EFFECT OF DRYING [boilerplate: PERPUSTAKAAN UMP 0000076046] NT CAPACITY OF *Cosmos*

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EFFECT OF DRYING METHOD ON THE ANTIOXIDANT CAPACITY OF *Cosmos caudatus* EXTRACT

ABSTRACT

Cosmos caudatus have high antioxidant capacity and able to contribute to the capability to scavenge free radical ions and reduce oxidative stress. However, the antioxidants may decrease during the drying process which further reduces the functionality of the herb. The purpose of this study was to investigate the effect of drying methods on the antioxidant capacity of *C. caudatus* extract. Three different types of drying treatment were set; freeze dryer (-40°C, 72 hours), spray dryer (150°C, 20 rpm of pump setting, 50 rpm of fan setting) and oven dryer (40°C, 72 hours). In this study, Soxhlet extraction method was used and distilled water and 70% acetone (v/v) as the extraction solvent. The determination of antioxidant and ascorbic acid content were tested by DPPH assay and HPLC analysis respectively. Freeze-dried sample of 70% acetone (v/v) exhibits highest concentration of antioxidant (59.00 mgAAE/g) followed by spray-dried and oven-dried sample (37.00 mgAAE/g and 36.00 mgAAE/g) respectively. HPLC analysis shows significant results of ascorbic acid content where freeze-dried sample of 70% acetone (v/v) exhibits highest ascorbic acid concentration followed by spray-dried and oven-dried samples (1.09 mgAAE/g, 0.69 mgAAE/g and 0.48 mgAAE/g) respectively. Freeze dryer was the efficient technique for the preservation of antioxidant compounds followed by spray dryer and oven dryer. Further study is proposed by differentiating pre-drying temperature and drying periods used for each dryer to discover the effect of pre-drying temperature and drying period on the antioxidant compounds preserved.

KESAN KAEDAH PENGERINGAN TERHADAP KAPASITI ANTIOKSIDAN DARIPADA PENGEKSTRAKAN *Cosmos caudatus*

ABSTRAK

Cosmos caudatus mempunyai kapasiti antioksidan yang tinggi dan mampu menyumbang kepada keupayaan menghapuskan ion radikal bebas dan mengurangkan tekanan oksidatif. Akan tetapi, nilai antioksidan akan berkurangan semasa proses pengeringan sekaligus mengurangkan fungsi herba tersebut. Tujuan kajian adalah untuk mengkaji kesan kaedah pengeringan terhadap kapasiti antioksidan daripada pengekstrakan *C. caudatus*. Tiga kaedah digunakan untuk pengeringan sampel ekstrak iaitu pengering beku (-40°C, 72 jam), pengering semburan (150°C, penetapan pam sebanyak 20 putaran seminit, penetapan kipas sebanyak 50 putaran seminit) dan pengering ketuhar (40°C, 72 jam). Kajian ini menggunakan kaedah pengekstrakan Soxhlet dan air suling serta 70% larutan aseton digunakan sebagai pelarut. Aktiviti antioksidan dan kandungan asid askorbik telah diuji dengan cerakin DPPH dan analisis kromatografi cecair berprestasi tinggi. Sampel pengering beku daripada 70% larutan aseton mempamerkan kepekatan tertinggi sebatian antioksidan (59.00 mgAAE/g) diikuti sampel semburan kering dan pengering ketuhar (37.00 mgAAE/g dan 36 mgAAE/g). Analisis kromatografi cecair berprestasi tinggi menunjukkan keputusan dimana sampel pengering beku daripada 70% larutan aseton mempamerkan kepekatan kandungan asid askorbik tertinggi diikuti sampel semburan kering dan pengering ketuhar (1.09 mgAAE/g, 0.69 mgAAE/g dan 0.48 mgAAE/g). Teknik pengeringan beku adalah cara yang paling berkesan dalam pemeliharaan sebatian antioksidan diikuti oleh pengering semburan dan pengering ketuhar. Kajian lanjut boleh dilakukan dengan membezakan suhu pra-pengeringan dan tempoh masa pengeringan ekstrak bagi mengetahui kesan suhu pra-pengeringan dan tempoh pengeringan terhadap pemeliharaan sebatian antioksidan.

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AAE	-	Ascorbic acid equivalent
C. caudatus	-	*Cosmos caudatus*
DPPH	-	2, 2-diphenyl-1-picrylhydrazyl
EtOH	-	Ethanol
hr	-	Hour
KH_2PO_4	-	Potassium dihydrogen phosphate
MeOH	-	Methanol
min	-	Minute
OD	-	Optical Density
rpm	-	Rotation per minute
T	-	Temperature
UV	-	Ultra-violet
UV-Vis	-	Ultra-violet Visible

LIST OF SYMBOLS

%	-	Percentage
µL	-	Micro-Liter
g	-	Gram
g/mL	-	Gram per milli-Liter
L	-	Liter
mAU.s	-	milliabsorbance units
mg/g	-	milli-gram per gram
mg/L	-	milli-gram per Liter
mg/mL	-	milli-gram per milli-Liter
mgAAE/g	-	milli-gram Ascorbic Acid Equivalent per gram of extract sample
mgAAE/mL	-	milli-gram Ascorbic Acid per milli-Liter
ML	-	Moisture lost
mL	-	milli-liter
nm	-	nanometer
°C	-	Degree Celsius
°F	-	Degree Fahrenheit
v/v	-	Ratio of solvent to water
w/w	-	Mass of sample per mass of dry extract

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Herbs are very significance with their advantages to improve healthiness and prevent diseases. One of herb that is rich in nutrients is *Cosmos caudatus. C. caudatus* belongs to the family of asteraceae, an edible plant having about 20 to 26 species worldwide (Md Rasdi *et al.,* 2010). It is an annual and short-lived aromatic herb with purple, pink or white ray florets, grows up from one to eight feet tall, hairless or sparsely hairy, and the leaves are finely dissected, 10 to 20 cm long (Shui, Lai & Shih, 2005).

C. caudatus is originated from tropical Central of America and already widespread in almost part of tropical regions including Malaysia, Thailand and other South-East Asian countries, Mexico, and South America. *C. caudatus* usually consumed freshly as salad or cooked. They are also used as an appetizer and natural food seasoning due to their unique taste and aroma. In Malaysia, *C. caudatus* is well-known as ulam raja (King's salad), in Indonesia, it is known as kenikir or randa midang, and in Thailand, it

is called daoruang-phama or khamhae (Global Information Hub on Integrated Medicine, 2010).

The freshness of herbs cannot last longer although been refrigerated for a long period of time and may affect the nutrient mainly antioxidants presence. Therefore, searching of technique that is suitable to be used to preserve the bioactive nutrients is strongly encouraged although is it not consumed freshly. One of the best methods is by drying process. There are various drying techniques that can be used in which give different results based on the method of drying, drying period and temperature used for the drying process.

1.2 Problem Statement

Drying is the oldest method of preserving food by removing water content inside foods. Examples of foods that usually been dried are fruits and herbs such as corn, apple slices and kiwi slices, ginger, peppermint, meats and fishes and others (Troftgruben, 1977). In addition, dried foods can last-longer and no microorganisms are able to grow because of low humidity of water content. However, drying will not preserve the taste, texture and appearance of the foods compared to canning and freezing methods. This is because of the changes of the water content inside the food that may affect the taste itself, the shrinkage of the dried product and also the change in dried foods colour. There are lots of methods to dry foods; by air drying, oven drying, spray drying and also freeze drying.

Although drying of foods helps in preserving and ensure long-lasting of foods' expiry date, there are problems in drying method especially for herbs processing. It is known that herb is rich in nutrients that can be easily affected by high temperature when drying process occurs. Active chemical compounds such as various types of antioxidants compounds and phenolic contents may decrease as the drying process occurred thus reducing the functionality of the herbs. The total antioxidant compounds preserved during drying process is affected by several factors. The factors include types of drying method used, temperature applied for drying process and types of solvent used for extraction process.

Therefore, it is essential to determine which drying methods that may be the best to be used to preserve the antioxidant capacity of *C. caudatus* in addition with type of solvent used as extraction medium so that the beneficial of its nutrients can be manipulated and used as supplement or food additive to increase nutrients intake.

1.3 Research Objective

The objective of this research is to study the effect of drying method on the antioxidant capacity of *C. caudatus* extract.

1.3 Scope of Study

The scopes of this study include:

1.4.1 Extracting antioxidant content from *C. caudatus* by using Soxhlet extraction method for eight hours for distilled water and 70% acetone solution.

1.4.2 Investigating the effect of freeze-drying, spray-drying and oven-drying method on antioxidant capacity of *C. caudatus* extract.

1.4.3 Identifying the ascorbic acid content from *C. caudatus* extracts by using High Performance Liquid Chromatography (HPLC).

1.5 Significance of Study

C. caudatus has been used traditionally in various scopes as remedy to cure diseases. The effectiveness of the remedies is studied so that the usefulness of this substance can be proving scientifically. Antioxidant contents of *C. caudatus* leaves were studied in relation to the processes involved in food and herbal medicine preparation. (Sukrasno *et al.*, 2011). Thus, the antioxidant capacity of the herb must be preserved so that the production yields of supplements or new applications can be perform. The best drying methods also must be known in search of the best preserved antioxidant capacity from *C. caudatus* extract. Fresh *C. caudatus* is rich in flavonoid and antioxidant activities. Therefore, it is best to manipulate this content by using drying method to

preserve the antioxidant capacity by selecting drying method which gives the best results.

CHAPTER 2

LITERATURE REVIEW

2.1 *Cosmos caudatus*

Cosmos caudatus (genus: Cosmos, family: Asteraceae) are plants that can be eaten raw mainly its leaves part as a form of local salad. It is a well-known traditional salad that got a high demand on market nowadays not only because of its unique aroma and taste, but also because of its high nutritional values. The main nutrients of *C. caudatus* are vitamins and minerals in which ensure their consumers' health with no need of modern supplements.

Natively, *C. caudatus* can be found in Northern and Southern Mexico, Southern America such as Costa Rica, El Salvador, Guatemala, and Panama, Northern South America such as Venezuela and Western South America like Colombia. Because of its ability to grow wild especially in tropical region, *C. caudatus* can be easily found in

South-East Asian region including Malaysia, Indonesia and Thailand, including Africa and Asia Pacific Country (GRIN, 2012).

Figure 2.1 *Cosmos caudatus* (Ulam raja)

2.2 Antioxidant Components in *C. caudatus*

Antioxidant molecules are used to neutralize the free radical ions inside body by supplying another electron to balance it. Free radical molecules which are created from oxidative process of chemical reaction, exposure to pollution, cigarette smokes and fatty foods in the body are unstable because of the loss of electron and able to react with other molecules to form other free radical. Omenn, Goodman and Thornquist (2009) identifies that massive amount of free radical molecules inside body may cause damage to the cell, and have relation to the development of heart and liver disease, cancers, arthritis, accelerated aging and cataract. Without antioxidant, our body cells are unable to rejuvenate them and can easily been damage. This may results in improper health for human.

C. caudatus plays an important role to nature to be used as remedy for various diseases. Based on previous research, it is indicated that the *C. caudatus* had extremely high antioxidant capacity especially proanthocyanidins and quercetin which is believed to have some medicinal functions (Shih, Lai & Koh, 2005). It contributes to the ability to scavenge free radicals and reducing oxidative stress (Sukrasno *et al.,* 2011). Analysis of the extractions of *C. caudatus* leaves shows highest linoleic acid oxidation, DPPH, ABTS, and ferric cyanide antioxidant capacities as compared to other vegetables tested (Andarwulan *et al.,* 2010). This results supported by Shui, Lai & Shih (2005) which prove that the *Cosmos caudatus* had the highest DPPH free radical scavenging activity.

It is reported that *C. caudatus* contains several types of bioactive components for examples, antimutagen and antifungal compounds. This is supported by Md Rasdi *et al.* (2010) that showed antimicrobial activity against microorganisms tested. This result shows the effectiveness and multiple functions of *Cosmos caudatus* in which beneficial to the society. It is necessary to search for new methods of preserving antioxidant components rather than depending on intake of fresh raw vegetables which are enrich in vitamins and minerals. Altering the fresh raw of *C. caudatus* as a new potent in dry condition and used as supplements or food additives may be useful for obtaining enough nutrients needed. By preserving the antioxidant contents of *C. caudatus* in dry state helps in prolonging the antioxidant compounds durability. Thus, is it important to determine the best drying method need to be used for this study to obtain highest amount of antioxidant compounds preserved from the drying process.

2.3 Ascorbic Acid

Ascorbic acid is one of many antioxidants present naturally in fruits and vegetables. The ascorbic acid with formula $C_6H_8O_6$ appears in white to yellowish colour in powder and crystal form with odourless characteristic. Ascorbic acid is a light sensitive substance but stable under normal temperature (Sigma-Aldrich, 2012; Sciencelab.com Inc., 2005). Ascorbic acid is a type of formerly known antioxidant presents richly in fruits and vegetables. Ascorbic acid also known as Vitamin C is water soluble vitamins which can only be obtained through consumptions of foods enriched in Vitamin C such as citrus fruits, dark leafy greens, broccoli and herbs. This type of vitamin is easily excreted from the body. Thus, continuous supply of Vitamin C is needed to ensure enough continuous supply to fulfill the requirement of body cells instead of depending on the cell-self production (The George Mateljan Foundation, no date).

Antioxidant such as Vitamin C is an essential nutrient required to help in reducing the oxidative stress of free radical ions inside the body. Vitamin C helps to reduce potency of some damages on the body caused by free radicals by which able to cause cancer, heart disease and also arthritis. In addition, higher free radicals are also responsible for the increase rate of aging process (U.S. National Library of Medicine, 2012). Vitamin C also helps to promote effective production of collagen. Collagen is a type of protein which is important to be used for healing wounds on skins, build up structure to bones, tendons cartilages, muscles and blood vessels. Furthermore, Vitamin C also helps for iron absorption inside the body. The United States Adequate Intake (AI) outlined the intake of Vitamin C is 90 milligrams per day and 75 milligrams per day for men and women respectively (Mosure, 2004).

Despite of lessen the oxidative stress, Vitamin C is also important for maintaining healthy body from diseases. Some common diseases related to the deficiency of Vitamin C are scurvy, weak immune functions and lung-infections. Scurvy is a disease where lacking of Vitamin C occur. Symptoms of scurvy are bleeding of gums, loosened teeth, loss of appetite, bleeding in eyes and others (Medical News Today, 2009). Other than that, peoples who lack of Vitamin C may have weak immune systems and easily affected by colds and other infections. Respiratory tracts in which depends a lot with Vitamin C for protection may affected by respiratory infection and lung-related conditions in lacking of Vitamin C (The George Mateljan Foundation, no date).

The ascorbic acid is widely used in food and beverages processing industry for its nutritional values and also for its ability to preserve product's quality. The ascorbic acid is added to the processed fruit juices to restore or as fortified nutrient and also helps to improve the colour and the appearance of products. According to Takeda U.S.A Incorporation (no date), ascorbic acid may act as an oxidizing agent in surrounding with low oxygen concentration. Thus, the oxidizing agent reduces the available oxygen present in environment. This action makes the ascorbic acid as an effective antioxidant.

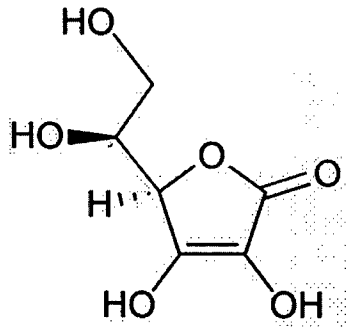

Figure 2.2 The structure of ascorbic acid molecule

2.4 The Drying Methods

One of methods that always been use to preserve foods like herbs is by using drying method. Drying is a process where the water content been removed from foods. Low of water content and humidity helps to ensure for long-lasting of foods because of less possibility for germination of microorganisms. There are lots of method to dry foods, some of well-known drying process including freeze drying, spray drying and also oven drying. For each drying method, there are pro and cons depending on what types of foods that we want to dry. Different method results in different quality of foods. Thus, the best method to be used is depending on the types of foods, water content, times taken and also preservation of foods' nutrients as well as appearance and colour.

2.4.1 Freeze Drying

Freeze drying process has been widely use in a number of applications especially in food and pharmaceutical industries. Freeze drying also known as lyophilization is a dehydration process which preserves perishable material. This process can be done by freezing material (food) and the reducing the surroundings pressure to allow sublimation of frozen water. Sublimation process occurs when a frozen liquid directly goes into gaseous state without turned into liquid phase first (Labconco Corp., 2010). There are three stages in the process of freeze drying; prefreezing, primary drying and secondary drying.

For the pre-freezing stage, fresh or cooked foods are flash frozen, and then placed in a vacuum chamber (Emergency Essentials, 2012). The methods of handling the pre-frozen and the final temperature of the products' frozen can affect the successfulness of freeze dry process. Next, during primary drying, about 98% of the foods moisture is evaporated by evaporating process of the ice. This is done at temperature as low as -50°F (Emergency Essentials, 2012). Conditions must be established in which ice can be removed from the frozen product. This step requires two important parameters, which are temperature and pressure (Labconco Corp., 2010). For secondary drying step, Continued drying is necessary at the warmer temperature to reduce the residual moisture content to optimum values. Heat or high temperature is applied to the frozen product to accelerate the sublimation process so that the remaining moisture can be removed. Next step, low temperature condenser plate remove the vaporize solvent from the vacuum chamber by converting it back to solid. This complete the separation process (Bellis, 2012).

Some advantages of using freeze drying method are freeze dried products maintain the nutrients, shape and size, colour, flavor, and also the texture of the food products. In addition, the frozen product reconstitute to its original state when placed in water, no requirement for cold storage and offers highest quality in a dry product. However, this method have its own disadvantages, including need expensive equipment for freeze drying process, cost effective for selective products and usually restricted to delicate, heat sensitive materials of high value (Emergency Essentials, 2012).