EFFECTS OF DRYING METHODS ON THE ANTIOXIDANT ACTIVITIES ON *BENINCASA HISPIDA* SEED EXTRACTS

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Thesis submitted in fulfilment of the requirements for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

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SUPERVISOR DECLARATION

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

Signature :.....

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Date :.....

STUDENT DECLARATION

I declare that this thesis entitled "Effect of Drying Methods on the Antioxidant Activities of Extracted from *Benincasa hispida* seeds'' is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature :.....

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Dedicated to my beloved father and mother

My friends, who gave me everlasting inspiration, never ending encouragements and priceless support towards the success of this research project.

Thanks for everything.

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ABSTRACT

The present study investigated the changes in total phenolic content (TPC) and antioxidant capacity of *B.hispida* seeds after three drying treatment (sun drying, oven drying and freeze drying) compared to fresh samples by DPPH radical scavenging method and total phenolic content. Ethanol is used as the solvent for extraction because ethanol can obtain the highest antioxidant activity due to its highest polarity. The analysis was conducted in University Malaysia Pahang laboratory using the ultraviolet-visible spectrophotometry. The results of this study showed that the water content, antioxidant activity and total phenolic content which are highest from *B.hispida* seeds were obtained by freeze drying. Sun drying was the worst in antioxidant capacity and phenolic content because the value obtained are less compared other methods. Overall, *B. hispida* may have high potential applications in the medical and food industries.

KESAN-KESAN KAEDAH PENGERINGAN TERHADAP AKTIVITI ANTIOKSIDAN KE ATAS PENGEKSTRAKAN BIJI *BENINCASA HISPIDA*

ABSTRAK

Kajian ini menyiasat perubahan dalam jumlah kandungan phenol dan kapasiti antioksidan dalam biji benih *B.hispida* selepas tiga kaedah pengeringan (pengeringan matahari, pengeringan ketuhar dan pengeringan sejukbeku) berbanding dengan sampel segar dengan kaedah DPPH radikal dan kandungan phenolik total. Etanol digunakan sebagai pelarut untuk pengekstrakan kerana etanol boleh mendapatkan aktiviti antioksidan tertinggi disebabkan kepolarannya adalah tinggi. Analisis telah dijalankan di makmal Universiti Malaysia Pahang dengan menggunakan spektrofotometri ultraungu. Keputusan kajian ini menunjukkan bahawa kandungan kelembapan, aktiviti antioksida dan kandungan jumlah phenol yang tertinggi telah didapati dalam biji benih *B.hispida* adalah daripada pengeringan sejukbeku. Pengeringan matahari adalah yang terburuk dalam kapasiti antioksidan dan kandungan phenolik kerana mempunyai jumlah yang sedikit. Secara keseluruhan, *B. hispida* mungkin mempunyai aplikasi potensi tinggi dalam industri perubatan dan makanan.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENT	iv
	ABSTRACT	v
	ABSTRAK	vii
	LIST OF TABLES	
	LIST OF FIGURES	
	LIST OF ABBREVIATION	
1	**INTRODUCTION**	
	1.1 Research Background	1
	1.2 Statement of Problem	3
	1.3 Research Objective	4
	1.4 Scope of Proposed Study	5
	1.5 Significant of Proposed Study	6
2	**LITERATURE REVIEW**	
	2.1 Oxidation	7
	2.2 *Benincasa hispida*	8
	2.2.1 The morphology of Seeds *B.hispida*	8
	2.2.2 The Uses and Benefits of *B.hispida* Seeds	10

2.2.3 Previous Studies Done on *B.hispida* Seeds 11

2.3 Natural Antioxidants From Plants 12

2.3.1 Source of Natural Antioxidants 12

2.3.2 Benefits of Antioxidants 14

2.4 Assays for Detecting Antioxidant Activity 15

2.4.1 DPPH Radical Scavenging Activity 15

2.4.2 Total Phenolic Content 16

2.5 Aqeous Solvent Extraction Method

2.5.1 Process of Extraction 18

2.5.2 Solvents 19

2.6 Effects of Drying Methods 22

2.6.1 Sun Drying 23

2.6.2 Oven Drying 24

2.6.3 Freeze Drying 25

2.6.4 Effect of Moisture Content 26

2.6.5 Effect of Drying Temperature 28

3 MATERIALS AND METHOD

3.1 Introduction 29

3.2 Materials 31

3.2.1 Plant Material 31

3.2.2 Chemicals 31

3.3 Methods 32

3.3.1 Drying Process 32

3.3.2 Moisture Content 33

3.3.3 Preparation of Plant Extracts 33

3.3.4 Antioxidant Activity Assay 34

3.3.4.1 Determination of DPPH Scavenging Activity 34

3.3.5 Assay for Phenolic Content 35

4 RESULTS AND DISCUSION

4.1 Introduction 36

4.2 Effects of Drying on *B.hispida* Seeds 37

4.3 Effects of Drying on DPPH Radical Scavenging Activity 40

4.4 Effect of Drying on Total Phenolic Content 42

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion 45

5.2 Recommendation for Future Work 46

REFERENCES 47

APPENDIX A 50

LIST OF TABLES

		PAGE
Table 2.1	Some common medicinal and pharmacological properties of different parts of Kundur (*Benincasa hispida* fruit)	14
Table 2.2	Solvents commonly used for the extraction of the plants	20
Table 2.3	Effect of moisture content and total vitamin C values of dried and fresh red delicious apple slices	27
Table 2.4	Analysis of dried Misai Kucing leaves extract	28
Table 4.1	Water content for different drying methods compare fresh of seeds *B.hispida*	38

LIST OF FIGURES

		PAGE
Figure 2.1	Half cut Kundur (*Benincasa hispisa*) fruit	9
Figure 2.2	Color changes in Kundur (*Benincasa hispisa*) fruit seed with maturity	10
Figure 2.3	DPPH structure	16
Figure 2.4	The structure of Phenol	16
Figure 2.5	Extraction Process	18
Figure 2.6	Solar drying	24
Figure 2.7	Oven drying	25
Figure 2.8	Freeze drying	26
Figure 3.1	Overview of overall methodology	30
Figure 4.1	The seeds of *B.hispida* before and after drying	38
Figure 4.2	DPPH radical scavenging acticity of sun, oven and freeze dried compare fresh of *Benincasa hispida* seeds	41
Figure 4.3	Total phenolic contents in fresh, oven and freeze dried seeds of *B.hispida*	43

LIST OF ABBREVIATIONS

AAE	Ascorbic acid equivalent
BH	*Benincasa hispida*
DPPH	1,1-diphenyl-2-picrylhydrazyl radical
DW	Distilled water
TAE	Tannic acid equivalent
TPC	Total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Research Background

Nowadays, oxidation is essential to many living organisms for the production of energy to fuel biological process. However, the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, genetic damage and the promotion of disease and aging. These free radicals are unstable and it can react with cells and destroy it. In order to reduce oxidation damage to the human, it is essential to develop and utilize effective natural antioxidants to protect the human body from free radicals and reduce risk of many diseases, cancer, arthritis and the aging process (Nandita *et al*., 2004).

As the alternative methods to reduce oxidation damage to the human, many of researchers do some research about the foods. Most of the foods high in antioxidants come from fruits and vegetables. Natural antioxidant from fruit is safer for human body because it is come from natural condition compared with the synthetic antioxidant (Weerasak *et al.*, 2008). According to an old Korean medical encyclopedia, the "Donguibogam" the *Benincasa hispida* is a good source of valuable nutrients including organic acids, natural sugars, amino acids, vitamin and mineral elements. Furthermore, *B.hispida* or Kundur fruit is one of the fruit vegetables currently gaining popularity among researchers due to its potential health benefits because of high nutritional value and functional properties such as antioxidant, anti-ulcer, anti-diarrheal and anti-angiogenic.

Benincasa hispida is mainly cultivated in China, and its seeds are obtained from the insideof dried, matured *B.hispida*, which is harvested during the months of August and September (Ji and Lee, 1988). Researchers from Taiwan found seeds have a higher capacity on anti-oxidation and inhibition of angiotensin-converting enzyme (ACE) activity than the fresh. It may be due to its high phenolic contents and superoxide dismutase activity (Huang *et al*., 2004). Thus, they believe, seed extracts of dong gua may offer a good health benefits on lowering risk of cardiovascular diseases and cancers. The seed extract is a good source of carbohydrate, amino acids, proteins and phenolic compounds. The antioxidant activity of the plant extract was affected by extraction solvents. Common solvents such as acetone, methanol, ethanol, water, hexane, chlorofoam, butanol and petroleum ether were used to extract antioxidant contained in fruits (Mohsen and Ammar, 2009).

Furthermore, the seasonal variations of plants can be eliminated using dried seeds and it can be kept for a long period without any damange. Seeds of *B.hispida* are generally dried to low moisture content for long-storage. Rapid and efficient drying techniques and methods that could minimize the nutrient loss have acquired considerable attention (Afzan *et al*., 1999), although dehydration is probably the oldest method of food preservation practiced by mankind (Antonio *et al*., 2008). Drying enhanced antioxidant activity by the formation of phenolics compounds. Phenolic compounds play a key contribution to the antioxidant property in dried of *B.hispida* seeds.

1.2 Statement of Problem

Fruits or herbs often require drying after harvested because they contained high moisture content which was the main factor contributed to the spoilage of highly perishable of fruits (Muller *et al*., 1989). Therefore, drying could improve shelf life, encapsulate original flavour, reduce storage volume and maintain nutritional values of fruits if compared to the fresh fruits (Gunhan *et al*., 2005). The antioxidant activity of the fruit was found to be reduced after dried under sun, oven or freeze dried (Chan *et al*., 2009). The degree of reduction of antioxidant activity in different drying methods was found to vary with different drying temperature and time (Katsube *et al*., 2008).

Thus, it is necessary to determine what effect drying has on the antioxidant activity of *B.hispida*. Michalczyk, Macura and Matuszak (2009) reported that freeze drying is much more effective in preserving valuable food compunds that traditional methods of drying.

However, information on changes in antioxidant activity of seeds *B.hispida* after drying is limited. For this reason, the present study investigated the effects of drying methods (oven drying, sun drying and freeze drying) on the antioxidant activity for seeking the potential drying process for the extracted from the seeds of *B.hispida*.

1.3 Research Objective

The main objective of this research project is to assess the effects of different drying method (oven dried, sun dried and freeze dried) on the antioxidant activity of the seeds extracted from *Benincasa hispida*.

1.4 Scope of Proposed Study

In order to achieve the research objectives, there are a few scopes of work that have been identified as follows:

1. Study on the effects of different drying methods on moisture content.

2. Determination of antioxidant activities from different drying method.

1.5 Significant of Proposed Study

Natural antioxidants are perceived safe, less toxic and beneficial for human health. However, it is very expensive and not widely commercialized. Source of antioxidants such as spices andherbs, and such material have been used troughout history for flavouring and preservative (Kikuzaki and Nakatani, 1993). The purposes why needed to find the alternative sources of natural antioxidants such as:

1. To make sure the price is low and value to commercialize.
2. Demand is very high, so it very easy for the antioxidant processes industry to get profit and market.
3. Dried of natural antioxidants may enhanced of antioxidant activity by the formation of phenolics compounds.
4. Reduce the energy consumed and cost of production with drying of natural antioxidants (Fatouh *et al*., 2006).

CHAPTER 2

LITERATURE REVIEW

2.1 Oxidation

Free radical is naturally produced in our body by the metabolism of amino acids and fats. Oxidation is essential to many living organism for the production of energy to fuel biological process. However the uncontrolled production of oxygen derived free radicals is involved in the onset of many diseases such as cancer, rheumatoid, arthritis, and arteriosclerosis as well degenerative processes associated with aging (Halliwel and Gutteridge, 2003). These free radicals are unstable and it can react with cells and destroy it. The bind of free radical with DNA structure will lead to mutation and it is cause of cancer. Free radicals formed in our body in several of type such as superoxide, hydroxyl, peroxyl and alkoxy.

To overcome this problem, the antioxidant is needed to inhibit all the free radical from react. They scavenge radicals by inhibiting initiation and breaking of chain reaction, suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide and quenching superoxide and single oxygen (Shi *et al*., 2001)

2.2 *Benincasa hispida*

2.2.1 The Morphology of Seeds *B.hispida*

The seeds of *Benincasa hispida* are defined as the seed and nutshell of *Benincasa hispida* Cogniaux, which belongs to a family of Cucurbitaceae, according to International Plant Name Index (IPNI) and the Korea Pharmacopoeia, the book of standard oriental medicine and herbal science (Ji and Lee, 1988). *Benincasa hispida* is one of the species of cucurbit family, which has a great potential for functional food production. One of the unique characterictics of Kundur fruit, if there is no injury to the fruits, in that it can be stored for many months, even for a full year in dry and cool atmospheres (Morton, 1971).

Kundur fruit is easier to grow than any of other cucurbits and mainly cultivated in China, and it seeds are obtained from the inside of dried, matured *B.hispida,* which is harvested during the months of August and September (Ji and

Lee, 1988). The seeds of Kundur fruit are flat, smooth and buff ranging from 1.0 to 1.5 cm in length and 0.5 to 0.8 cm in width, depending on the type and shape of fruit (Raveendra and Martin, 2006). White or yellowish white seeds (Figure 2.1) are filled in the center of the fruit (Morton, 1971). From Figure 2.2, as the fruit matures, the seeds color changes from white to yellowish brown. The seeds, which also have considerable amount of oil, can be fried and used as a snack.

Figure 2.1 Half cut Kundur (*Benincasa hispida*) fruit

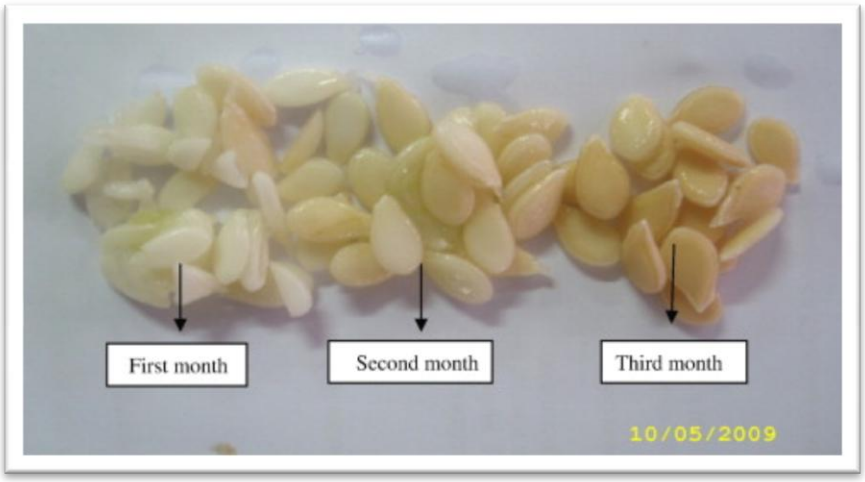


Figure 2.2 Color changes in Kundur (*Benincasa hispida*) fruit seed with maturity

2.2.2 The Uses and Benefits of *B.hispida* Seeds.

Due to high nutritional value and its growing demand, it could be suggested that Kundur fruit might be explored for uses in different food commodities such as jams, juices, beverages, cakes and ice cream for value-addition. Although some preliminary data on nutritional value of Kundur fruit is available, there is lack of information especially on nutritional profile of other parts of Kundur fruits such as seeds, skin and core which could be investigated. Especially, there is much need to characterize the Kundur fruit seeds. Lee *et al*.(2005) found that there is a potential angiogenic inhibitor in Kundur fruit seeds against tumor growth and obesity. Besides, Morton (1971) reported that the seed and seed oil have been in use to expel tapeworms. The seeds also help to reduce internal heat of body in summer and very useful in kidney stones. The seeds which also have considerable amount of oil, can be fried and used as a snack. Furthermore, the seed extract of *Benincasa*

hispida facilitates mucus secretion and also prevents gastrics ulcer (Grover *et al*., 2000).

2.2.3 Previous Studies Done on *B.hispida* Seeds.

Mingyu *et al.* (1995) studied about the different parts of Kundur fruit such as pulp, seed and skin stated that protein and free amino acids are present in high amounts in seed, with 5714.017 and 264.366 mg/100 g fresh weight. Total protein and free amino acids are the lowest in amount in the pulp, having concentrations of 216.400 and 92.549 mg/100 g fresh weight.Beside that, Grover *et al.* (2001) stated that *Benincasa hispida* seeds are mainly composed of saponin, urea, citrulline, linoleic acid, oleic acid and fatty acids. The seeds also contains minute amounts of a triterpenoid known as isomultiflorenol, proteins such as cucurbitacin B (Uchikoba *et al*., 1998).

Regarding the chemical composition of Kundur fruit seed, Martin (1984) suggested that Kundur fruit seed is perhaps one of the best cucurbit seed oil sources for the hot and humid tropics. It is further supported by Sew *et al.*(2010) that Kundur seed oil contains a high proportion of an essential fatty acid, linoleic acid, accounting for 67.37% of the total fatty acids. The oil from the seeds is soporific, good for the brain and liver, and also useful in the treatment of syphilis (Qadrie *et al*., 2009).

2.3 Natural Antioxidants From Plants

2.3.1 Source of Natural Antioxidants

Natural antioxidant, particularly in fruits and vegetables have gained interestamong consumers and the scientific community because epidemiological studies haveindicated that frequent consumption of natural antioxidant is associated with a lowerrisk of cardiovascular disease and cancer (Renaud *et al*., 1998). During this recent year, the use of fruit and vegetable juice has been increasing due to their health benefit to human beings. Fruits are rich with antioxidant that helps in lowering incidence of degenerative disease such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich *et al*., 2000; Gordon, 1996; Halliwell, 1996). This alternative sources of naturalantioxidants are needed to make sure the price is low and value to commercialize.

Antioxidant is defined as the molecule that can slow or prevent theoxidation process of other molecules. To get the natural antioxidants we should extract it from fruits or vegetables first. For those who need the antioxidant they can get it through the fruit when they eat. But for 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, spies and herbs. Seeds of *Benincasa hispida* is the material for the purpose ofextraction. Before extraction process, seeds of *B.hispida* must be dried first to low moisture of content.

Dried seeds from B.hispida could be used as a source of natural antioxidants in the food industry.

A study by Huang *et al*. (2004), on the in vitro antioxidant activity of Kundur fruit demonstrated that the seed has the highest capacity for inhibition of linoleic acid oxidation and scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals compared to the peel, pulp and core of the fruit. This may be due to higher total phenolic contents and superoxide dismutase (SOD) activity of the seeds. In addition, the fruit may provide protective effects against the development of atherosclerosis and also exhibits anticarcinogenic effects in vitro (Huang *et al*., 2004).

Beside that, Gill *et al*. (2010) investigated the anti-inflammatory effects of Kundur fruit seed. He suggested that free radical scavenging activity of Kundur fruit seed might have been responsible for the reduction of inflammation in the carrageenan-induced paw edema in rats. Some research about the common medicinal and pharmacological properties of different parts of Kundur (*Benincasa hispida*) fruit have also been summarized in Table 2.1.

Table 2.1 Some common medicinal and pharmacological properties of different partsof Kundur (*Benincasa hispida*) fruit

Part	Medicinal and pharmacological properties.	References
Pulp	Anti-inflammatory, anti-ulcer, anti-depressant, anti-histaminic, antioxidant, used for Alzheimer disease treatment	Grover and Rathi (1984), Mingyu *et al*.(1995), Huang *et al*.(2004)
Seed	Anti-angiogenic, anti-tumor, antioxidant, beneficial effects for brain and liver, used for the treatment of syphilis, cardiovascular diseases and softening or soothing the skin.	Qadrie *et al*.(2009), Lee *et al*.(2005), Huang *et al*. (2004)
Peel	Antioxidant activity, inhibition of angiotensin converting enzyme (ACE)	Huang *et al*. (2004)

Reference: Zaini *et al.* (2011)

2.3.2 Benefits of Antioxidants

The interest in studying antioxidant activity had increased recently due to the increased public awareness on the benefits of antioxidant in disease prevention (Kaefer and Milner, 2008). Beside that, oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants will 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.

Furthermore, natural antioxidants are widely used in the pharmaceutical and food processing. Antioxidants play a significant role to prevent many several diseases like Alzheimer disease, cancer, and even aging. As we know that natural

antioxidant from fruit is safer forhuman body because it is come from natural condition compared with the synthetic antioxidant. (Weerasak *et al*., 2008). Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease.

2.4 Assays for Detecting Antioxidant activity

2.4.1 DPPH Radical Scavenging Activity

The DPPH radical ($DPPH^{+}$) scavenging capacity assay is a decolorization assay that measures the capacity of antioxidant to directly react with (scavenge) DPPH radicals by monitoring its absorbance at 517 nm with a spectrophotometer. The solution (in absolute ethanol) appears as a deep violet colour and shows a strong absorption band at 517 nm. DPPH radical can accept an electron or hydrogen radical to become a stable diamagnetic molecule and has pale violet. If substance for testing antioxidant activity is mixed with DPPH solution and gives rise to pale violet, it suggests that this substance has antioxidant effect by mechanism of free radical scavenging activity. So, it is means DPPH radical is a stable organic nitrogen centered free radical (Figure 2.3) with a dark purple color that when reduced to its nonradical form by antioxidant become colorless. The use of $DPPH^{+}$ is to measure the antioxidant properties of compounds by quantify scavenging capacities against this radical using a spectrophotometer (Brand and Williams,1995).

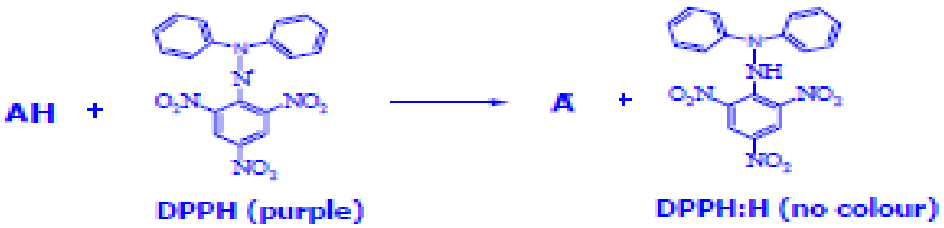


Figure 2.3 DPPH structure

Reference: Kaefer and Milner (2008)

2.4.2 Total Phenolic Content

Phenols or Phenolics is a class of chemical compounds that have one or more aromatic rings with one or more hydroxyl groups (-OH). The simplest way to draw the structure of phenol is shown as Figure 2.4.

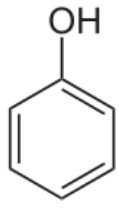
Phenol	
	

Figure 2.4 The structure of phenol (C_6H_5OH)

Reference:Eberhardt *et al*.(2000)

The phenolic compounds prove the importance of antioxidant behaviour and contribute significantly to the total antioxidant activity of medicinal and aromatic samples (Eberhardt *et al*., 2000). Total phenolic content of plants is an important parameter for their antioxidant properties. The Folin-Ciocalteu procedure of Singleton (1965) has been used as a measure of total phenolics in natural products for many years. On the other hand, analytical techniques have been used to isolate, identify, and determine individual phenolic compounds by gas chromatography and mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) and liquid chromatography and mass spectrometry (LC-MS).

Phenolics substances can be category as phytonutrients that produce strong antioxidant properties. It can prevent our body from oxidative stress associated diseases such as cancer. The antioxidants activity of phenolics is mainly due to their redox properties. Redox properties in phenolics function as reducing agents, hydrogen donors and singlet oxygen quenchers. Besides that, antioxidants activity of phenolics also plays an important role in the adsorption and neutralization of free radicals (Dutra, Leite and Barbosa, 2008). Plant phenolics also involved in defense against ultraviolet radiation and aggression by pathogens, parasites and predators (Dai and Mumper, 2010). Phenolic compounds contain an antioxidant potential which give benefits to us. Nowadays it has been used in various fields such as food, chemistry and medicine.

2.5 Aqueous Solvent Extraction Method

2.5.1 Process of Extraction

Many herbs and plant cannot be extracted from distillation method but compare others process of extraction, aqueous solvent extraction is the safest method for extracting high quality oil. In this process, the plant or herbs plants are immersed in the solvent and the 'separation' is performed chemically. These include pigments, volatile molecules and non-aromatic waxes. The herbs and spices are then subjected to low pressure distillation and the volatile oil is then separately collected. In aqueous solvent extraction, solvent selection is most important.

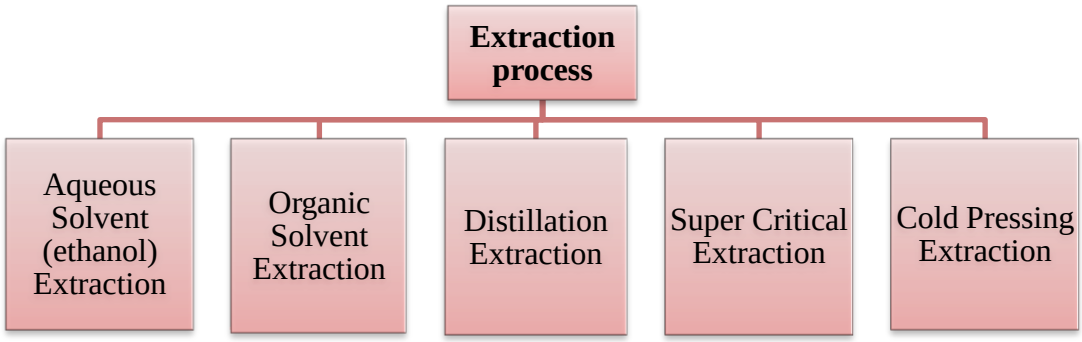


Figure 2.5 Extraction Process

Reference:Li *et al.* (2001)

2.5.2 Solvents

Extraction is the process in which the plant tissues are treated with specific solvents whereby the medicinally active constituents are dissolved out, cell tissues and most of inactive or inert components remain undissolved. The plant material used for extraction should be properly identified. The choice of the plant material for extraction depends on its nature and the components required to be isolated.

From the stand point of pharmacy, the purpose of a solvent is to remove from a solid, either in part or in its entirety such substances that may be rendered to a liquid. Solvents differ widely from each other, not only in differing boiling points, but how they act or react with substances in which they come in contact. An ideal solvent for the extraction of the plant should meet the following criteria:-

1. It should be non-toxic and selective, example it should dissolve only the required constituent with minimum amount of the inert materials.
2. It should not cause the extract to complex or dissociate.
3. It should be preservative in action.
4. It should promote rapid physiologic absorption of the extract.
5. It should be easily evaporated at low heat.

There are large number of solvents used for extraction of herbs, but the selection of the suitable solvents capable of extracting the active constituents depends upon the chemical properties of active constituents as well as the qualities of the solvent. The solvents commonly used for the extraction of the plants include water, alcohol and there different dilutions.

Table 2.2 Solvents commonly used for the extraction of the plants

SOLVENTS	CATEGORY OF EXTRACTION
Water	Extraction of many types of active constituents such as alkaloidal salts, colouring agents, glycosides and tannins. Not a suitable solvents for constituents like waxes, fats, fixed oil and alkaloidal bases due to there insolubility in water.
Ethanol	Dissolve a large number of chemical constituents such as alkaloids, and resins, but constituents like albumin, gums, waxes, fats, fixed oils and sucrose are insoluble in alcohol.
Ether	Soluble constituents are oils, fats, waxes, resins and alkaloidal bases. Highly inflammable produces physiologically effects.
Chlorofoam	Soluble constituents are oils, fats, waxes, resins and alkaloidal bases. Non inflammable.
Glycerin	Soluble constituents are tannins. Non inflammable and viscous liquid.
Light petroleum	Soluble constituents are oils, fats, waxes, resins and alkaloidal bases. Highly inflammable and very volatile.
Propylene glycol	Soluble constituents are progesterone, phenobarbitone sodium. Clear, colorless and odourless

Reference: Matt *et al*.(2010)

By using ethanol as the main solvent in extraction fruiting seeds of antioxidant from *B.hispida* can deactivate the endogenous enzymes and remove some soluble materials, and extracted with ethanol to obtain highest antioxidant activity (Li *et al*., 2001).

Ethanol is a very polar molecule due to its hydroxyl (OH) group, with the high electronegativity of oxygen allowing hydrogen bonding to take place with other molecules. Ethanol therefore attracts polar and ionic molecules. The ethyl (C_2H_5) group in ethanol is non-polar. Ethanol therefore attracts non-polar molecules. Thus, ethanol can dissolve both polar and non-polar substances. In industrial and consumer products, ethanol is the second most important solvent after water. Ethanol is the least toxic of the alcohols (it is only poisonous in large amounts), which makes it more suitable for use in industry and consumer product (Matt *et al*., 2010).

Generally dilute alcohols (hydroalcoholic solutions) are used for many extractions, but in some cases stronger alcohol may be used to prevent the extraction of unwanted substances such as gums. It is non-toxic in the quantities present in medicinal substances. It is reasonably selective. In a herb containing a number of chemical substances such as alkaloidal salts, glycosides, albumin and gum, water will dissolve all the substances. Whereas dilute alcohol will dissolve only the alkaloidal salts and glycosides.

2.6 Effects of Drying Methods

Dried fruits have the advantage of being very easy to store and distribute, they are readily incorporated into other foods and recipes, relatively low cost and present a healthy alternative to sugary snacks. The scientific state that individuals who regularly eat generous amounts of these foods have lower rates of cardiovascular disease, obesity, several cancers, diabetes and other chronic diseases.

Dried fruits are an excellent source of polyphenols and phenolic acids. These compounds make up the largest group of phytochemicals in the diet and appear to be, at least in part, responsible for the potential benefit associated with the consumption of diets abundant in fruits and vegetables. Different dried fruits have unique phenolic profiles. For example, the most abundant in raisins are the flavonols quercetin and kaempferol and the phenolic acids caftaric and coutaric acid (William and Carughi, 2010). Furthermore, by virtue of their high polyphenol content, dried fruits are an important source of antioxidants in the diet (Wu *et al*., 2004). These phytochemicals are believed to account for a major portion of antioxidant capacity in plant foods. Antioxidants can lower oxidative stress and so prevent oxidative damage to critical cellular components. Beside that, different methods of drying also give impact to give excellent source of polyphenols, phenolic acids, antioxidant acticity and also the cost to dry the fruits.

2.6.1 Sun Drying

Sun drying is the old-fashioned way to dry food because it uses the heat from the sun and the natural movement of the air.The high sugar and acid content of fruits make them safe to dry out-of-doors when conditions are favorable for drying. Vegetables (with the exception of vine dried beans) and meats are not recommended for out-of-doors drying. It is because vegetables are low in sugar and acid (Elizabeth and Judy, 2006). This increases the risks for food spoilage. Beside that, meats are high in protein, making them ideal for microbial growth when heat and humidity cannot be controlled. It is best to dry meats and vegetables indoors using controlled conditions of an oven or food dehydrator (Elizabeth and Judy, 2006).

To dry fruits out-of-doors hot, dry, breezy days are best. A minimum temperature of 85ºF is needed with higher temperatures being better. It takes several days to dry foods out-of-doors. Because the weather is uncontrollable, drying fruits out-of-doors can be risky. At night, fruits dried out-of-doors must be covered or brought under shelter. The cool night air condenses and could add moisture back to the food, thus slowing down the drying process. To dry the fruits, the only equipment that need is racks or screens to placed on blocks allow for better air movement around the food (Figure 2.6). Because the ground may be moist, it is best to place the racks or screens on a concrete driveway or if possible over a sheet of aluminum or tin. The reflection of the sun on the metal increases the drying temperature.

Figure 2.6 Solar drying

Reference:Elizabeth and Judy (2006).

Screens need to be safe for contact with food. The best screens are stainless steel, Teflon-coated fiberglass and plastic. Avoid screens made from "hardware cloth." This is galvanized metal cloth that is coated with cadmium or zinc. These metals can oxidize, leaving harmful residues on the food. Also avoid copper and aluminum screening. Copper destroys vitamin C and increases oxidation. Aluminum tends to discolor and corrode (Elizabeth and Judy, 2006).

2.6.2 Oven Drying

Oven drying is the simplest way to dry food because it needs almost no special equipment (Figure 2.7). It is also faster than sun drying or using a food dryer. But oven drying can be used only on a small scale. The concept of this appliance is using electric to heat the heating medium to produce heat forcook and heat. The temperature can be set for various heating process. In this research, oven is used for drying raw material.

Figure 2.7 Oven drying

2.6.3 Freeze Drying

Freeze drying is a drying alternative to obtain high quality food, with good aroma retention and rehydration capacity (Figure 2.8). The freeze drying process is used to stabilise, concentrate and increase the shelf-life of these products without destroying their chemical structure (Chou and Chua, 2001). Freeze dryer requires that both the temperature and pressure be controlled during the drying process. The product to be dried can be either in vials or in trays. When the vials are placed on the trays, the stoppers are closed only partially so that water vapor can escape.

The first step in the freeze drying process is the cooling of the product to a sufficiently low temperature to allow complete solidification. The pressure in the chamber is then reduced to below the vapor pressure at the triple point of water so that drying can occur by sublimation. Due to the absence of liquid water and the low temperature required for the process, most of deterioration and microbiological reactions are stopped which gives a final product of excellent quality (Ratti, 2001).

This process also concentrates the product, increasing their potency compared with their natural form. It is easy to store and transport as their volume and weight can be reduced significantly.

Figure 2.8 Freeze dryer

2.6.4 Effect of Moisture Content

During the drying process, fruit loses its water content, leaving only vitamins, minerals and other nutritional content inside of the edible piece. In many cases, a chemical additive gets added to the fruit in order to maintain freshness, color and some vitamin content. Through this process, some fruits actually lose vitamins and minerals.The ascorbic acid content of fresh apple and that of apple dried by the four drying methods are shown in Table 2.3 (Cui *et al*., 2004).

Table 2.3: Effect of moisture content and total vitamin C values of dried and fresh red delicious apple slices.

Drying methods	**Moisture content (% w.b.)**	**Vitamin C (ug/100g dry wt) Mean ±standard error**	**Retention of vitamin C (%)**
Fresh carrots	87.31	47.4±0.8	100
Freeze drying	6.91-6.30	43.5±2.1	91.8
Microwave drying	6.83-6.22	42.2±1.8	89.0
Hot air drying	6.75-5.98	30.2±2.4	63.7

Reference: Cui *et al*. (2004)

From table 2.3 above adapted from Cui *et al*.(2004) shown the moisture removal of red delicious apple slices by freeze drying is higher comparing others drying. Jimoh *et al*.(2009) proved that freeze drying is the most effective way to extend the life of food stuff or plants because the microorganism and enzymes that reduce food quality over time require a sufficient amount of water to exist. However, the fresh carrots are most highest ascorbic acid content. It is because only less removal of water content from carrots. Besides that, loss of water during a drying process originates a reduction in the size of the cellular tissue, which is usually referred as shrinkage phenomenon. The shrinkage depend on the drying method applied (Krokida and Maroulis, 1997) and on drying conditions. Shrinkage affects mass and heat transfer parameters and it is a relevant factor to be accounted for establishing drying models.

2.6.5 Effects of Drying Temperature

The production of *B.hispida* seeds starts with a drying process as initial treatment after harvesting process is done and before any other processing. As the drying aspect plays an important role to ensure a high quality product, the study of its parameter such as temperature needs to be explored to prevent over-drying and thus it can decrease drying time, energy cost, mass losses and the risk of quality deterioration. Drying at higher temperature shortened the drying time and increased the drying rate. The quality of dried misai kucing leaves after treatment and different temperature used shown in Table 4 according to Arslan *et al.* (2008).

Table 2.4: Analysis of dried Misai Kucing leaves extract (Arslan *et al*., 2008)

Temperature (^{o}C)	**Period (hours)**	**DPPH (%)**	**TPC (ppm)**
40	2	35.482	58.042
55	3	39.714	58.000
70	6	50.456	48.333

From the result showed above, the leaves of Misai Kucing take only 2 hours to dry at 70^{o}C and almost 3 hours for 55^{o}C while, at 40^{o}C the leaves took 6 hours to dry. So, because of that the fastest drying rate is occurred when misai kucing leaves are dried at higher temperature. The best drying method for this research is oven because the temperature used are range 50 until 80^{o}C. For DPPH result at higher temperature is greater than lowest temperature. However, for total phenolic content (TPC) at lowest temperature is greater than higher temperature.

CHAPTER 3

MATERIALS AND METHOD

3.1 Introduction

This chapter discussed the materials and method adopted in the experimental work. A schematic structure of the whole process flow is illustrated in Figure 3.1.

1. Pretreatment method

- Washed the seeds of kundur fruit by salt solution to remove all pulp.

↓

2. Dried 10 g of seeds in different drying methods

- Sun drying : three days with about 36 hr of daylight at 26^{o}C.
- Oven drying : the seeds was dried in a oven at 50^{o}C for 8 hr
- Freeze drying : the seeds dry by freezing at -80^{0}C in a freezer and then carried out in a freezer dryer for -50^{o}C.

↓

3. The dried seeds was blended and weighed.

↓

4. Extraction Process with ethanol (95%)

- 2 g of each sample with 30 ml ethanol was used by using aqueous solvent extraction method.

↓

5. Separation the mixture of antioxidant and solvent using rotary evaporator

↓

6. Analysis of the sample by using uv-vis spectrophotometer.

Figure 3.1 Overview of overall methodology

3.2 Materials

3.2.1 Plant Material

The seeds of *B.Hispida* used in this study was purchased from Warisan Kundur Resources, Pekan, Pahang Darul Makmur.

3.2.2 Chemicals

1,1-diphenly-2-picrylhydrozyl (DPPH), ascorbic acids, tannic acids, folin-ciocalteau (FC) reagent, sodium carbonate was obtained from Sigma Chemicals. Ethanol (95%) was readily available in FKKSA (UMP) lab.

3.3 Methods

3.3.1 Drying Process

The seeds of *B.Hispida* were selected and dried with the three different drying methods such as sun drying, oven drying and freeze drying. For each drying method, 10 g of fresh seeds was used. Sun drying involved drying for three days with about 36 h of daylight. Mid-day temperature can reach 26^{o}C. For each of the above drying methods, the materials were spread out evenly on aluminium foil. In oven drying, seeds were dried in a oven at 50^{o}C for 8 hours. Stepwise, in freeze drying, the samples were dried by freezing at -80^{0}C in a freezer and then carried out in a freezer dryer for -50^{o}C. The total drying time was 12 hours. All the dried materials were then powdered in a blender and stored under -20^{0}C until analysis to prevent from contaminated of surrounding.

3.3.2 Moisture Content

The moisture content of the seeds sample was determined by using a weighing balance according to the Equation (3.1) :

$$\text{Moisture content (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ x } 100\% \qquad (3.1)$$

3.3.3 Preparation of Plant Extracts

The seeds were cleaned and dried, and once their weight was stable, the dried seeds were ground into powder forms, using commercial blender. Then, the powders were extracted using ethanol with different beakers. The solution was required to be heated on hot plated at temperature 80^{o}C for 10 min to allow the reaction occurred.10 ml of the solution were transferred into the centrifuge tube and vigorously shaken for 30 second, cooled and centrifuged at 4500 rpm for 15 min at 4^{o}C. Then, the final volume of clear supernatant was added to 50 ml with ethanol and remove using rotary evaporator at 50^{o}C under vacuum. The extracts were stored at -20^{o}C prior to further analyses.

3.3.4 Antioxidant Activity Assay

3.3.4.1 Determination of DPPH Scavenging Activity

Free radical scavenging activity against 1,1-diphenyl-1-picrylhydrazyl (DPPH) radical was measured according to the Sun and Ho (2005). Each of sample was diluted with ethanol solution to obtain different concentration 2.5, 5.0, 10.0 and 20.0 mg/ml. Each diluted sample (2.5 ml) was mixed with 2.5 ml of DPPH solution (0.2 mmol/L in ethanol). The reaction mixture was vortexed for 15 to 30 second and allowed to stand without any disturbance for 30 minutes at room temperature. The absorbance was read at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the control, while the inhibition ratio for DPPH scavenging activity was calculated from the Equation (3.2):

$$\text{Scavenging activity (\%)} = \left(1 - \frac{A_1}{A_0}\right) \times 100\% \qquad (3.2)$$

Where A_o is the absorbance of the control (ascorbic acid), and the A_1 is the absorbance of the samples.

3.3.5 Assay for Phenolic Content

Total phenolic content of the extracts were measured using the Folin-Ciocalteau assay developed by Singleton et al. (1999) with some modification. This method is based on measuring color changes that caused by reduction of Folin-Ciocalteau reagent by phenolates in the presence of sodium carbonate (Na_2CO_3).

In this method, 0.1 ml of each extracts was added to 0.25 ml of 0.1 mg/ml Folin-Ciocalteau reagents. Then, it was required to stand about 5 minutes for proper reaction between extract and reagents. 1.25 ml of 40 g/Lof sodium carbonate (Na2CO3) were added and made upto 2.0 ml by using distilled water. The above solution was kept for incubation at room temperature for 30 minutes. Absorbance was measured at 760 nm using 1 cm cuvette in a perkin-elmer UV-VIS lambda 25 spectrophotometer.

Besides that, tannic acid (2 – 10 ug/mL) was used to obtain standard calibration curve for the phenolic content. For the blank, 0.50 ml of distilled water was mixed with 0.25 ml of 0.1 mg/ml Folin-Ciocalteau reagents and 1.25 ml of 40 g/Lof sodium carbonate (Na_2CO_3).The absorbance value for each concentration was obtained by using UV-Vis with 760 nm as their wavelength.Those absorbance values can estimate the phenolic in sample.The total phenolic content was expressed in ug of Tannic acid equivalents (TAE) / g of distilled water (DW).

CHAPTER 4

RESULTS AND DISCUSION

4.1 Introduction

This chapter discussed the experimental results that carried out in the research work. The material discussed in this chapter includes the effect of drying method on antioxidant activity of *B.hispida* seeds by observing the water content, DPPH radical scavenging activity and total phenolic content. Then, each of the results are discussed throughly and justified accordingly.

4.2 Effects of Drying on *B.hispida*Seeds

The key elements that should be considered in this research studyis water content because from here the production capacity sought will be the factors to consider in selecting a suitable drying method. The initial weight of the seeds and final weight of the seeds after dried were measured to calculate the water contents of each sample according to the Equation (4.1):

$$\text{Water content } (\%) = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \qquad (4.1)$$

Table 4.1 Water content for different drying methods compare fresh of seeds *B.hispida*

SAMPLE NAME	WET WEIGHT (g)	DRY WEGHT (g)	WATER CONTENT (%)
Fresh	-	-	-
Freeze	2.0	0.408	84.2
Sun	2.0	0.354	79.6
Oven	2.0	0.316	82.3

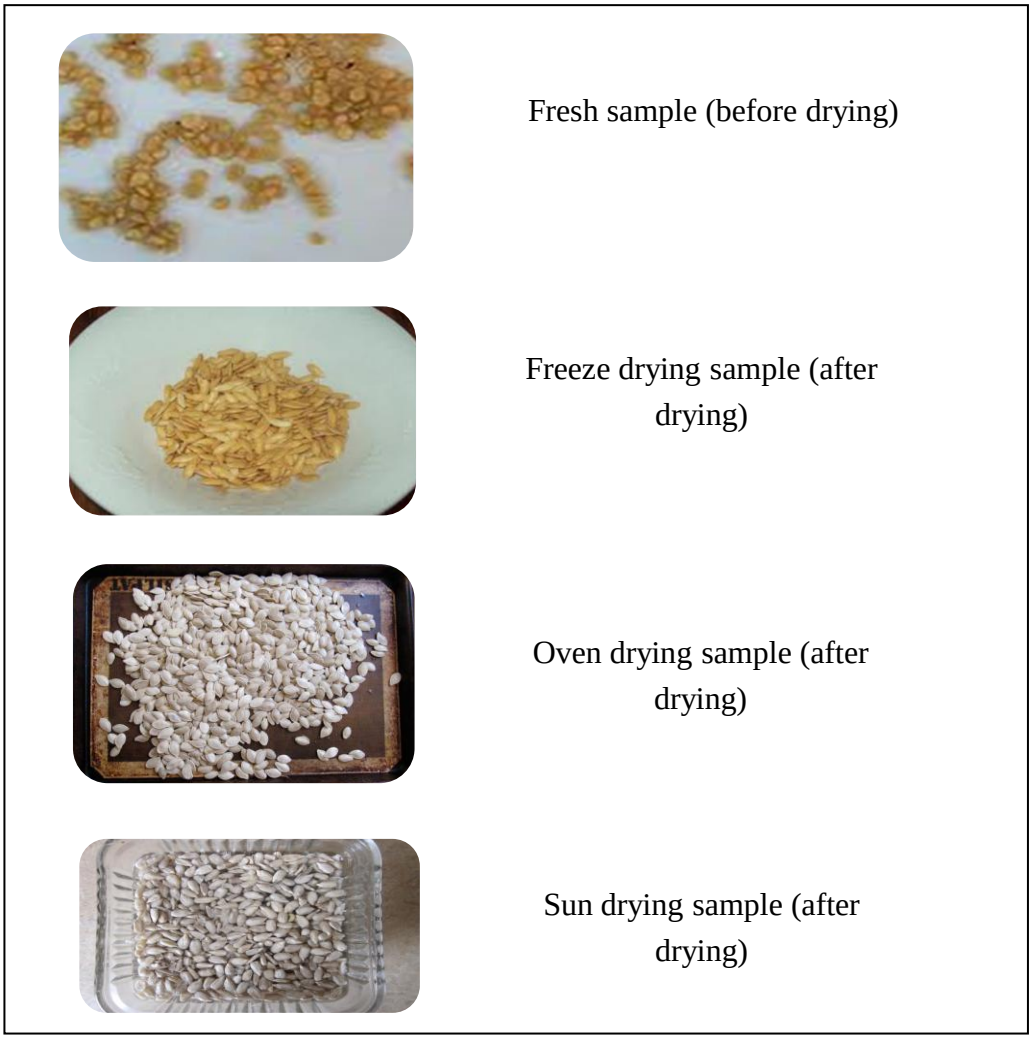


Figure 4.1 The seeds of *B.hispida* before and after drying

Results of water content for different drying methods compare fresh sample and also the picture of seeds *B.hispida* before and after drying seeds are showed in Table 4.1 and Figure 4.1.Three different drying methods resulted in drastic weight loss due to loss of water (moisture content). The water contents of dried sample obtained by freeze drying has significantly higher percent water content compared with those of the other dried samples. It is because freeze drying were absent of air and light and also dried at very low temperatures which inhibit oxidation, so antioxidant or ascorbic acid as well as other nutrient contents in the product can be largely preserved. On the other hand, from Table 4.1, freeze drying and fresh have higher moisture content because their samples contained more water than the sun drying and oven drying samples. Sun drying is lowered in water content because its method was good dried product as temperature used was higher compared oven drying and freeze drying. However, low moisture content may enhance deterioration for antioxidant of the product.

In fact, Ratti (1994) found that good quality is defined as lower moisture content for the dried product.Besides, drying except freeze drying method resulted in considerable shrinkage of the materials and caused them to become in nature, thus making them easier to blend during extraction. In this research study, dry weight values were similar to those reported by Figuerola *et al.* (2005) and Vergara-Valencia *et al.* (2007) for different fruits.

4.3 Effects of Drying on DPPH Radical Scavenging Activity

DPPH radical scavenging activity was observed to increase rapidly with sample concentration from 2.5 to 20 mg/ml at Figure 4.2. The antioxidant activity of seeds extracts from *B.hispida* was determined using ethanol solution of DPPH reagents and expressed in terms of percentage of inhibition (%). Parallel to examination of the antioxidant activity of seed extracts, the values for standard compounds were obtained and compared to the values of the antioxidant acitity. The standard substance was ascorbic acid (the standard curve equation: y = 0.0295x – 0.00333, r^2= 0.9997). The absorbance was read at 517 nm using a UV-VIS spectrophotometer. Ascorbic acid was used as the control, while the inhibition ratio for DPPH scavenging activity was calculated from the Equation (4.2):

$$\text{Scavenging activity (\%)} = \left(1 - \frac{A_1}{A_0}\right) \times 100 \qquad (4.2)$$

Where A_o is the absorbance of the control (ascorbic acid), and the A_1 is the absorbance of the samples.

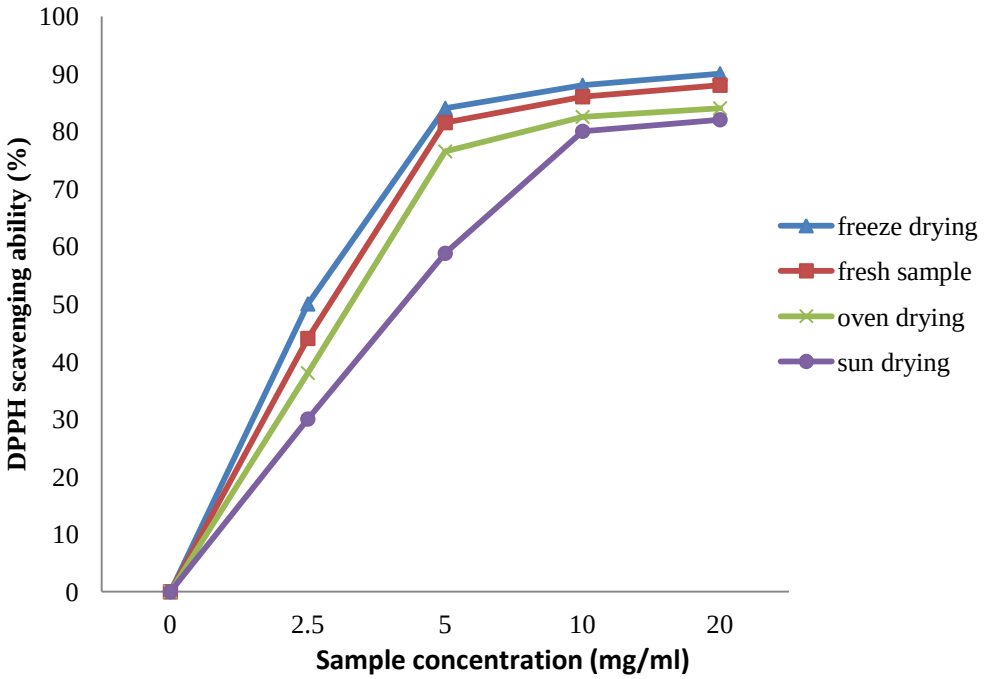


Figure 4.2 DPPH radical scavenging activity of sun, oven and freeze dried compare fresh of *B.hispida* seeds.

Drying process caused an increase in DPPH radical scavenging activity in *B.hispida* seeds. DPPH activity was observed to increase rapidly with sample concentration from 0 to 5 mg/ml and then reached a high plateau between 5 to 20 mg/ml for three samples (fresh, oven drying and freeze drying) while for sun drying is 10 and 20 mg/ml have reached a high plateau. Additionally, at 2.5 to 20 mg/ml, DPPH radical scavenging activity of freeze dried sample was much higher than that of fresh sample, oven dried sample and sun dried sample.

From the result above, freeze drying was higher compare others because for fresh sample, the cause of depletion of antioxidants could be due to operations such as cutting and slicing as they induce a rapid enzymatic oxidation of natural antioxidants according to Lim and Murtijaya (2007). Freeze-drying is higher

antioxidant because there is no thermal degradation and neither dose the process allow degradative enzymes to function (Chan *et al.*, 2008).

Sun drying showed the lowest antioxidant value in the present study. The high DPPH scavenging activity of oven-drying can be explained that high temperature might disrupt the cell wall and liberate antioxidant compounds from insoluble portion of the formation of novel compound that having powerful donating ability (Peleg *et al.*, 1991; Choi *et al.*, 2006). The effect of antioxidants on DPPH radical scavenging was thought to result from their hydrogen donating ability (Baumann and Bruchlarusen, 2002).

Besides, drying enhanced antioxidant activity by the formation of phenolics compounds. Phenolics compunds play a key contribution to the antioxidant property in dried of *B.hispida* seeds.

4.4 Effects of Drying on Total Phenolic Content

B.hispida seeds could be considered as good source of natural antioxidants, due to their richness in phenolic compounds. The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of tannic acid equivalent (TAE) (the standard curve equation: $y= 0.0537x + 0.0027$, $R^2=0.9999$. The values obtained for the concetration of total phenols are expressed as ug/g of distilled water (DW).

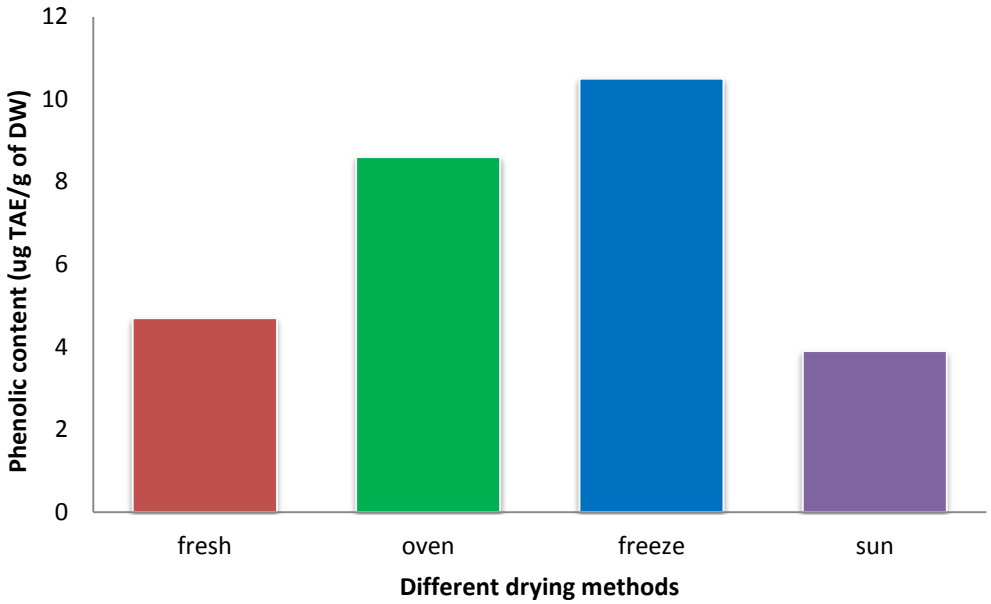


Figure 4.3 Total phenolic contents in fresh, oven, freeze and sun dried seeds of *B.hispida*.

The total phenolic contents in the examined extracts ranged from 0 to 12 ug TAE/g of DW. From the result in Figure 4.3, the phenolic content amount in the fresh seeds is 4.7 ug TAE/g of DW. Then, after drying in three different drying methods (sun drying, oven drying and freeze drying), the phenolic content was increased. It was shown that freeze dried *B.hispida* seeds contained the highest content of phenolic content amount with 10.5 ug TAE/g of DW. However, the sun dried sample had the lowest phenolic content amount compared than oven dried and fresh samples.

Sun drying demonstrated the lowest total phenolic content compare others. It is because, for sun-drying method, loss of TPC may be caused by enzymatic processes that ocurred during sun drying. Sun drying did not immediately deactivate degradative enzymes such as phenolic oxidases, which are able to degrade phenolic compounds before the *B.hispida* seeds are completely dry. It have been proved that

Okuda *et al.* (1989) have mentioned that rosmarinic acid was degraded when it is dried under direct sunlight and Mueller-Harvey (2001) has reported that some phenolic compounds decomposerapidly in direct sunlight.

On the other hand, the total phenolic content resulted from oven drying treatment was lower than the total phenolic content from the freeze drying. Oven drying temperature at 50℃ was capable to cause rapidly inactive polyphenol oxidase present in plant materials, however, some of their initial activities may have occurred earlier and caused some polyphenols to be degraded.

Besides that, freeze drying showed higher value in total phenolic content because freeze-drying is known to have high extraction efficiency because ice crystals formed within the sample matrix can rupture cell structure, which allows exit of cellular components and access of solvent, and consequently better extraction (Asami and Hong, 2003).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The results of this study showed that the highest moisture content, antioxidant activity and total phenolic content were achieved by freeze drying the seeds. In this research study, *B.hispida* seeds produced by different drying methods (sun drying, oven drying and freeze drying) can enhanced antioxidant activity by the formation of phenolics compounds. This is because phenolic compounds play a key contribution to the antioxidant property in dried of *B.hispida* seeds.It have been proved that freeze drying could be suitable drying method because it showed higher antioxidant value from DPPH study due to no thermal degradation and have high extraction efficiency due to formation of ice crsytals that allows exit of cellular components and access of solvent. Therefore, considering the antioxidant activitiy, phenolic content and moisture content, freeze drying is potentially suitable for drying the *B.hispida* seeds in order to achieve high antioxidant activity and total phenolic content.

5.2 Recommendation For Future Work

In conjugation with the results and conclusion obtained, the following recommendations were identified as to further improve the future work of this study:

1. Perform of some physical properties of different drying methods on antioxidant activity.
2. Further research about the antioxidant activty in vivo and antioxidant mechanism.
3. Study of each parts of plant material on antioxidant activity.
4. Optimise the extraction conditions with the indication of TPC using orthogonal array design matrix.

REFERENCE

Afzan T M, Abe T, Hikida Y. (1999). Enegy and quality aspects during combined FIR- convection drying of barley. *Journal of Food Engineering*, 42, 177-182.

Antonio G C, Alves D G, Azoubel P M, Murr F E X, Park K J. (2008). Influence of osmotic dehydration and high temperature short time processes on dried sweet potato (Ipomoea batatas Lam.). *Journal of Food Engineering*, 84, 375-382.

Chan,H.X., Zhang,M., Qu, Z.S. & Xie, B.J. (2009). Antioxidant activities of different fractions of polyaccharide conjugates from green tea (Camella sinensis). *Food Chemistry*, 106(2),559-563.

Chou,SK., Chua, KJ. (2001).New hybrid drying technologies for heat sensitive foodstuffs. *Trends in Food Science and Technology*, 12 (10), 359–369.

Fatouh, B., Cross, C.E .,Gutteridge, J.M.C. (2006). Free radicals, antioxidants and human disease: where are we now? *Journal of Laboratory and Clinical Medicine* 119: 598-620.

Gunhan, H.Y., Park, Y.K., Kim, D.I., Kim, Y.H., Yoon, G.B., Noh, Y.G., Lee, E.S., Heo, H.R.,Park, J.K., Han, S.M., Lee, R.S., Kang, Y.L., (2005). Donguibogam. Yeogang Press, Seoul, pp. 374-2002.

Halliwell, B., Gutteridge, J.M.C. and Cross, C.E. (1992). Free radicals, antioxidants and human disease: where are we now? *Journal of Laboratory and Clinical Medicine* 119: 598-620.

Huang,H.Y.,Huang,J.J.,Tso,T.K.,Tsai,Y.C.,Chang,C.K.(2004).Shih-Chien University, Taiwan Antioxidant and angiotension-converting enzyme inhibition capacities of various parts of Benincasa hispida (wax gourd). 48(3):230-3.

Ji, H.J., Lee,S.I.(2008). The Korean Pharmacopoeia.The Korea Medical Index C0, pp.121-122

Martin, F. W. (1984). Cucurbit seed as possible oil and protein sources. Echo Technical Note. Retrieved 31 October 2009, from

http://www.echotech.org/network/modules.php?name=News&file=article&sid=592

Mingyu, D., Mingzhang, L., Qinghong, Y.,Weiming, F., Jianxiang, X., &Weiming, X. (1995). A study on Benincasa hispida contents effective for protection of kidney. Jiangsu Journal of Agricultural Sciences, 11, 46−52.

Muller,K.O., Olurin,T.O., & Aina,J.O.(1989). Effect of drying method on the rheological characteristics and colour of yam flours. *African Journal of Biotechnology,* 8(10),2325-2328.

Mohsen, J. K., Ammar, G.(2009). Extracts of *Benincasa hispida* prevent development of experimental ulcers. *Journal of Ethnopharmacology*, 78, 159-164.

Nandita, S. & Rajini, P. S. (2004). Free radical scavenging activity of an aqueous extract of potato peel. *Food Chemistry*, 85,611-616.

Pietta, P.G. (2000). Flavonoids as antioxidants. *Journal of Natural Products* 63: 10350- 1042**.**

Qadrie, Z. L., Hawisa, N. T., Khan, M. W. A., Samuel, M., & Anandan, R. (2009).Antinociceptive and anti-pyretic activity of Benincasa hispida (Thunb.) Cogn. inWistar albino rats. *Pakistan Journal of Pharmaceutical Sciences*, 22, 287−290.

Ratti, C. (2001). Hot air and freeze-drying of high-value foods: A review. *Journal of Food Engineering*, 49, 311-319.

Schiller,M. (2010). *Ethanol as a Solvent : EasyChem.com.au during the 2010-2012.* Retrieved fromhttp://www.easychem.com.au/production- of materials/renewable-ethanol/ethanol-as-a-solvent.

Sew, C. C., Zaini, N. A. M., Anwar, F., Hamid, A. A. & Saari, N. (2010). Nutritionalcomposition and oil fatty acids of Kundur [Benincasa hispida(Thunb.) Cogn.] seed.*Pakistan Journal of Botany*, 42(5), 3247–3255.

Shi, B.V., Arjuman, A., Jorapur, A., Samanth, R., Yadav, S.K., Valliammai,N.,(2001). Kasturba Medical College, Effect of extract of Benincasa hispida on oxidative stress in ratswith indomethacin induced gastric ulcers. Indian J Physiol Pharmacol.;52(2):178-82.

Singleton,V.L., Orthofer,R., & Lamuela-Faventos,R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Cicolteau reagent. *Methods in Enzymology*, 299, 152-178.

Weerasak, B., Gutteridge, J.M.C. and Cross, C.E. (2008). Free radicals, antioxidants and human disease: where are we now? *Journal of Laboratory and Clinical Medicine* 119: 598-620.

Zhao,M., Hall,M. (2008).An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Advances in Sonochemistry*, vol. 8, 303–313.

APPENDIX A Effects of drying on *B.hispida* seeds

A.1 Calculation for Moisture Content

For sun drying:

$$Initial\ weight = 2\ g$$

$$Final\ weight = 0.408\ g$$

$$Moisture\ content\ (\%) = \frac{(2 - 0.408)g}{2} \times 100$$

$$= 79.6\ \%$$

For oven drying:

$$Initial\ weight = 2\ g$$

$$Final\ weight = 0.354\ g$$

$$Moisture\ content\ (\%) = \frac{(2 - 0.354)g}{2} \times 100$$

$$= 82.3\ \%$$

For freeze drying:

$$Initial\ weight = 2\ g$$

$$Final\ weight = 0.316\ g$$

$$Moisture\ content\ (\%) = \frac{(2 - 0.316)g}{2} \times 100$$

$$= 84.2\ \%$$

A.2 Calculation for DDPH scavenging activity

Standard curve for ascorbic acid

Standard curve for ascorbic acid to determine the concentration of DPPH of each sample.

Table A.1 Concentration of each sample

vitamin c-Ascorbic acid (mg/ml)	**Absorbance at 517 nm**
0	0
2.5	0.071
5	0.143
10	0.286
20	0.59

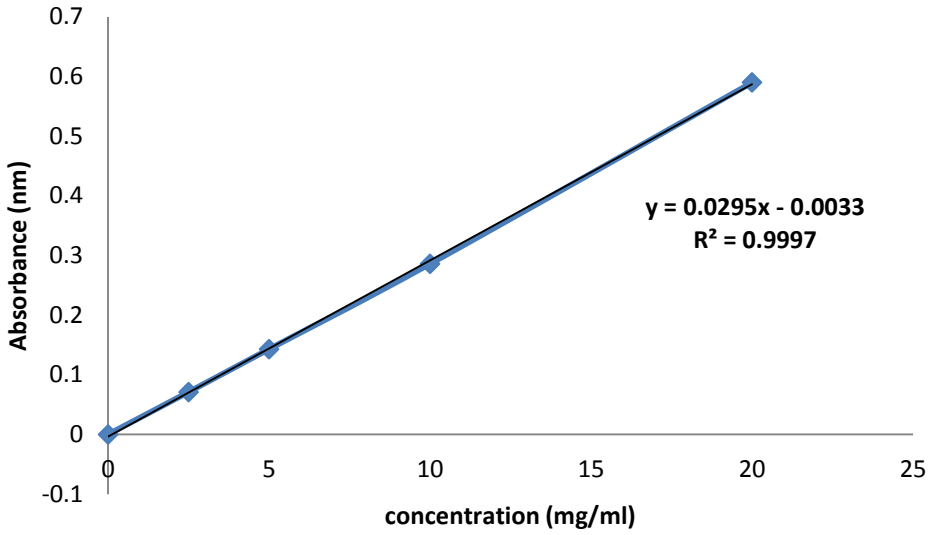


Figure A.1 Graph for standard curve for ascorbic acid

DPPH scavenging ability (%)

For sun drying:

At 2.5 mg/ml: A_O = 0.071 nm; A_1 = 0.0497 nm

$$Scavenging\ ability\ (\%) = \left(1 - \frac{0.0497}{0.071}\right) \times 100$$

$$= 30.0\ \%$$

At 5 mg/ml: A_O = 0.143 nm; A_1 = 0.0589 nm

$$Scavenging\ ability\ (\%) = \left(1 - \frac{0.0589}{0.143}\right) \times 100$$

$$= 58.8\ \%$$

At 10 mg/ml: A_O = 0.286 nm; A_1 = 0.0572 nm

$$Scavenging\ ability\ (\%) = \left(1 - \frac{0.0572}{0.286}\right) \times 100$$

$$= 80.0\ \%$$

At 20 mg/ml: A_O = 0.59 nm; A_1 = 0.1062 nm

$$Scavenging\ ability\ (\%) = \left(1 - \frac{0.1062}{0.59}\right) \times 100$$

$$= 82.0\ \%$$

Table A.2 Calculation for Total Phenolic Content

Tube	**Tannic acid solution (0.1 mg/ml) (ml)**	**Distilled water (ml)**	**Folin reagent (ml)**	**Sodium carbonate (ml)**	**Absorbance at 725 nm**	**Concentration tannic acid (ug/ml)**
Blank	0.00	0.50	0.25	1.25	0.000	0
T_1	0.02	0.48	0.25	1.25	0.112	2
T_2	0.04	0.46	0.25	1.25	0.218	4
T_3	0.06	0.44	0.25	1.25	0.327	6
T_4	0.08	0.42	0.25	1.25	0.432	8
T_5	0.10	0.40	0.25	1.25	0.538	10

Table A.3 Absorbance of sample compare tannic acid

Different drying methods	Absorbance at 725 nm	Concentration (ug/ml) compare tannic acid
fresh drying	0.2551	4.7
oven drying	0.4645	8.6
freeze drying	0.5666	10.5

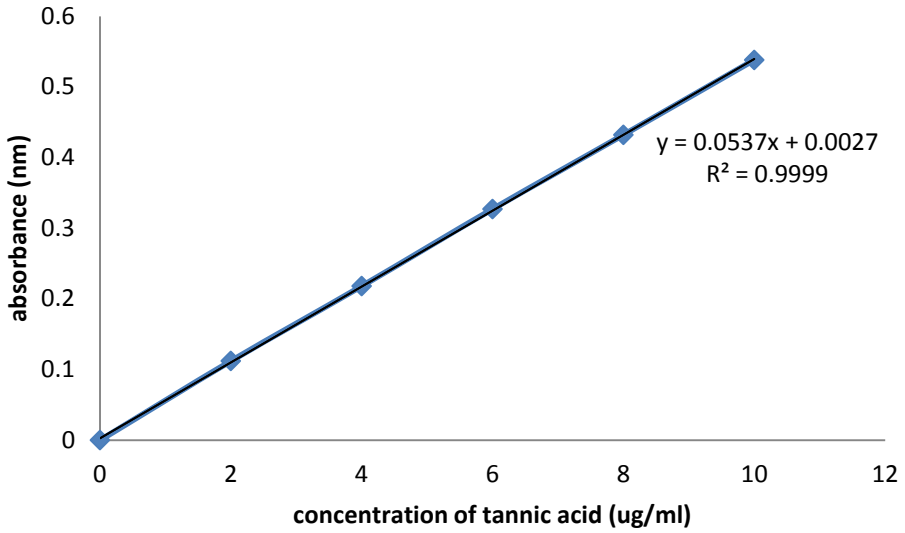


Figure A.2 Calibration curve for total phenolic content

Calculation

For fresh:

$$\text{Absorbance of sample, y} = 0.2551 \text{ nm}$$

By using this linear equation : $y = 0.0537x + 0.0027$

Find x:

$$\text{Total phenolic content}\left(\frac{\text{ug TAE}}{\text{mL}}\right) = \frac{0.2551 - 0.0027}{0.0537}$$

$$= 4.7 \frac{ug\ TAE}{mL}$$

For oven:

$$\text{Absorbance of sample, y} = 0.4645 \text{ nm}$$

By using this linear equation : $y = 0.0537x + 0.0027$

Find x:

$$\text{Total phenolic content}\left(\frac{\text{ug TAE}}{\text{mL}}\right) = \frac{0.4645 - 0.0027}{0.0537}$$

$$= 8.6 \frac{ug\ TAE}{mL}$$

For freeze:

$$\text{Absorbance of sample, y} = 0.5666 \text{ nm}$$

By using this linear equation : $y = 0.0537x + 0.0027$

Find x

$$\text{Total phenolic content}\left(\frac{\text{ug TAE}}{\text{mL}}\right) = \frac{0.5666 - 0.0027}{0.0537}$$

$$= 10.6 \frac{ug\ TAE}{mL}$$

For sun:

$$\text{Absorbance of sample, y} = 0.2121 \text{ nm}$$

By using this linear equation : $y = 0.0537x + 0.0027$

Find x

$$\text{Total phenolic content}\left(\frac{\text{ug TAE}}{\text{mL}}\right) = \frac{0.2121 - 0.0027}{0.0537}$$

$$= 3.9 \frac{ug\ TAE}{mL}$$