THE EFFECT OF EXTRACTION PARAMETERS ON ANTIOXIDANT ACTIVITY OF *Cosmos caudatus*

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ABSTRACT

The present of natural antioxidant in plant is well known and *Cosmos caudatus* is one of the plants that have high content of antioxidant. The objective of this study was to investigate the effect of operating conditions on extraction of total antioxidant compound (TAC) from *C. caudatus.* TAC was extracted using 70 % (v/v) acetone as the solvent for 4, 8 hour and 12 hours. *C. caudatus* was then extracted at different to solid to solvent ratio of 1:20, 1:25 and 1:30 (w/v). Finally, *C. caudatus* was extracted at different extraction temperature (60, 80 and 100 ^oC) for 30 min. The results show that the best extraction time to extract the TAC was after 8 hour extraction using solvent to sample ratio of 1:30 (w/v) and 66 ^oC of extraction temperature. The TAC obtained was 28.8 mg AAE/g with the corresponding of 10.1% of its antioxidant activity. However, when considered the extraction temperature, the highest value of TAC (19.5 mg AAE/g) was observed in extraction at 80^o C after 30 minute and for temperature 60 and 100 ^oC the TAC were 16.4 and 17.1 mg AAE/g. In HPLC analysis using the ratio sample to solvent at 8 hour, the highest TAC was at 1:30 (2.368 mg AAE/g) and also for the extraction temperature 80 ^oC has highest TAC (1.84611 mg AAE/g). This trend was similar with the result obtained from DPPH assay where 1:30 (w/v) and at 80 ^oC extraction has highest TAC.

KESAN PENGEKSTRATAN PARAMETER KE ATAS ANTIOKSIDAN AKTIVITI *Cosmos caudatus*

ABSTRAK

Pada masa kini antioksida semula jadi dari tumbuhan diketahui umum dan ulam raja merupakan salah satu tumbuhan yang mempunyai kandungan antioksida yang tinggi. Objektif kajian ini adalah untuk mengkaji kesan pengekstratan parameter ke atas antioksidan aktiviti *Cosmos caudatus*. Jumlah sebatian antioksidan menggunakan 70 % aceton sebagai pelarut untuk 4, 8 dan 12 jam. Ulam raja kemudiannye diekstrak pada nisbah pepejal kepada pelarut yang berbeza sebanyak 1:20, 1:25 dan 1:30 . Akhirnya ulam raja diekstrak pada suhu yang berbeza (60, 80 dan 100 ^{o}C) selama 30 minit. Keputusan menunjukkan bahawa pengekstrakan terbaik untuk mengekstrak jumlah sebatian antioksidan adalah pada pasa 8 jam menggunakan nisbah kepada pelarut 1:30 dan pada suhu 66 ^{o}C. Jumlah sebatian antioksidan yg diperolehi adalah 28.8 mg AAE/g dengan bersamaan 10.1% aktiviti antioksida. Walau bagaimanapun, apabila diekstark menggunakan suhu, nilai tertinggi jumlah sebatian antioksida adalah 19.5 mg AAE/g diperhatikan pada suhu 80 ^{o}C selepas 30 minit. Dalam analisis HPLC menggunakan sampel nisbah pelarut pada masa 8 jam pada setiap nisbah 1:20,1:25 dan 1:30, hasilnya 1:30 mempunyai keputusan jumlah sebatian antioksida yang tertinngi iaitu 2.368 mg AAE/g dan juga pada suhu 80 ^{o}C mempunyai nilai jumlah sebatian tertinggi iaitu 1.8461 mg AAE/g. Hasil daripada analisis HPLC menunjukkan bacaan yang sama seperti analisis menggunakan DPPH dimana 1:30 dan suhu 80 ^{o}C mempunyai nilai kandungan antioksidan yang tinggi.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

mAU.s	milliabsorbance units
mg	miligram
mg/g	milligram/gram
min	minute
min	minute
nm	nanometer
^{o}C	degree Celsius
ppm	part per million
v/v %	volume of solvent/volume of solute
w/v	weight of sample/ volume of solvent

LIST OF ABBREVIATIONS

AAC	Ascorbic acid content
AAE	Ascorbic acid equivalent
AC	Absorbance control
AS	Absorbance sample
C.caudatus	*Cosmos caudatus*
DPPH	2, 2, diphenyl-1-picrylhyrazyl
HPLC	High Performance Liquid Chromatography
KH_2PO_4	Potassium dihydrogen phosphate
TCA	Total antioxidant content
TPC	Total Phenolic Content

CHAPTER 1

INTRODUCTION

1.1 Research Background

Malaysia, being a tropical country, is blessed with a variety of plants which according to ethno botanical record, can be categorized as medicinal plants. Some of the plants are used as vegetables and spices. Those which are consumed raw or after a short blanching are collectively called "ulam". These include 'Selom', 'pegaga' 'Kesum and 'Ulam raja''. Usually these vegetables are mixed with other ingredients such as chilies, grated coconut and rice (Noor zaleha *et al*., 2003). Most of these herbs are believed to be associated with antioxidant activities and have many beneficial effects (Huda-Faujan *et al*., 2007).

Cosmos caudatus is one of type of 'ulam' that contains high antioxidant content. It is an annual, short-lived, perennial, aromatic herb found to be containing extremely high antioxidant capacity (Shui *et al*., 2005). The young leaves are often eaten raw with chili or coconut paste and are used in dishes such as kerabu. They are also used as an appetizer and food flavouring due to their unique taste and aroma.

Traditionally, *C.caudatus* been used for improving blood circulation, as anti-aging agent, reducing body heat, strengthening bone marrow and also been used to treat infection associated with pathogenic microorganism (Rasdi *et al*, 2010).

To determine the antioxidant capacity of the plant, various methods and their extraction parameters need to be considered. Various researches have been studied such parameters in the extraction of TAC from various plant. The Extraction efficiency is influenced by various factors such as method of extraction, solvent type, solvent concentration, extraction time and temperature, particle size and solid to solvent ratio (Thoo *et al.* 2010). It also said that the parameter such as extraction time is crucial in solvent extraction because the compound may be affected by the equilibrium concentration of antioxidant and phenolic compound been reached. Hence, excess extraction time may reduce the yield of antioxidant content in sample.

According to Durling *et al.,* (2007); Silva *et al.,* (2007), heat being found to enhance the recovery of antioxidant capacity and phenolic compound in the extraction. In the study by Thoo *et al., (2010)* found increasing extraction temperature had a positive effect to both ABTS and DPPH radical-scavenging capacities but once the extraction reach it equilibrium temperature, the antioxidant capacity reach it maximum that can be extracted. Liyana- Pathirana and Shahidi (2005) reported that the rate of extraction of thermally stable the antioxidant at elevated temperature is higher than the rate of decomposition of less soluble antioxidant.

While study by Tan *et al.,* (2011) state high solid to solvent ratio could promote an increasing the concentration gradient which will increase the diffusion rate that allow greater extraction of solid by solvent. Interaction of the compound could have modified the activity coefficient and thus solubility of the compound to the solvent.

According to Frank *et al.*, (1999) a solid solubility is affected by change in the activity coefficient which varies with the temperature and composition of the solution. Therefore, in the extraction solid to sample ratio, the antioxidant content it been affected by solid to solvent ratio used.

1.1 Problem Statement

There are many researches that been conducted on study the extraction of various plants and herb in determining the antioxidant compounds contain in the plant. *C. caudatus* is one of the plant that containing high antioxidant compound. Extraction yield of total antioxidant compounds from plant materials are typically depending on different extraction parameter. Different extraction parameter such as temperature, time and sample to solvent ratio are some of the important parameter to be study in determining the antioxidant content from plant.

1.3 Research Objectives

The aim of this research is to investigate the effect of extraction parameter on antioxidant activity from *Cosmos caudatus.*

1.4 Scope of Study

To achieve this objective, four scopes have been identified in order to achieve the objective of this research:

i. Determining the effect of extraction time on antioxidant activity from *C. caudatus* for 4, 8, and 12 hour using Soxhlet extraction method. 70 % (v/v) acetone was used as the solvent by using ratio 1:20 at temperature 66 ^oC.
ii. Identifying the effect of sample to solvent ratio on the extraction of antioxidant activity from *C. caudatus.* The chosen sample to solvent ratio were 1:20, 1:25 and 1:30 (w/v) at temperature 66 ^0C.
iii. Determining the effect of temperature (60, 80 and 100 ^oC) on the extraction of antioxidant activity from *C. caudatus* using solvent extraction at ratio of 1:20 (w/v).
iv. Identifying the ascorbic acid content from *C. caudatus* extracts by using HPLC.

1.5 Significant of Study

C. caudatus has high antioxidant compounds among other plant and herb (Almey *et al*., 2010). The high level antioxidant content in *C. caudatus* has the potential to be used as natural antioxidant. By study the operating condition of *C. caudatus* give benefit in the other research that related to extraction of *C. caudatus.* Beside that it will give additional on operating condition in extracting *C. caudatus.* High content of antioxidants contained in *C. caudatus* could be partly responsible for its ability to reduce oxidative stress.

CHAPTER 2

LITERATURE REVIEW

2.1 *Cosmos caudatus*

Plants are the potential sources of natural antioxidant because they can produce various antioxidative in order to counteract with reactive oxygen species (ROS) (Huda-Faujan *et al.*, 2007). Most of work on local herbs, vegetables and "ulam" were concentrated on the total polyphenols content and antioxidant activities (Amin *et al*., 2004; and Asmah *et al*., 2000). 'Ulam' is a traditional Malay salad, they are consumed raw, however it depending on the type of 'ulam'.

C.caudatus is a popular herb in Malaysia and is locally known as ulam raja (Ong and Norzalina, 1999). This plant is native to tropical regions of the Americas and has been naturalized in Java, where it is often cultivated as an ornamental plant (Fuzzati *et al.*, 1995). It is found worldwide in tropical areas including Mexico, United States (Arizona and Florida), Central America, South America, Malaysia and Thailand (Samy *et al*., 2005). According to Plant Resources of South East Asia (PROSEA) *C. caudatus* was first been introduced by Philippines via the Spaniards.

It is annual, short lived, perennial, and aromatic herb. It young leaves is often eat raw with chili or coconut paste and been used in dishes as kerabu. Beside that, it also used as food flavouring and an appetizer due to its unique taste and aroma (Shui, *et al.*, 2004). *C.caudatus* is easy to grow. It thrives in a sunny spot outdoor with well draining, fertile and moist soil. Under optimal condition, it grows quickly, flower and sets seed readily**.** It is an edible plant having 20 - 26 species Worldwide.

C.caudatus is bearing purple, pink, or white ray florets, grows up to about 1 - 8 ft tall, hairless or sparsely hairy and leaves are finely dissected, 10 - 20 cm long. It flowers from June to November (Shui *et al*., 2005). Harvesting of leaves can commence once plant are 6 weeks old and subsequent once can be done every 3 weeks. With regular harvesting will stimulate the production and help to delays flowering.

In Malaysia, traditionally *C. caudatus* is been used as herbal medicine. Hassan,2006 state *C. caudatus is* used for improving blood circulation, as anti-aging agents, reducing body heat, strengthening bone marrow because in contain high calcium content and also to treat infection associated with pathogenic microorganism (Rasdi et al,2010). Beside that *C. caudatus* also been used for appetizer, carminative and insect repellent (Shui, *et al*, 2005). Figure 2.1 shows the picture of *C. caudatus* plant.

2.2 Extraction Parameters

Extraction is the initial and the most important step in the recovery and purification of bioactive compounds from plant materials. The extraction method can be affected by various conditions of parameter such as solvent used, temperature, time, and sample to solvent ratio. This been supported by Thoo *et al*., (2010) which said that the extraction efficiency is influenced by various factors such as method of extraction, solvent type, solvent concentration, extraction time and temperature, particle size and solid to solvent ratio. Therefore many researchers have been studied this operating condition for various plant, fruit and food.

Dent *et al.*, (2012) has studied on the effect of the extraction solvent, temperature and time on the composition and mass fraction of the *Salvia officinalis* L. Besides, Wong *et al*., (2012) have also studied the effect of solid to solvent ratio on phenolic and antioxidant capacities of *Phyllanthus niruri*. From their studies, it has shown the best parameter and condition that needed to be used to extract the plant in order to get highest yield of antioxidant activity or phenolic content of the plant or herbs. From their studied, solid to solvent ratio of 1:20 (w/v) show the highest amount of antioxidant activity and TPC.

2.2.1 Solid to Solvent Ratio

One of the effects that influenced the extraction of antioxidant activity is the solid to solvent ratio. According to Tan *et al.*, (2011), the antioxidant activity is affected by the extraction of solvent used. Beside that, Al- Farsi and Chang, (2007) said that a high solid to solvent ratio could promote an increasing of concentration gradient which resulting in an increase of diffusion rate that allow greater extraction of the solid by the solvent.

While Zhang *et al.*, (2007) also state that there is a possibility of bio-active component coming into contact with the extracting solvent and it will not increase as equilibrium is reached. It means that, the extraction of solvent will not increase as all the solvent has react with the solid. The optimum yield of the antioxidant and phenolic content can reach when the solid to solvent ratio reach it optimum condition. There was study by Tan *et al*., (2011) on *Cantella asiatica* which it yield the antioxidant capacities that increase gradually with the increase of solid to solvent ratio and achieved the highest antioxidant capacities and DPPH radical scavenging.

2.2.3 Extraction Time

The extraction of antioxidant from plant and fruit is affected by the time of extraction and temperature to be used for the extraction in order to maximize the yield of antioxidant compound. According to Shui *et al*., (2005), the best extraction parameter needs to be identified such as time beside method of extraction that needs to be used. For each method of extraction there are time and temperature parameter that need to be consider. It is also observed that the optimum extraction time for antioxidant compounds varies with antioxidant capacity. This phenomenon has been postulated that the estimation of ABTS and DPPH radical-scavenging capacities are not solely dependent on a single group of antioxidant compounds; indeed it is based on the ability of any compounds present that could scavenge ABTS or DPPH radicals (Prior *et al.,* 2005).

The studied by Benhammou *et al*, (2008) state that as the time of the extraction increase the antioxidant yield will also increase but according to Herodež *et al*., (2003) state that the active component yields will not continue to increase once equilibrium is reached. According to Chew *et al*., (2011) extraction time is a crucial in solvent extraction because appropriate extraction time can result in time and cost saving. Beside that, the value of antioxidant capacities is significantly decreased as the maximum time for the extraction is reached. Therefore, to study the extraction time and temperature is definitely important recognize the suitable parameter to be used in the extraction for each plant or fruit. This is because; excess extraction time will reduce the yield of antioxidant compounds.

2.2.4 Effects of Temperature on Extraction

Temperature is one of the parameter used in extraction. This parameter will affect the result of antioxidant yield from the extract because different extraction from plant needed different temperature. Beside that, according Durling *et al.,* (2007) said that heat being found to enhance the recovery of antioxidant capacity and phenolic compound in the extraction. While the studied Dent *et al*., (2012) has been study the effect of extraction parameter on time and temperature from *Salvia officinalis* L. from the study it been found that as the temperature is been increase, the antioxidant activity also increase.

However, further raise in extraction temperature could degrade phenolic compounds, due to the interference compound stability caused by chemical and enzymatic degradation, or reaction with other plant components reducing extraction efficiency (Durling *et al.*, 2007). Increasing in extraction temperature will be helpful in the increasing the extraction rate, as well as reducing the extraction time (Cacace and Mazza, 2003).

It was confirmed that the rate of extraction of thermally stable antioxidants at elevated temperature was higher than the rate of decomposition of less soluble antioxidants (Liyana-Pathirana and Shahidi, 2005). The temperature during extraction influences the compound stability due to chemical and enzymatic degradation and losses by thermal decomposition; these have been suggested to be the main mechanisms causing the reduction in polyphenol content (Corrales *et al*., 2008).

2.3 Review Related Research on Operating Parameter used for Extraction

Table 2.1: The extraction Operating Condition Used by some researchers in Determining the Antioxidant Yield

Operating parameter	**Herbs**	**Antioxidant yield (mg AA/100g)s**	**References**
Solid to sample ratio: 1:10 Temperature: 40°C Time: 8 hour Solvent: ethanol	*Artemisia argyi* *Centella asiatica* *C. caudatus* *Polygonum hydropiper*	654.43 ± 17.22 717.68 ± 46.46 3200.37 ± 54.11 5867.54 ± 164.13	Lee and Vairappan ,2011
Solvent: methanol Time: 24 hour	*Centella asiatica*	8.13± 11.7	Zainol *et al*,2003
Solvent : water Time: 12 hour Temperature: 100°C	*C. domestica* *B. racemosa* *C. caudatus* *P.sarmentosum* *K. galangal*	78.92 81.49 15.44 24.81 83.27	Sumaizian *et al*,2010
Solvent To Sample Ratio:1:5,1:10,1:15 and 1:20 (w/v) Solvent: 60.4 % ethanol Time:45 min	*Phyllanthus niruri*	1906.5	Wong *et al*.(2012)
Solvent :70% acetone Temperature :56.6°C	*C. caudatus*	7.77 mg AAE/g	Nur Ain, (2012)

Table 2.1 shows the antioxidant yield for various plants from previous studies. There were different types of parameter used such as solvent, sample to solvent ratio, temperature and time for the extraction. The studied by Nur Ain (2012) shows that 70% acetone extract the highest antioxidant yield (7.77mg AAE/g) from *C. caudatus* compared to 50% and 100 % acetone. Moreover, from this studies also state that acetone give highest yield of antioxidant compare to other solvent which was ethanol. Acetone system provided the extract with greater content of phenolic and antioxidant compounds. Moreover, boiling point of acetone was approximately 22 °C lower than that of ethanol (78.37 ^{o}C), lower temperature for solvent evaporation could be used, which could save energy and reduce thermal degradation of the antioxidants during evaporation process (Lied, 2008).

2.4 Antioxidant

Herbs and spices that are usually used to flavor dishes are among the tremendous sources of phenolic compounds, which have been reported to show good antioxidant activity (Zheng & Wang, 2001). Antioxidants are widely found in nature especially in plant. Antioxidant also can be produced within in the body and from the fruit, vegetables, seed, meat and oil. The examples of plant that have antioxidant content are *Murraya koenigii*, *Citrus hysrix*, *Pandanus odurus* and other various plants.

Most of the extracts from fruits and vegetables exhibit some antioxidant properties. One of the most widely studied antioxidants in fruits and vegetables is L-ascorbic acid (vitamin C). It has numerous biological functions, which include the synthesis of collagen, some hormones and certain neurotransmitters. It is believed that the role of L-ascorbic acid in disease prevention is due to its ability to scavenge free radicals in the biological systems. The majority of the antioxidant capacity of a fruit or vegetable may be contributed by compounds other than vitamin C. For example, carotenoids, another big family of compounds with antioxidant activities, are the most common and most important natural pigments in fruit and vegetables (Guan *et al.*, 2005).

Antioxidant is a protection agent which can terminate the initiation of oxidizing chain reactions and it can inhibit or delay oxidation by other molecules (Suchandra *et al.*, 2007). Oxidation processes are important because it can control the production of free radicals and the unbalanced mechanism of antioxidant protection that can cause diseases and accelerated ageing (Dawidowicz *et al.*, 2006).

According to the definition by Britton, an effective antioxidant is a molecule able to remove these radicals from the system either by reacting with them to yield harmless products or by disrupting or inhibiting free radical chain reactions (Cadenas, 2000). Halliwell and Gutteridge define an antioxidant as “any substance that, when present at low concentrations compared with those of an oxidizable substrate significantly delay or prevent oxidation of that substrate (Halliwell and Gutteridge, 1999).

2.5 Equipment

2.5.1 Soxhlet Extractor

Solid liquid extraction is also known as solvent extraction is one of the processes of extraction. The extraction process consist two steps. First the solvent is contact with solid. After that, the residual solid is separated from the solution. It also process of removing a solute from solid using liquid solvent. Soxhlet is one the technique used to extract solid medium into organic solvent (Luque de Castro and Garcia-Ayuso, 1998). Figure 2.1 shows schematic diagram of soxhlet extractor.

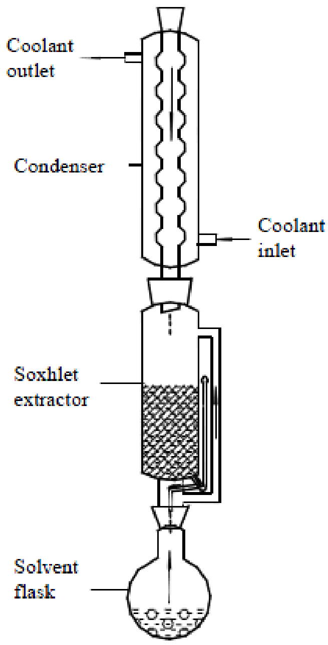

Figure 2.1 Soxhlet extractor