amount of gallic acid for each sample is expressed as in mg GAE/g.

4.2.1 Effect of Solvent types on extraction of TPC

Table 4.1 shows a list of the total phenolic content (TPC) for different type of solvent extraction from *Cosmos caudatus*. The amounts of total phenols were expressed as mg GAE/g using Gallic acid standard calibration curve in Figure 4.1.

Table 4.1 : Total Phenolic Content of *Cosmos caudatus* extract

Solvent	**Total phenols (mg GAE/ 4g)**
Distilled Water	12
Ethanol	
50%	7.21
70%	10.66
100%	15.61
Acetone	
50%	9.91
70%	6.53
100%	13.96

Based on the result in Table 4.1, different solvents show different values of total phenols. The *Cosmos caudatus* was extracted using three different polarities of the solvent system that are acetone, ethanol and distilled water. The concentrations of solvent used in this experiment were 50%, 70% and 100% of ethanol and acetone respectively. Then, in extracting polyphenolics from *Cosmos caudatus* was quantitatively measured and compared according to different type of solvents. Phenolic compounds in plants act as free radical scavengers and most of the plant the antioxidant activity produced is mainly due to the presence of phenolic compounds (Skerget *et al*., 2005). The usage of Folin- Ciocalteu reagent was measured based on the colour measurement which was non-specific on phenol. The measurement of colour changes after 30 minutes could be used to determine the existence of phenol in samples. This may due to the antioxidant properties of plant extract that react as reductant agent and known as redox action. Usually, for the antioxidant mechanism of polyphenolic compounds is based on their hydrogen donating and metal ion chelating abilities (Lee *et al*., 2004). Polyphenolics is a type of natural chemical in plant. Actually, they are part of a wider group of compounds called antioxidant and can prevent cellular damage in the body. In human, polyphenols is helping to eliminate free radicals from the body. It can stabilize free radical molecules and turning them into harmless waste by products.

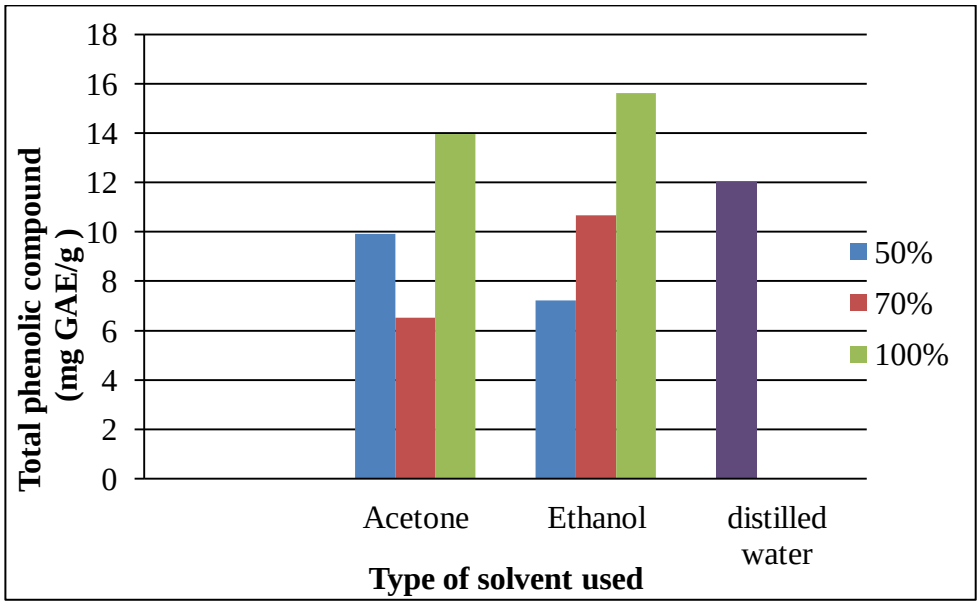

Figure 4.2: Effect of solvent types on Total phenolic compound from *Cosmos caudatus* extract

Based on Figure 4.2, 100% of ethanol extract exhibited the highest value of total phenolic compounds and followed by water extract. In the sample extracted with 100% of ethanol, the amount of total phenols was 15.61 mg GAE/g . Meanwhile for the 100% of acetone extract was 13.96 mg GAE/g. For the distilled water extract the amount of total phenols recovered was 12 mg GAE/g.

From the above data, 70% of acetone indicated the lowest level of total phenols with 6.53 mg GAE/g. There were differences in comparing the results obtained with the literature data due to the sample used, extracting procedures and solvent systems. The extraction yield of phenolic content is strongly depending on the solvent polarity. In this study, it was considered ethanol extract as the most efficient solvent system for extracting phenolic compounds. According to the polarity index water solvent exhibited the highest value of 9 and followed by acetone with 5.1 and ethanol is 5.2. Although water extract yield the highest polarity but it was indicated the lower value of total phenolic content compared to 100% ethanolic extract. Water is not good to extract polyphenols. Water extract is only the water-soluble bioactive compound and more other residual substances and impurities are present in the aqueous extract. Alcoholic solution provides good result

for the extraction process (Zohra *et al*., 2011). For details refer to Appendix A. The polarity of water molecule arises due to its peculiar molecular structure. The molecule is made up of one oxygen atom covalently bonded with two H atom. Ethanol is an alcohol containing polar group in its structure that is OH and it is a polar group. Acetone is a carbonyl group compound contain two CH_3 group which are non polar and carbonyl group is slightly polar compared to alcoholic group so ethanol is highly polar than acetone. The polar group exhibited the better extraction yield of total phenolic compounds while non polar produce least extraction yields of plant sample. Moreover, the difference in polarities of extracting solvents might influence the solubility of chemical constituents in a sample and also its extraction yield. Therefore, in selection of an appropriate solvent system are the most relevant steps in optimizing the recovery of total phenolic compound from a sample (Zhao *et al*., 2006).

4.3 ASCORBIC ACID STANDARD CALIBRATION CURVE

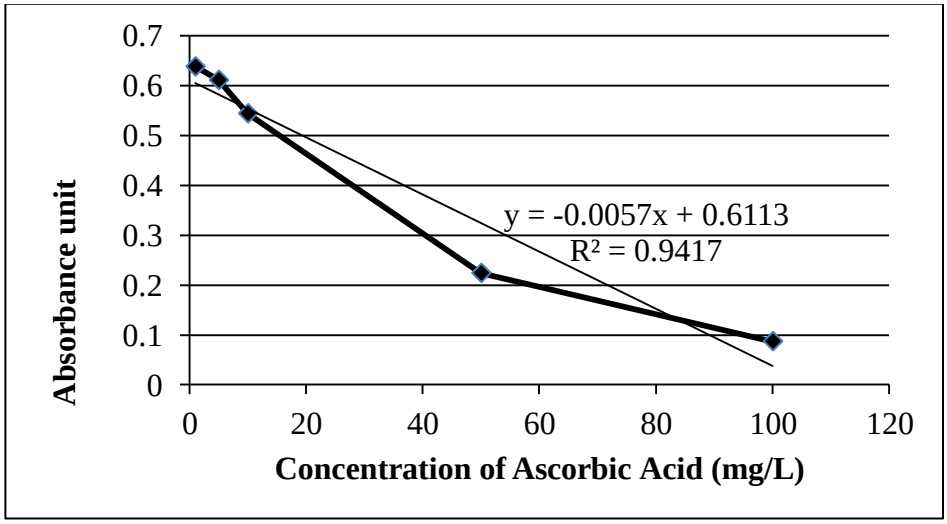

Figure 4.3 : Ascorbic acid standard curve

Figure 4.3 shows standard curve of ascorbic acid. When the concentration of ascorbic acid was increased, the absorbance unit decreased. Both of these variables were indirectly proportional. The concentration of 100 mg/L ascorbic acid indicated the lowest absorbance unit with 0.087 whereas at 1mg/L of ascorbic acid showed the highest value with 0.638 absorbance unit. Then, these were followed by the concentration of 5mg/L ,10mg/L, and 50 mg/L with the values of absorbance unit were 0.611, 0.544 and 0.224 respectively.

4.3.1 Antioxidant activity of *Cosmos caudatus* with various solvent

Table 4.2 shows DPPH inhibition and the percentage of antioxidant activity of *Cosmos caudatus* extracted by different types of solvent.

Table 4.2 : Antioxidant activity of *Cosmos caudatus* extract

Solvent	DPPH inhibition (mg AAE/g)	Inhibition of DPPH (%)
Water	2.87	8.82
Ethanol		
50%	3.60	21.58
70%	4.46	36.89
100%	3.17	14.15
Acetone		
50%	7.03	44.2
70%	7.77	84.78
100%	3.00	11.14

The antioxidant activities of the *Cosmos caudatus* extracts were measured using DPPH free radical scavenging assays. Based on the Table 4.2 when the DPPH inhibition was increased, the percentage inhibition of DPPH also increased. According to Moon and Shibamoto (2009), an antioxidant donates hydrogen or electron which is accepted by the DPPH radicals. The colour changes from purple to yellow because of the reduction of DPPH radical to stable diamagnetic molecule. For DPPH radical it was measured at 517 nm by using spectrophotometer.

4.3.1.1 Effect of different types of solvent on Antioxidant activity from *Cosmos caudatus* extract

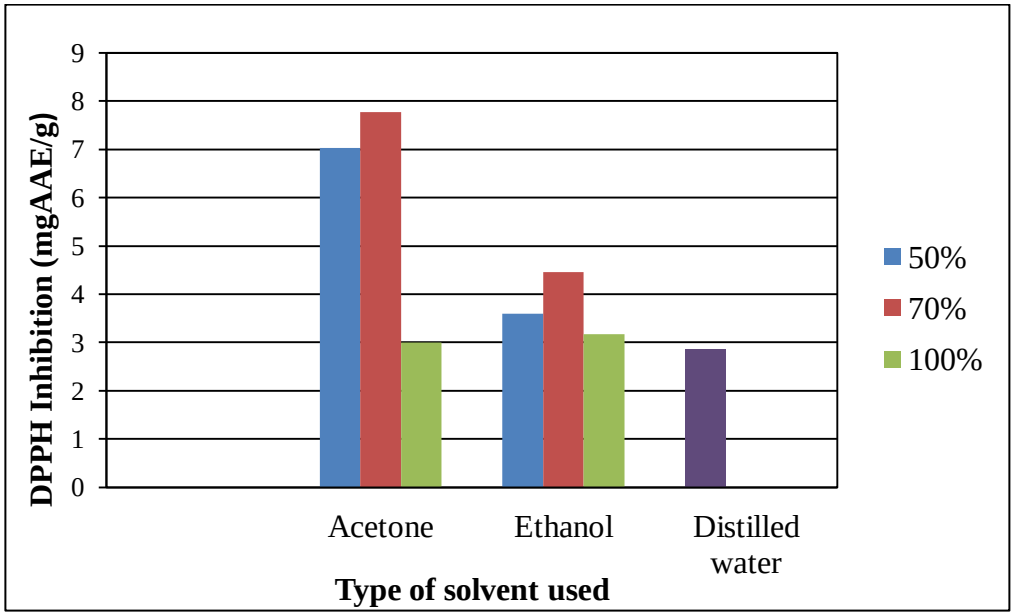

Figure 4.4 : Effect of different types of solvent on antioxidant activity from *Cosmos caudatus* extract

According to the Figure 4.4, 70% acetone extract exhibited the highest DPPH inhibition with 7.77 mg AAE/g and followed by 50% acetone extract mg/L that was 7.03 mg AAE/g . For 70 % ethanol extract was indicated as 4.46 mg AAE/g. On the other hand, distilled water extract showed the lowest of DPPH inhibition among the other types of solvent with 2.87 mg AAE/g. In DPPH experiment, distilled water could be considered as the least effective solvent for extraction of antioxidant compound for *Cosmos caudatus*. In these findings, it found that DPPH assay may give the true picture of total amount antioxidant compared to total phenolic content with acetone extract as the most effective solvent. DPPH is a stable of free radical and one of the most effective methods for evaluating the concentration of radical scavenging materials active by chain breaking mechanism (Niki *et al*., 1987). The polarity of the solvent with different antioxidant compounds affects the efficiency of the extraction thus the antioxidant activity for each solvent will differ.

4.3.1.2 Relationship of solvent types on Antioxidant activity from *Cosmos caudatus* extract

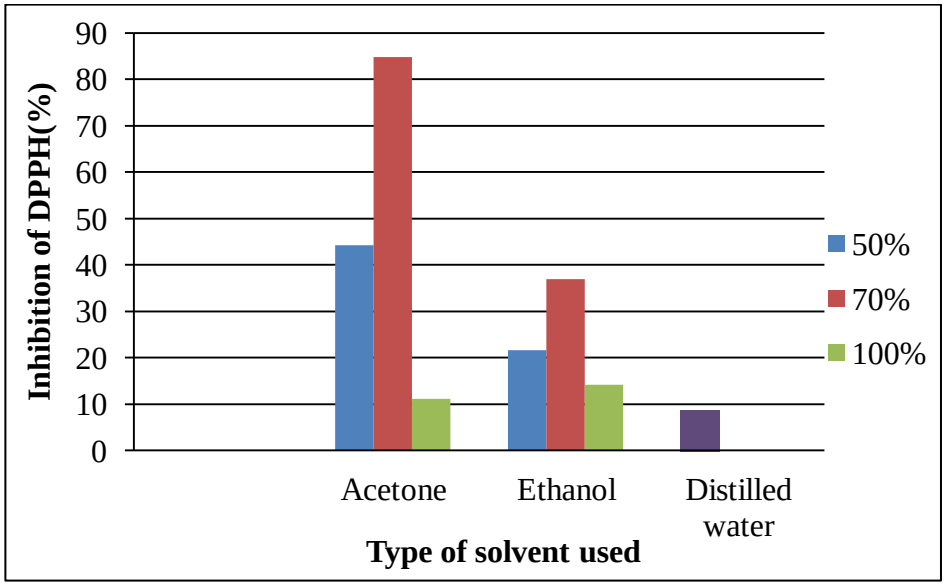

Figure 4.5 : Relationship of solvent types on Antioxidant activity from *Cosmos caudatus* extract

Figure 4.5 shows the relationship of solvent types on antioxidant activity from *Cosmos caudatus* extract. Of all the solvent systems studied, 70% acetone extract had the highest percentage inhibition of DPPH with 84.78%. It was followed by 44.2% of 50% acetone extract. Similarly as in Figure 4.4, distilled water was exhibited as the lowest percentage of DPPH (8.82%). For ethanol solvent 50%, 70% and 100% ethanol extract were 21.58%, 36.89% and 14.15% inhibition of DPPH respectively. In conclusion the type of antioxidant compound that being dissolved in the solvent was different with the change of solvent polarity, vapour pressure and viscosity. The solvent polarity of acetone is lower than ethanol and water with 5.1, 5.2 and 9 respectively. In fact, the acetone solvent has low viscosity with 0.0003075 Pa.s compared with ethanol and water. So with low viscosity, acetone solvent can diffuse into the pores of *Cosmos caudatus* easier than ethanol and water to leach out the bioactive from plant. Naczk and Shahidi (2006) were said solvent with low viscosity have high diffusivity and low density that allows them to

easily diffuse into the pores of the plant materials to produce the bioactive compound. For details refer to Appendix A.

4.4 CORRELATION BETWEEN TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY

Table 4.3 shows the total phenolic content antioxidant activity of *Cosmos caudatus* extract for each solvent.

Table 4.3: Data for TPC and DPPH of *Cosmos caudatus* extract

Solvent	**Total Phenolic content (mg GAE/ 4 g)**	**Antioxidant activity (%)**
Ethanol		
50%	7.21	21.58
70%	10.66	36.89
100%	15.61	14.15
Acetone		
50%	9.91	44.2
70%	6.53	84.78
100%	13.96	11.14
Distilled water	12	8.82

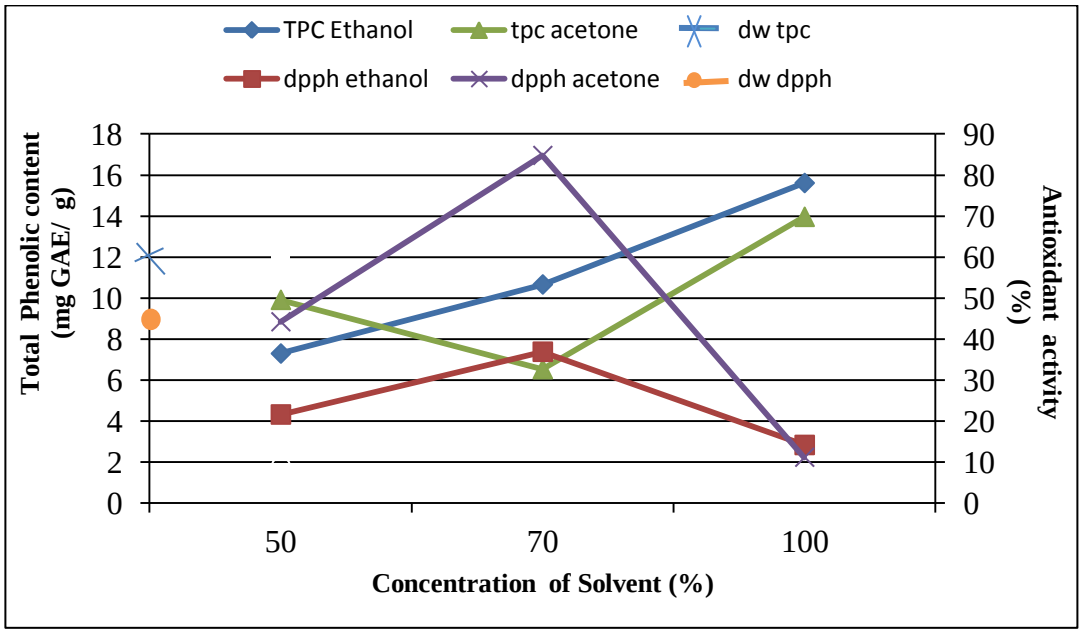

Figure 4.6: The correlation between antioxidant activity and total phenolic content

Figure 4.6 illustrates the correlation between total phenolic content and antioxidant activity of *Cosmos caudatus* extract. At 70% acetone extracts indicated the better correlation between antioxidant activity and total phenolic content and followed by 70% ethanol extracts. The quantitative estimation of total phenolic content and DPPH values is influenced by two variables that are extracting solvent and species of plant (Sulaiman *et al.*, 2011). In this study, 100% concentration of solvent had the highest of total phenolic content but demonstrated weak correlation with the lower antioxidant activity. It shows here that the total phenolic content is not the only contributor to its antioxidant activity. Actually the existence of other components in the fresh extracts such as enzymes and vitamins can directly react with free radicals in addition to polyphenolic compounds (Sulaiman *et al.*,2011).The higher level of TPC not necessary correspond to the higher antioxidant respond. Since phenolic compounds contribute directly to antioxidant activity (Duh *et al.*, 1999) so there is a correlation between total phenolic content and antioxidant activity.

4.4.1 Content of Vitamin C in *Cosmos caudatus* extract

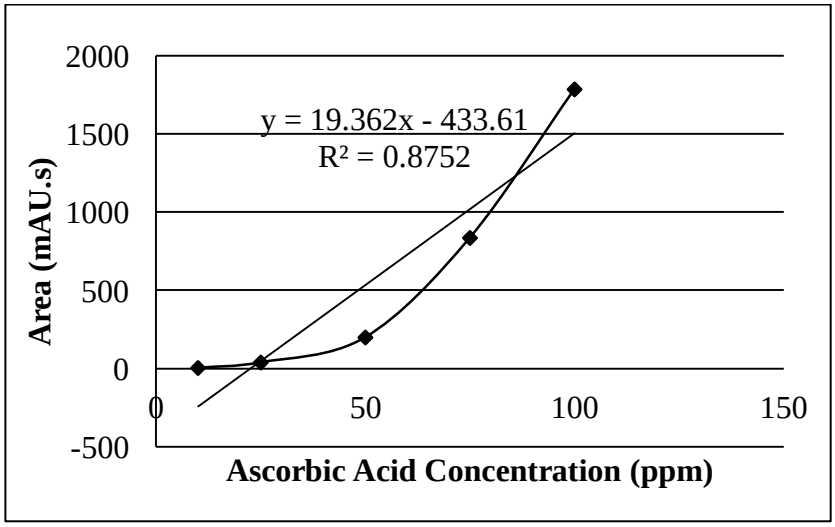

Figure 4.7: Ascorbic acid standard curve (HPLC)

The peak area was directly proportional with the amount of vitamin C as shown in Figure 4.7. The increasing vitamin C concentration showed the higher of peak area. From the results obtained 70% acetone extract showed the highest value of antioxidant activity. Thus, the amount of vitamin C was 2.38 mg AAE/g (181.59019 mAU*s) after analyzing using HPLC. For the result refer to Appendix B. In addition, the vitamin C is a polar molecule. The polar solvent is needed for the highest extraction yield. Solvent polarity influences the amount of extraction yield.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

In conclusion, the total phenolic and antioxidant compound are depending on the extracting solvent used and the species of plant. From the results obtained 100% ethanol extract showed the highest total phenolic content with 15.61 mg GAE/ g. Meanwhile, 70% acetone extract exhibited the higher DPPH free radical scavenging activity with 7.77 mg AAE/g and its percentage was 84.78%. By considering all the solvent system,70% acetone extract was found to be the most effective solvent for antioxidant activity and 100% ethanol extract was the best for total phenolic content. The quantitative estimation of total phenolic content and antioxidant activity are greatly influenced by two variables that are extracting solvent and species of plant (Demiray *et al*., 2009). The effectiveness of phenolic as antioxidant is not only of their composition but also the degree of polymerization, concentration and interaction of chemical structures. The increasing polarity of solvent will increase the polarity of antioxidant. Last but not least, the efficiency of phenolic extraction depends on the type of solvents used as well as antioxidant compound.

5.2 RECOMMENDATIONS

There are some recommendations need to be proposed for improvement for the future study. Further research is warranted to explore the individual or major polyphenolic groups and other bioactive compounds in the *Cosmos caudatus* and their contribution to the health. The *Cosmos caudatus* will be a potential source of natural antioxidants, so the toxicity of plant extracts with high antioxidant activity need to be tested first to confirm of their safety for used as food additives. Besides that, add more parameters such as use different conditions of temperature, extraction time, and types of solvent instead of distilled water, acetone and ethanol. In fact, use various sources of *Cosmos caudatus* and plant parts such as fruit, fruit peel, fruit flesh, seed skin, bud flower and tree parts. The characteristics of the phytochemicals and antioxidant mechanisms of extract should be further studied to gain more information and understanding of antioxidant activity in food systems. In fact, the result obtained on a fresh weight basis should be confirmed with future work for more standardized and comparable quality.

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APPENDIX A

Physical properties of solvents

i. Properties of Ethanol

Properties	
Molecular formula	C_2H_6O
Molar mass	46.7
Exact mass	46.041864814 g mol^{-1}
Appearance	Colorless liquid
Density	0.789 g/cm^3
Melting point	−114 °C, 159 K, -173 °F
Boiling point	78 °C, 351 K, 172 °F
Vapor pressure	5.95 kPa (at 20 °C)
Acidity (pK_a)	15.9
Basicity (pK_b)	1.36
Reftractive Index (n_D)	1.36
Viscosity	0.0012 Pa s (at 20 °C)
Dipole moment	1.69 D
Polarity index	5.2

ii. Properties of Acetone

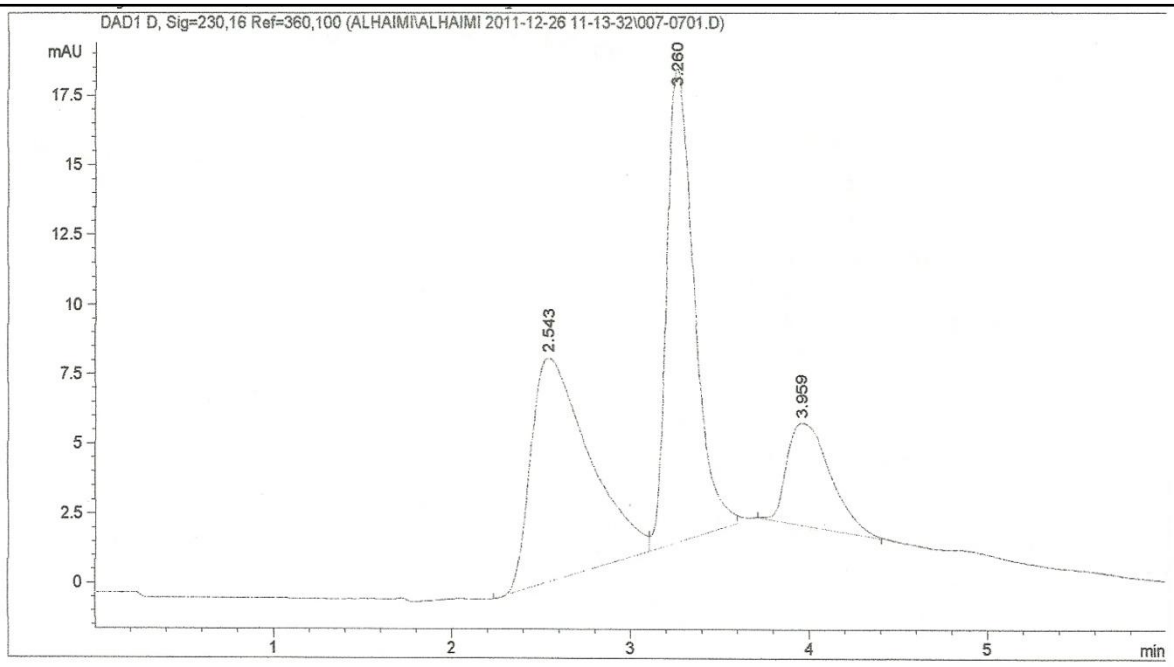
Properties	
Molecular formula	C_3H_6O
Molar mass	58.08 g mol^{-1}
Exact mass	58.041864814 g mol^{-1}
Appearance	Colorless liquid
Density	0.791 g cm^{-3}
Melting point	-95--93 °C, 178-180 K, -139--136 °F
Boiling point	56-57 °C, 329-330 K, 133-134 °F
Vapor pressure	24.46-24.60 kPa (at 20°C)
Acidity (pK_a)	24.2
Basicity (pK_b)	-10.2
Reftractive Index (n_D)	1.35900
Viscosity	0.3075 cP
Dipole moment	2.91 D
Polarity index	5.1

iii. Properties of Water

Properties	
Molecular formula	H_2O
Molar mass	18.01528(33) g/mol
Appearance	white solid or almost colorless, transparent, with a slight hint of blue, crystalline solid or liquid
Density	1000 kg/m^3, liquid (4 °C) (62.4 lb/cu. ft) 917 kg/m^3, solid
Melting point	0 °C,(273.15 K)
Boiling point	99.98 °C, 211.97 °F (373.13 K)
Vapor pressure	2.338 kPa (20 °C)
Acidity (pK_a)	15.74 ~35–36
Basicity (pK_b)	15.74
Reftractive Index (n_D)	1.3330
Viscosity	0.001 Pa s at 20 °C
Dipole moment	1.85 D
Polarity index	9

APPENDIX B

Analysis of Vitamin C content from HPLC



Area Percent Report

Sorted By : Signal
Multiplier: : 1.0000
Dilution: : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 D, Sig=230,16 Ref=360,100

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.543	BV	0.3307	179.62212	8.03831	42.3933
2	3.260	VB	0.1667	181.59019	16.98829	42.8578
3	3.959	BB	0.2654	62.49190	3.65574	14.7489
Totals :				423.70421	28.68234	

*** End of Report ***