# PROXIMATE ANALYSIS AND ANTIOXIDANT ACTIVITY OF WATERMELON RIND POWDER

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**DECLARATION**

I hereby declare this thesis entitled "Proximate analysis and antioxidant activity of watermelon rind powder" is the results of origin research except that I have cited in the reference.

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**Proximate Analysis and Antioxidant Activity of Watermelon Rind Powder**

## ABSTRACT

Watermelon is a summer plant and it belongs to the Cucurbit family (*Citrullus lanatus*). Watermelon is high in nutrients even though it is low in calories. It contains Vitamin C and Vitamin A in the form of beta – carotene which is powerful antioxidant to protect the body from free radical and fight disease. The watermelon rind has natural source of antioxidant nutrients including flavonoids, flavones and polyphenols. Therefore, this research aimed to determine the proximate analysis and to analyze antioxidant activity in the watermelon rind powder. The proximate analysis was performed to find moisture, ash, crude fat and crude protein. Then, the antioxidant activity was performed to find antioxidant activity using DPPH. The results of proximate analysis showed that the ash, moisture, crude fat and crude protein content in watermelon rind powder were 43.33 ± 19.73, 16.71 ± 0.55, 0.70 ± 0.26 and 1.89 ± 0.66 respectively. Moisture content and crude protein were significantly ($p<0.05$) higher than control. The result of antioxidant activity show watermelon rind powder was significant ($p\leq0.05$) higher compared to control. The result also showed the moisture content in the watermelon rind was high compared to control, thus to increase shelf life, the moisture level of the watermelon rind must be reduced. In conclusion, the watermelon rind powder was excellent source of important nutrients that may be used to enhance human diets and the antioxidant activity in watermelon rind powder has lower antioxidant in every concentration compared to control. Further studies may be performed to lower the watermelon rind powder moisture content with using osmotic dehydration machine

Keyword: Watermelon, Watermelon rind, Proximate analysis, Antioxidant, Shelf life

**Analisis Proksimat dan Aktiviti Antioksidan Serbuk Kulit Tembikai**

## ABSTRAK

Tembikai ialah tumbuhan musim panas dan ia tergolong dalam keluarga Cucurbit (Citrullus lanatus). Tembikai kaya dengan khasiat walaupun rendah kalori . Ia mengandungi Vitamin C dan Vitamin A dalam bentuk beta – karotena yang merupakan antioksidan yang kuat untuk melindungi tubuh daripada radikal bebas dan melawan penyakit. Kulit tembikai mempunyai sumber semulajadi nutrien antioksidan termasuk flavonoid, flavon dan polifenol. Oleh itu, kajian ini bertujuan untuk menentukan analisis proksimat dan menganalisis aktiviti antioksidan dalam serbuk kulit tembikai. Analisis proksimat dilakukan untuk mencari kelembapan, abu, lemak kasar dan protein kasar. Kemudian, aktiviti antioksidan dilakukan untuk mencari aktiviti antioksidan menggunakan DPPH. Keputusan analisis proksimat menunjukkan bahawa kandungan abu, lembapan, lemak kasar dan protein kasar dalam serbuk kulit tembikai adalah masing-masing 43.33 ± 19.73, 16.71 ± 0.55, 0.70 ± 0.26 dan 1.89 ± 0.66. Kandungan lembapan dan protein kasar adalah ketara ($p<0.05$) lebih tinggi daripada kawalan. Hasil aktiviti antioksidan menunjukkan serbuk kulit tembikai adalah signifikan ($p\leq0.05$) lebih tinggi berbanding kawalan. Hasil kajian juga menunjukkan kandungan lembapan dalam kulit tembikai adalah tinggi berbanding kawalan, justeru untuk meningkatkan jangka hayat, tahap lembapan kulit tembikai mesti dikurangkan. Kesimpulannya, serbuk kulit tembikai merupakan sumber nutrien penting yang sangat baik yang boleh digunakan untuk meningkatkan diet manusia dan aktiviti antioksidan dalam serbuk kulit tembikai mempunyai antioksidan yang lebih rendah dalam setiap kepekatan berbanding kawalan. Kajian lanjut boleh dilakukan untuk mengurangkan kandungan lembapan serbuk kulit tembikai dengan menggunakan mesin dehidrasi osmotik.

Kata kunci: Tembikai, Kulit Tembikai, Analisis Proksimat, Antioksidan, Jangka Hayat

**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURE

## LIST OF DIAGRAM

## LIST OF SYMBOLS

| Symbol | Meaning |
|---|---|
| ± | Plus/minus |
| ˚C | Degree Celsius |
| % | Percentage |
| g | Gram |
| ml | Millilitre |
| µL | Microliter |
| > | Greater than |
| x | Times |
| mm | Millimeter |
| = | Equal |

## LIST OF ABBREVIATIONS

| Abbreviation | Meaning |
|---|---|
| nm | Nanometer |
| DPPH | 2,2-diphenylpicrylhydrazyl |
| MeOH | Methanol |
| rpm | Revolutions per minute |
| CO2 | carbon dioxide |
| N2 | Nitrogen Gas |
| pH | Potential hydrogen |

# CHAPTER 1

# INTRODUCTION

## 1.1 Research Background

Watermelon (Citrullus lanatus) is fruit that can be consumed as sweet and refreshing low calorie snack during summer as it provides hydration as well as essentials nutrients. Watermelon belongs to the family known as *cucurbitaceae* and it is a warm season crop like squash, cucumber and pumpkin. The whole parts of watermelon is consumable, including the rind. Watermelon is high in nutrients even though it is low in calories. It contains vitamin C and vitamin A in the form of beta – carotene which is powerful antioxidant to protect the body from free radical and fight disease (Milczarek, Olsen & Sedej, 2020). Watermelon has high perishable properties due to high water content which accounts for about 99 per cent of the total weight, mostly affected by post-harvest deterioration (PHD). Watermelon is nutritious and rich in carotenoids, includinglycopene and photogene (Li et al., 2007).

The rind on plants and fruits, as a natural source of antioxidants rich in compounds of free radical activity, and this is highly considered in the present (Ciou et al., 2008). However, there are relatively large quantities of antioxidant nutrients in the rind of the fruit, including flavonoids, flavones and polyphenols. It can also be used as fertilizer in many countries (Lee et al., 2010). Therefore, it has several components such as pectin, flavonoids, carotenoids and limonene and polyethoxic flavones (El-Adwy & Taha, 2001).

## 1.2 Problem Statement

The part of watermelon that is always eaten is the red or yellow flesh. Most people will discard watermelon rind because it tastes less palatable without realizing that watermelon rind is rich in nutrients. Many people discard watermelon rind because they think watermelon rind has no value for commercialization. In general, rind contains protein, fat, hydrocarbons, crude fiber and ash (Koocheki, et al., 2007). Thus, this study aimed to commercial the watermelon rind by converting it into powder from thus prolong its shelf life which in turn can reduce the disposal problems that are commonly caused by fruit waste.

## 1.3 Objectives of Study

1. To determine a nutritional value of the watermelon rind powder.
2. To determine antioxidant activity of watermelon rind powder.

## 1.4 Research Questions

1. What are the nutritional value in term of content of ash, moisture, crude fat and crude protein of watermelon rind powder?
2. How much antioxidant activity does watermelon rind powder have?

## 1.5 Hypothesis of Study

$H_0$: There was no difference on nutritional value in term content of ash, moisture, crude fat and crude protein of watermelon rind powder.

$H_1$: There was difference on nutritional value in term of content of ash, moisture, crude fat and crude protein of watermelon powder.

$H_0$: There was no difference of antioxidant activity in watermelon rind powder compared to commercial agar-agar powder.

$H_1$: There was difference of antioxidant activity in watermelon rind powder compared to commercial agar-agar powder.

## 1.6 Scope of Study

The study attempts to determine nutritional content of watermelon rind powder in term of content of ash, moisture, crude fat and crude protein using proximate analysis. The study also covers the analysis of antioxidant activity in watermelon rind powder. The study covers the collection of sample in Jeli since the availability of the watermelon waslimited during the duration of study from August to December 2022 since the end of theyear is monsoon season.

## 1.7 Significance of Study

The data obtained from this study were useful to another research. The data of this study can be used to determine nutritional value of watermelon rind. This is because the anthocyanin and flavonoid contained in watermelon rind serve a good antioxidant effect for protecting human body from free radical damage. In addition, the antioxidant content in watermelon rind can be used to make health supplement for human consumption.

## 1.8 Limitations of Study

Limited previous research on watermelon rind is limitation in this study. There is several research on this study about watermelon rind powder but not many. Next, is limitation of the food sample. This is because November and December is a rainy season which it not suitable to grow watermelon. Thus, it is difficult to find watermelon in Jeli.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Watermelon

Watermelon (*Citrullus lanatus*) is in the cucurbit family (*Cucurbitaceae*) (Edwards et al., 2003). With 6.8% of the world's plant grown area, watermelon is a large crop. These crops are grown commercially in old and frost free areas during the summer (FAO, 2012). Due to the long and trailing trees, the plants need to be planted in large quantities. The exception is dwarf crops, where fruits can be grown faster. On transplanting or planting containers the seeds can be used for planting. The fruits are then harvested manually by more experienced workers such as removing the fruits and placing them in baskets or trucks from the remaining watermelon trees. The fruit will remain for two to three weeks after harvest when the watermelon fruit is stored properly at a temperature of 10-15 ° C and 90 percent humidity (Paltrinieri, n.d.). In addition, there are popular cuts on watermelons while selling with various parts such as pre-cut, quarters, chunks and slices. Fruits are usually cut in the store with cold and aseptic conditions because the cut fruit is not stored properly. Semi-free watermelons are very popular for pre-cut sales because they can show seedless quality and can attract customers (Wehner, 2008).

Watermelon crops are not only hot weather but require more heat than other vegetables for better growth. Watermelons are best grown on well drained sandy soil. Poorly drained soils should be avoided (Kumar et al., 2013). Due to disease problems, watermelons cannot be grown in the same soil year after year. Three years is supposed to wait for watermelons to be planted in the same land (Agri Farming, 2020). Watermelon seeds will germinate well and thrive at a temperature of 25 ° C to 30 ° C. The best ripe fruit is at a temperature of 30 ° C. Watermelons need dry weather and lots of sunlight to grow. However, during rain or cloudy weather, the plant will not only stop, but will also reduce the flora and fruit environment. The sugar content is greatly reduced when the watermelon juice matures during the rainy season (Asfaw, 2021).

### 2.1.1 Watermelon Composition

Watermelon is mostly eaten while fresh especially in hot weather where it is suitable as a tasty and cold dessert. In the red flesh, watermelon has a high lycopene content which is 60% higher than tomatoes (Guner & Wehner, 2004). However, watermelon fruit is non - climacteric and grows slowly during ripening in its quality profile and its physiological maturation ends during harvesting and does not produce a very significant harvest maturity index (Kyriacou & Roupheal, 2018). Watermelons account for 4.7% of the acreage and 7.8% of the world's vegetables for production, including root crops. It is a cold food with very few calories and also contains certain minerals and vitamins (Oms-Oliu et al., 2010). There is very rich in water and it contains no fat or protein, so its calories are low. In addition, vitamin A and potassium are important sources (Interempresas Media, 2021).

Fruit coat, mesocarp, and seeds make up the fruit. White, green, yellow, orange, pink, or red mesocarps are all possible (Jyoti & Shashi, 2016). Watermelon seeds are high in protein, oil, dietary fibre, and micro and macronutrients including magnesium, calcium, potassium, iron, phosphorus, zinc, and vitamins (Perkins-Veazie & Collins 2006). Omega 3 and omega 6 fatty acids are abundant in the seed. Watermelon seed has a high quantity of magnesium, which offers health advantages, particularly in terms of heart function. Watermelon biomass is made up of three basic components as flesh, seeds, and rinds. The flesh accounts for around 68% of the total weight of the fruit, the seeds for 2%, and the rinds for 30% (Romdhane et al, 2017).

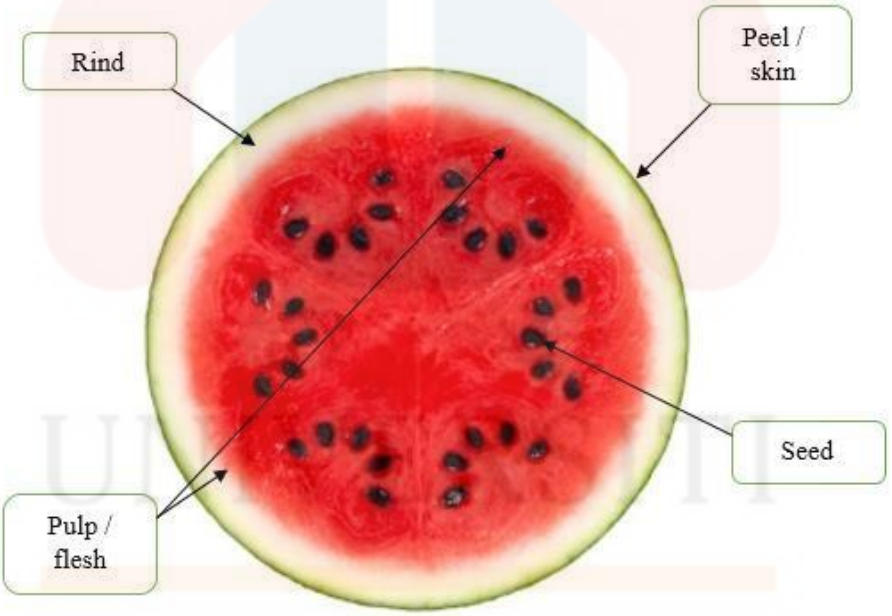



Figure 2.1 Transverse section of various part of watermelon.

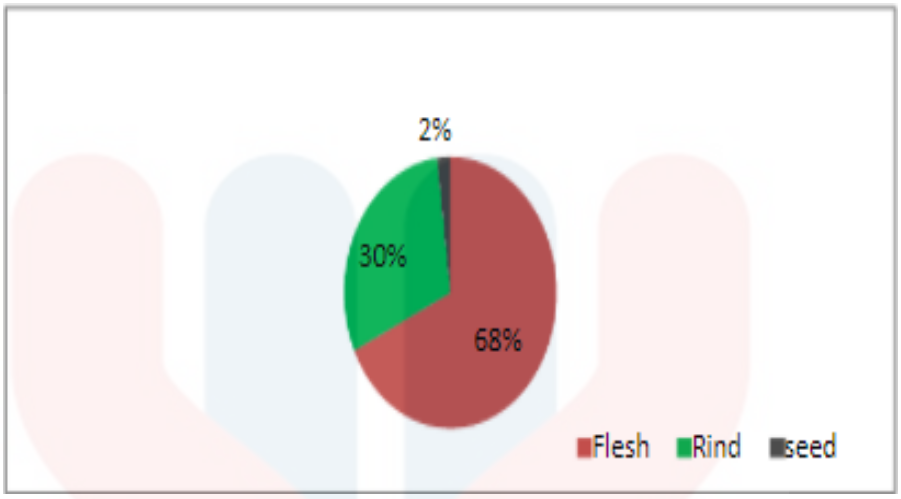



Figure 2.2 Composition watermelon (Source: Zubairu et al., 2018)

## 2.2 Watermelon rind

Usually, watermelon pulp and juice are consumed by people, however the rind and seeds, which account for 30% of the total weight of the fruit, are substantial solid wastes (Ho & Che Dahri, 2016). Even though watermelon skins are edible, almost 36 million tonnes of them were thrown in 2013 (Petkowicz et al., 2017). This shows watermelon skin is contributing to disposal problems and a lot of wastage which is getting higher as watermelon production is increasing day by day. Few people are aware that watermelon peels are edible and may be consumed as a vegetable. In China, watermelon skins are peeled and the fruit is discarded before being cooked with olive oil, garlic, chili pepper, scallions, sugar, and rum. Watermelon skin is also available in powder form in China, and the Chinese utilize it extensively as a traditional medicine to remove heat from the body and eliminate poisonous chemicals. Watermelon peels are also available pickled and are popular in the southern United States, Russia, Ukraine, Romania, and Bulgaria. In Nigeria, watermelon skins are fermented, mixed, and consumed as a juice (Ibrahim et al., 2017). The therapeutic effects of watermelon are associated with antioxidants such as

citrulline, which prevent free radical damage to the body. The amino acid, arginine which is very important for heart function, circulatory system and immune system, is also converted into citrulline. Thus, watermelon rind can cause blood vessels to become loose (Akshaya et al., 2018).

Figure 2.3 Watermelon rind. (Source : Ibrahim et al., 2017)

### 2.2.1 Watermelon rind composition

Watermelon rind is made up of cellulose, hemicellulose, pectin, and lignin, as well as lycopene, carotenoids, citrulline, and phenolic (Rimando and Perkins-Veazie, 2005). The mineral salts, fat, protein, carbohydrate, vitamins, and phytochemicals are also found in watermelon rind (Al-Sayed and Ahmed, 2013). Watermelon rind's major constituents are carbohydrate, and it has been stated that it may be utilized as a raw material for pectin extraction (Maran et al., 2014). The pectin are polysaccharides that are

widely employed as gelling, thickening, and stabilizing agents in the food industry. It are found in all live plants' cell walls as well as as the adhesive substance between cells (Petkowicz et al., 2017).

## 2.3 Agar – Agar Powder

Agar-agar from red seaweed (Rhodophyta) was used as a polymerizing agent. Agar-agar, a vegetable gelatin, is a denatured collagen protein derived from red algae of the family Rhodophyceae (Basumatary et al., 2018). Agar is a gelatinous material derived from seaweed and manufactured into flakes, powders, and sheets. It's popular in Asian cuisines and as a flavourless vegan alternative for gelatin. Agar aids in the gelation, stabilization, texturization, and thickening of drinks, baked goods, confectioneries, dairy products, dressings, meat products, and sauces (Marcus, 2013).

## 2.4 Proximate analysis

Proximate Analysis is a method for determining the levels of macronutrients in food samples (Jayasinghe et al., 2019). Proximate composition comprises moisture, ash, fat, protein, and carbohydrate content. These food components may be of interest in the food business for product development, quality control (QC), or regulatory purposes. Analyses employed may be quick QC procedures or more accurate yet time-consuming official methods. Sample collection and preparation must be carefully examined to guarantee analysis of a homogenous and representative sample and reliable results (NIH, 2016).

### 2.4.1 Ash content

Ash is the inorganic residue that remains after the ignition or full oxidation of organic materials in a food sample (Franco et al., 2016). In addition, ashing is the initial step in preparing a sample for particular elemental analysis. It employs the dry ashing process with a muffle furnace to determine the ash level of a range of food items. Water and volatiles evaporate, and organic matter burns and converts to $CO_2$ and $N_2$ oxides in the presence of oxygen. Wet ashing, on the other hand, is based on oxidizing organic materials using acids, oxidizing agents, or a mix of the two (Mathew et al., 2019). As a result, minerals are solubilized without being oxidized. Vegetables, for example, are frequently dried before ashing. Prior to ashing, foods with a high fat content, such as meat, may need to be dried and their fat removed (Nielsen, 2017).

### 2.4.2 Moisture content

The moisture content of the powder is a significant component in determining its quality (Sanwiriya & Suleiman, 2019). The moisture content of a product influences its processibility, shelf life, usefulness, and quality. Accurate moisture content determination therefore plays a critical role in guaranteeing quality in numerous industries, including food, pharmaceuticals, and chemicals. Furthermore, the maximum allowable moisture level in certain items may be limited by regulation. The loss on drying of sample, in which the sample is heated and the weight loss owing to moisture evaporation is recorded (Yang, Lai & Song, 2019). Moisture determination give enhancement the overall quality of the completed product at various phases of the manufacturing process, including raw

materials, goods-in, storage, in-process control, QC, packaging, and others (Nielsen,2017).

### 2.4.3 Crude Fat

The fat refers to a class of substances that are very slightly soluble in water but have varying solubility in a variety of organic solvents such as ethyl ether, petroleum ether, acetone, ethanol, methanol, and benzene (Cheng et al., 2021). The fat content of a meal measured by extraction with one solvent may differ significantly from the fat content by extraction with another solvent with a different polarity. Fat content is frequently determined using solvent extraction methods such as Soxhlet, Goldfish, and Mojonnier, but it can also be determined using non solvent wet extraction methods such as Babcock and Gerber, as well as instrumental methods that rely on the physical and chemical properties of fat such as infrared, density, and X-ray absorption. The technique of choosing is determined by a number of criteria, including the type of the sample dry or wet, the goal of the analysis as official nutrition labelling or fast quality check, and the technology available (Ellefson ,2017).

### 2.4.4 Crude Protein

Protein is known as a nutritional element that can reduce or even prevent muscular strength and mass loss, although human dietary intervention research on muscle health to date have mostly focused on animal-sourced protein (Hackney et al., 2019). There is a scarcity of data on new proteins, including those found in terrestrial and marine plants. Standardized analytical procedures are required to quantify the amount of protein in

meals. There are several method that can be used in the food industry to quantify protein content, including the Kjeldahl method, Lowry method, Bradford protein assay, and total amino acid content methods (Hayes, 2020). The precise assessment of the protein content of foods is crucial since it frequently dictates the economic worth of the food product, as in the case of milk and wheat. Protein nutritional quality may be defined as numerous things, including protein to promote optimum development, amino acid balance, the degree of protein digestion and absorption, or indispensable amino acids compared to amino acid needs (Hayes, 2020).

## 2.5 Antioxidant Activity

The term antioxidant has been defined in a variety of ways, such as substances that in small quantities, can inhibit or greatly slow down the oxidation of easily oxidizing substances and any substances that when present in low concentrations compared to substrates can be oxidized (Bajpai, 2018). It is responsible for the organism's defense system against diseases caused by free radical attack. Thus, the use of plant - derived antioxidants helps in the prevention of degenerative diseases caused by oxidative stress, such as cancer, Parkinson's disease, Alzheimer's disease, and atherosclerosis (Pisoschi & Negulescu, 2011). There are chemicals that prevent the oxidation of other molecules. Oxidation is a chemical process that transfers electrons or hydrogen from a compound to an oxidizing agent (Vishnoi et al., 2018). Free radicals can be produced through an oxidation process. These radicals can initiate a chain reaction, which can lead to cell damage or death. Antioxidants stop these chain events by eliminating free radical intermediates and inhibiting other oxidative processes. Antioxidants are frequent reducing agents such as thiol, ascorbic acid or polyphenols (Moharram, & Youssef,

2014). According to the research of Hue et al. (2011), to reduce the risk of chronic diseases such as cancer and heart disease, antioxidant molecules in food play an important role as a health protective element.

Due to differences in molecular structure, various antioxidants have significantly different antioxidant efficiencies in different nutritional systems. Antioxidants should not have an inappropriate taste or color. It must be easily integrated into the food or food system and must be stable at the pH of the food system throughout food processing. Antioxidant activation energy, pH redox potential stability, processing and stability are some of the elements that influence antioxidant efficiency (Sharma & Singh, 2013).

There are a lot natural free radical scavenging antioxidant found in all sections of plants, including fruits, vegetables, nuts, seeds, leaves, roots, and bark (Akbarirad, et al, 2016). Fruits provide a variety of vitamins and minerals, as well as dietary fibre. Most fruits are high in vitamin C, carotenoids, and polyphenolic substances (Ellong, et al, 2015)

The table 2.1 Summary of the most common natural antioxidants and their typical sources

| Compound name | Natural source |
|---|---|
| Ascorbic acid | Most fruits (particulary citrus fruits), some vegetable, tomatoes. |
| Beta - carotene | Vegetables as kale, red paprika, spinach, parsley, and tomatoes, carrots, sweet potatoes, apricots, papayas. |
| Tochopherols | Broccoli, brusseles sprouts, cereal grains, cauliflower, cooking oils as olive, sunflower, safflower, almonds, hazelnuts. |
| Flavonoids a type of polyphenol | Tomatoes, lettuce, onions, wheat, potatoes, concord grapes, black tea |
| Anthocyanisms a type of flavonoid | High content in red wines |
| Lycopene | Papaya, watermelon, guava, pink grapefruit, tomatoes, the skin of red grapes. |
| CoQ10 | Wheat brain |
| Various | Teas as well as many red/purple hued fruits or vegetable such as Concord grapes, red cabbage, blueberries, blackberries and another. |

Source: (Akbarirad et al., 2016).

# CHAPTER 3

# METHODOLOGY

## 3.1 Material and Apparatus

Micropipette tips, measuring cylinder , test tube, test tube rack, glass filter funnel, beaker, cuvette, stainless steel spatula, 90 mm SMITH Filter Papers, aluminium foil, conical flask, tube centrifuge, desiccator, volumetric flask, crucible, funnel, cotton, analytical balance. Micropipette 100 - 1000 µL was supplied by Eppendorf (Selangor) , Waterbath shaking was supplied by Memmert™ WNE14 (United Kingdom) , Oven drying was obtained from Jeio Tech OF-12G Oven (Gaithersburg, USA) , Spectrophotometer was obtained from Biochrom Libra S4+ (USA) , Centrifuge 5810 R was supplied by Eppendorf (Cambridge, USA) , Soxlet Foss 2055 was obtained from Matrix Analytical Technologies (Petaling Jaya) , Kjeldahl Gerhadt was obtained from Gerhadt Malaysia Sdn Bhd (Selangor) and Muffle furnace ECO 110/15 was obtained fromProtherm (Turkey).

### 3.2 Chemicals and reagent

Petroleum ether (A.R) 40-60 °C 8032-32-4 was purchased from Sigma-Aldrich (USA), 1000 Kjeltabs Cu/3,5 was purchased from United Kingdom, Hydrochloric acid min 37% was purchased from HmbG Chemical (Ireland) , Sodium hydroxide 97% was purchased Sigma-Adrich (USA) and Boric acid was purchased from Sigma-Aldrich (USA). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (New Jersey, USA). The Methanol was also purchased from Merck (MeOH). All chemical and reagent were analytical grades.

### 3.3 Collection and preparation of sample

Watermelon (Citrullus lanatus) was obtained from market Pantai Timur Tanah Merah. Then, watermelon was cut and the watermelon rinds were collected. The green part of the rind or watermelon peel was removed and the rind was kept. Watermelon rinds was clean with running tap water to get rid of any dirt. The watermelon rind was weighed and recorded. Control used was commercial agar- agar powder obtained from market Pantai Timur Tanah Merah.

#### 3.3.1 The production of watermelon rinds powder (WRP)

Watermelon rind was placed in airtight plastic and kept in-18 °C for 24 hours in Ultra-low temperature freezer (MDF-U55V-PE) by Panasonic. Then the sample watermelon rind was freezer dried for 72 hours by using the Freeze dryer Model CoolSafe 4-15L that was supplied by LaboGene (Denmark). The freese dried watermelon rind was

transferred onto airtight plastic and the watermelon rind was subsequently grounded using lightweight blender (Dry Mill) Model MX-M210SSL from Panasonic. The sample was saved a little bit for further analysis.

### 3.4 Proximate analysis.

The proximate analysis of watermelon rind powder was conducted using the standard method described by Association of Official Analytical Chemist 15th Edition. (Ooi et al., 2012). The method is outlined as follows:

#### 3.4.1 Determination of Moisture Content

Oven drying was used for moisture content determination, in which the temperature was kept at 150 ° C. 5g sample was collected in a crucible 30ml and placed for 6 hours in the oven at 150 ° C until the weight was constant. The percentage of moisture content was calculated using the equation (1):

$$\text{Percentage of moisture (\%)} = \frac{(X+Y)-Z}{Y} X\ 100, \qquad (1)$$

where, X = Initial weight of crucible, Y = Weight of sample and Z= Final weight of the sample.

### 3.4.2 Determination of Ash Content

The samples were weighed into crucible and place in muffle furnace at 550°C for overnight to remove the organic constituents through high temperature. The remaining inorganic waste after organic matter has been destroyed is ash content. The difference of sample weight before heating and final weight after heating were used to calculate of percentage of ash content using the equation below (1):

$$\text{Percentage of ash (\%)} = \frac{W2-W1}{WS} X\ 100, \qquad (2)$$

where, W2 = The sample weight after heating, W1= The sample weight before heating and WS= Weight of sample.

### 3.4.3 Determination of Crude Fat.

Weight of 1g of watermelon rind powder with petroleum ether were extracted to the soxhlet extractor to determine the fat content. . The percentage of moisture content was calculated using this equation (1):

$$\text{Percentage of fat (\%)} = \frac{W2-W1}{WS} X\ 100\ , \qquad (3)$$

where, W2 =weight of final cup sample, W1= weight of intial of cup and WS= weight of sample.

### 3.4.4 Determination of Crude Protein

The Kjeldahl method was used for crude protein content. Digestion was carried out using a digester and distillation machine. The analysis consist of three steps which was digestion, distillation and titration step. The kjeldahl method was performed by hydrolysing 1 gram of both sample with 12 mL concentration sulfuric acid ($H_2SO_4$) and two tablets of kjeltabs Cu/3,5 (potassium sulphate, $K_2SO_4$) then heating in kjeldahl digection machine at 420° C for 1 hours. In digestion step, the polypeptides bond was breaked down and convert into simpler chemicals such as ammonia, carbon dioxide and water by catalyst kjeltabs Cu/3,5 (potassium sulphate, $K_2SO_4$) to up the reaction. Then, the ammonia was separated from the digestion mixed in distillation steps. In titration steps, the ammonia trapped will determine by titration with standard solution and amount of the total nitrogen multiplied with conversion factor of 6.25 to identify the total of crude protein content (Mæhre et al.,2018).

### 3.5 Antioxidant Activity

The capacity of antioxidants to reduce 2,2 - diphenylpicrylhydrazyl (DPPH), another radical not typically present in biological systems, was measured using a spectrophotometer. A variety of procedures for DPPH antioxidant tests have been used, resulting in variations of results in other reashes. According to Sharma & Bhat (2009) DPPH was sensitive to light, pH, and solubility of the chemical when using spectrophotometry.

### 3.5.1 2,2 - diphenylpicrylhydrazyl (DPPH)

Weighed of 1 gram sample into 250ml of conical flask. Then, 25ml of 99% methanol were added in the same conical flask and shaked. The conical flask was closed with aluminium foil and parafilm. Then, it was placed in waterbath shaker at 300 rpm for 2 hours and 30 minutes.

For the preparation of DPPH solution, 0.004 g of DPPH was dissolved into 100ml of methanol in volumetric flask. The methanol were filled until it reached the mark and shaked. After that, the DDPH solution was covered with aluminium foil and was left at room temperature (25$^{o}$C) in the dark room. The sample was centrifuged at 8000 rpm for 15 minutes and the sample was filtered

Then, the extracted sample was mixed with methanol and DPPH solution in volumetric flask. Then, the volumetric flask were covered with aluminium foil and shaked. The extracted of sample was left at room temperature in the dark room for 30 minutes. Then, the solution was put in the cuvette and absorbance was measured at 517 nm by spectrophotometer. The sample was repeated.

From the DPPH radical scavenging activity, the following formula determine the inbition percentage was determine using equation (1) (Ho et al., 2018) :

$$\text{Inhibition (\%)} = [\ (\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}} \times 100 \quad (4)$$

The concentration of extract needed for 50% scavenging activity which is called IC50,

is value parameter used to measured the antioxidant activity. The calculated IC50 is from the dose – inhibition linear regression equation of each extract (Rivero-Cruz et al., 2020).

### 3.6 Statistical Analysis

The result of proximate analysis and antioxidant activity of watermelon rind powder was expressed as mean, ± standard deviation and p-value using IBM SPSS Statistic 20. The obtained data were analysed for the statistical significance of the results using a T-test and ANOVA test.

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 Analysis of Proximate

The proximate analysis was conducted to determine the ash content, moisture content, crude fat and crude protein. Table 4.1 shows the result of proximate analysis for watermelon rind powder with agar-agar powder (control). The mean and ± standard deviation for ash content was not significant different between watermelon rind powder with agar-agar powder ($p>0.05$). The mean and ± standard deviation of moisture content was significant difference between watermelon rind powder with agar-agar powder ($p<0.05$). Next, the mean and ± standard deviation of crude fat was not significant difference between watermelon rind powder with agar-agar powder ($p>0.05$). Lastly, for the mean and ± standard deviation of crude protein was significant difference between watermelon rind powder with agar-agar powder ($p<0.05$).

Table 4.1 show the proximate analysis of agar-agar powder (control) and watermelon rind powder.

| **Proximate Analysis (%)** | **Food sample** | |
|---|---|---|
| | Agar – agar powder | Watermelon rind powder |
| **Ash Content** | 33 ± 9.53 | 43.33 ± 19.73 |
| **Moisture Content** | 9.11 ± 0.24 | 16.71 ± 0.55* |
| **Crude Fat** | 1.29 ± 0.51 | 0.70 ± 0.26 |
| **Crude Protein** | 0.23 ± 0.05 | 1.89 ± 0.66* |

The result in Table 4.1 shows, there was significant different in moisture content between watermelon rind powder and control. Watermelon rind powder contains around 95% water, making it prone to degradation. As a result, in order to make goods with a stable shelf life, the moisture level of the watermelon rind powder must be reduced (Athmaselvi et al., 2012). Although water is a suitable medium for microbial development, a high value implies a general rise in the likelihood of microbial assault on watermelon (Lee, K. H, 2000).

The result in Table 4.1shows, there was significant different in crude protein between watermelon rind powder and control. The protein content in watermelon rind powder can be used food consumption for diabetic patiens (Abdulazeez A, 2020). However, if watermelon rind powder been exposed of time may lose their nutritional value (Asif-Ul-Alam et al., 2014).

The result in Table 4.1 shows, there have no significant different in ash content between watermelon rind powder and control. Samples with a high proportion of ash are predicted to aid peristaltic movement as well as accelerate metabolic processes required for growth and development enhancement (Bello et al., 2008). Watermelon rind powder is less tolerant to heat and has lower volatile characteristics due to the minerals (Omotoso and Adedire, 2007). According to Lakshmipathy and Sarada (2013) the main minerals found in watermelon rind powder include sodium, potassium, calcium, magnesium, and another trace minerals as zinc and iron.

The result in Table 4.1 shows, there have no significant different in crude fat between watermelon rind powder and control. This shows the high moisture in watermelon rind powder can be the cause of low content of the crude fat (Olayinka and Etejere, 2018).

Thereby, this results discover that watermelon rind can give good impact towards food industry since it can help to reduce the waste disposal. It was because the watermelon rind powder has high protein content which is good for human consumption. Proteins are an essential nutrient for human diet that is required for survival especially for diabetes patient (Maurya and Kushwaha, 2019). Furthermore, the result of moisture content for watermelon rind powder shows a good value. Moisture content has an impact on shelf life since more water in a product makes it more susceptible to bacteria and enhance product quality (Nielsen, 2010).

## 4.2 Analysis of Antioxidant Activity.

The antioxidant activity was conducted to determine the antioxidant activity of nutrient oxidation from the control and watermelon rind powder. Based on the Table 4.2, the result T-Test of antioxidant activity in control and watermelon rind powder were slightly different. According to Asghar, Shahzad, Nadeem, & Ashraf. (2013), the rind extracts from watermelon have significant free radical scavenging activity.

Table 4.2 show the T-Test of antioxidant activity in control and watermelon rind powder.

| **Antioxidant Activity** | **Food sample** | |
|---|---|---|
| **(%)** | Agar – agar powder | Watermelon rind powder |
| **Inhibition %** | 44.77 ± 4.70 | 44.23 ± 4.66* |

The results showed that the antioxidant content of agar-agar powder was significant ($p \leq 0.05$) equal to watermelon rind powder. Antioxidants can protect the body from the oxidative damage produced by reactive oxygen species (ROS). Antioxidants were mostly found in plant-based diets. Phytochemicals, in particular, phenolic compounds, flavonoid, alkaloid, and terpenoids, are important antioxidant compounds with a variety of nutraceutical effects (Luckner, 2013).

The Figure 4.1 show the curve percentage of DPPH radical scavenging versus by concentration (mg/ml). The calibration curved showed a linear graph for control with equation $y = 0.7145x + 38.196$ and the coefficient ($R^2$) value of 0.9234. The calculation of $IC_{50}$ for control was 16.52 where the result shows less than in watermelon rind powder.

Thus, a low value of IC50 represents a good activity of a tested sample. This has supported by Irondi, Anokam, & Ndidi (2013) that DPPH assay influences the stability of antioxidant activity. The result is compared based on IC50 values which is the concentration of extract needed for 50% of scavenging activity, it is calculated from dose-inhibition linear regression equation of each extract.

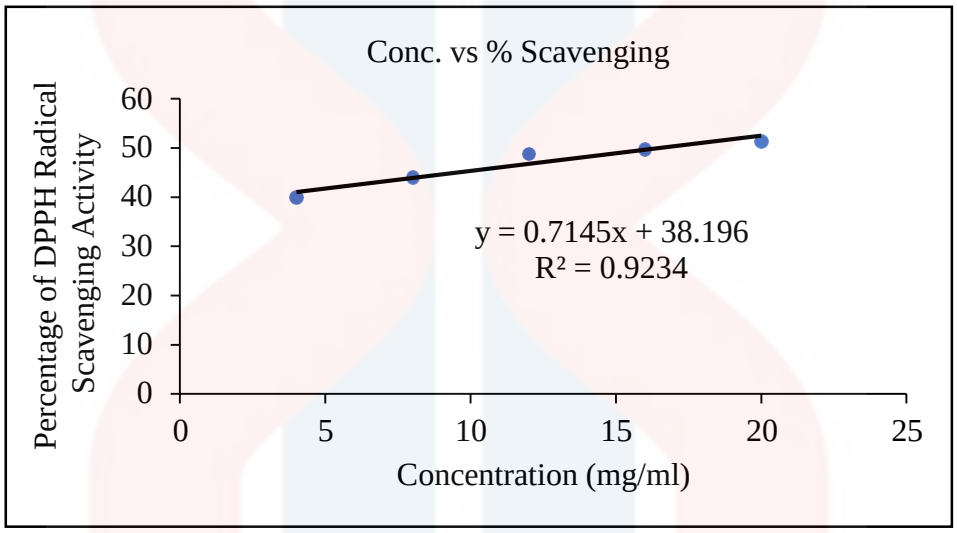
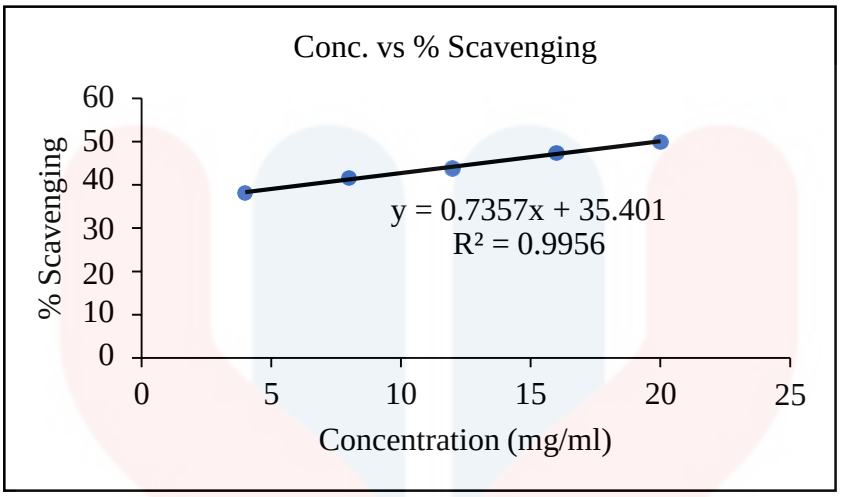



Figure 4.1 show the curve percentage of DPPH radical scavenging versus by concentration (mg/ml) in control.

Figure 4.2 show the curve percentage of DPPH radical scavenging versus by concentration (mg/ml). The calibration curved showed a linear graph for watermelon rind powder with equation y = 0.7357x + 35.401 and the coefficient ($R^2$) value of 0.9956. The calculation of $IC_{50}$ for watermelon rind powder is 19.84. According Rivero-Cruz et al, (2020) the lower IC50 value the higher antioxidant activity.



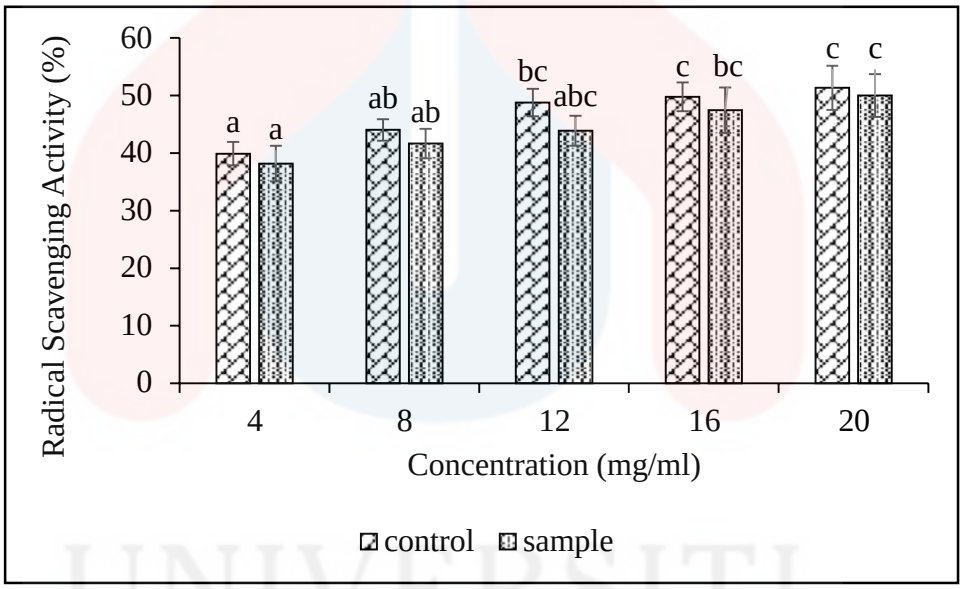

The Figure 4.2 show the curve percentage of DPPH radical scavenging versus by concentration (mg/ml).

Based on the Table 4.3, the result T-Test for antioxidant activity in control and watermelon rind by using Dpph radical scavenging activity. Each sample was performed in a concentration of 4, 8, 12, 16 and 20 mg/ml. Overall, the result showed that the higher the concentration of samples, the higher the antioxidant activity. According Rahman et al., (2015), the methanol extract with increased concentration, increased the total antioxidant activity. The result show the higher concentration of methanol in sample watermelon rind powder was 20 mg/ml and the mean and standard deviation value was 49.99 ± 3.72%. Then, the higher concentration of methanol in control was 20 mg/ml and the mean and standard deviation value was 51.34 ± 3.84 %. There was a significant value between control and watermelon rind powder when p-value was lower ($p<0.05$). Then, the diagram 4.1 show the error bars standard deviation of the mean valie of control and watermelon rind powder analysis.

Table 4.3, show anova test for antioxidant activity in control and watermelon rind by using Dpph radical scavenging activity.

| Concentration | Control | Watermelon rind powder |
|---|---|---|
| **4** | 39.91 ± 2.02 % | 38.17 ± 3.09 % |
| **8** | 44.04 ± 1.88 % | 41.66 ± 2.57 % |
| **12** | 48.80 ±2.38 % | 43.88 ± 2.60 % |
| **16** | 49.76 ± 2.50 % | 49.99 ± 3.94 % |
| **20** | 51.34 ± 3.84 % | 49.99 ± 3.72 %. |

Diagram 4.1 show percentage of radical scavenging activity in control and watermelon rind powder.




Watermelon rind powder was compared commercial agar-agar powder at different concentration. [a-c] all values are mean ± standard deviation (*n=3*). Different letters in some row indicates significant differences at p≤0.05.

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

In conclusion, this research has achieve objective to determining proximate analysis value. The findings of this study revealed that watermelon rind powder is an excellent source of important nutrients that may be used to enhance human diets. The nutritional value of watermelon rind powder has been determined by using proximate analysis. It shows that the value in terms of ash, crude protein, crude fat and moisture content of watermelon rind powder is higher compared to control. In this research, the second objective also achieved to analyze antioxidant activity in watermelon rind powder. The antioxidant activity analysis using DPPH radical scavenging activity resulted that the watermelon rind powder has lower antioxidant in every concentration compared to control. Watermelon rind powder contains antioxidants, flavonoids, and phenolic compounds. If consumed fresh or used in food stuff, these watermelon rind might give significant medical, health, and economic advantages.

## 5.2 Recommendation

The watermelon rind powder has higher nutritional value but antioxidant activity is lower. To increase IC50 value watermelon rind powder, it can be analyzer with difference method such as using 2-2' –azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay to strengthen the antioxidant activity and Ferric Reducing Antioxidant Power (FRAD) assay method to find various result in determining antioxidant properties for the food sample produced. Next, osmotic dehydration machine also can be implement in this research. This can help to eradicate water effectively thus the moisture content will be lower.

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## Appendix A

**Table A. 1. Statistical analysis of T-Test for moisture content.**

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Control | 9.1100 | 3 | .24880 | .14364 |
| | Sample | 16.7133 | 3 | .55645 | .32126 |

**Paired Samples Correlations**

| | | N | Correlation | Sig. |
|---|---|---|---|---|
| Pair 1 | Control & Sample | 3 | .899 | .289 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Control - Sample | -7.60333 | .35019 | .20218 | -8.47325 | -6.73341 | -37.606 | 2 | .001 |

**Table A. 2. Statistical analysis of T-Test for crude protein**.

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Control | .2300 | 3 | .05196 | .03000 |
| | Sample | 1.8933 | 3 | .66666 | .38490 |

**Paired Samples Correlations**

| | | N | Correlation | Sig. |
|---|---|---|---|---|
| Pair 1 | Control & Sample | 3 | .758 | .453 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Control - Sample | -1.66333 | .62820 | .36269 | -3.22386 | -.10280 | -4.586 | 2 | .044 |

**Table A. 3. Statistical analysis of T-Test for ash content**.

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Control | 33.00 | 3 | 9.539 | 5.508 |
| | Sample | 43.33 | 3 | 19.732 | 11.392 |

**Paired Samples Correlations**

| | | N | Correlation | Sig. |
|---|---|---|---|---|
| Pair 1 | Control & Sample | 3 | -.191 | .877 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Control - Sample | -10.333 | 23.502 | 13.569 | -68.715 | 48.048 | -.762 | 2 | .526 |

**Table A. 4. Statistical analysis of T-Test for crude fat**.

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Control | 1.2933 | 3 | .51675 | .29835 |
| | Sample | .7000 | 3 | .26458 | .15275 |

**Paired Samples Correlations**

| | | N | Correlation | Sig. |
|---|---|---|---|---|
| Pair 1 | Control & Sample | 3 | -.318 | .794 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Control - Sample | .59333 | .65118 | .37596 | -1.02428 | 2.21095 | 1.578 | 2 | .255 |

**Table B. 1. Statistical analysis of T-Test for Antioxidant Activity**

**Paired Samples Statistics**

| | | Mean | N | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|---|
| Pair 1 | Control | 46.7700 | 5 | 4.70262 | 2.10307 |
| | Sample | 44.2300 | 5 | 4.66361 | 2.08563 |

**Paired Samples Correlations**

| | | N | Correlation | Sig. |
|---|---|---|---|---|
| Pair 1 | Control & Sample | 5 | .956 | .011 |

**Paired Samples Test**

| | | Paired Differences | | | | | t | df | Sig. (2-tailed) |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Std. Deviation | Std. Error Mean | 95% Confidence Interval of the Difference | | | | |
| | | | | | Lower | Upper | | | |
| Pair 1 | Control - Sample | 2.54000 | 1.39633 | .62446 | .80622 | 4.27378 | 4.068 | 4 | .015 |

**Table A. 1. Statistical analysis of ANOVA test for Antioxidant Activity**

**Descriptives**

| | | N | Mean | Std. Deviation | Std. Error | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| Control | 4 | 3 | 39.9133 | 2.02609 | 1.16976 | 34.8803 | 44.9464 | 37.61 | 41.42 |
| | 8 | 3 | 44.0467 | 1.88982 | 1.09109 | 39.3521 | 48.7412 | 42.62 | 46.19 |
| | 12 | 3 | 48.8033 | 2.38500 | 1.37698 | 42.8787 | 54.7280 | 46.42 | 51.19 |
| | 16 | 3 | 49.7600 | 2.50863 | 1.44836 | 43.5282 | 55.9918 | 47.14 | 52.14 |
| | 20 | 3 | 51.3433 | 3.84928 | 2.22238 | 41.7812 | 60.9055 | 47.85 | 55.47 |
| | Total | 15 | 46.7733 | 4.88592 | 1.26154 | 44.0676 | 49.4791 | 37.61 | 55.47 |
| Sample | 4 | 3 | 38.1733 | 3.09797 | 1.78862 | 30.4775 | 45.8691 | 35.00 | 41.19 |
| | 8 | 3 | 41.6600 | 2.57659 | 1.48759 | 35.2594 | 48.0606 | 38.80 | 43.80 |
| | 12 | 3 | 43.8867 | 2.60962 | 1.50667 | 37.4040 | 50.3693 | 42.38 | 46.90 |
| | 16 | 3 | 47.4567 | 3.94473 | 2.27749 | 37.6574 | 57.2559 | 43.33 | 51.19 |
| | 20 | 3 | 49.9967 | 3.72011 | 2.14781 | 40.7554 | 59.2379 | 45.71 | 52.38 |
| | Total | 15 | 44.2347 | 5.11376 | 1.32037 | 41.4028 | 47.0666 | 35.00 | 52.38 |

**Test of Homogeneity of Variances**

| | Levene Statistic | df1 | df2 | Sig. |
|---|---|---|---|---|
| Control | .533 | 4 | 10 | .715 |
| Sample | .277 | 4 | 10 | .886 |

**ANOVA**

| | | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|---|
| Control | Between Groups | 265.261 | 4 | 66.315 | 9.618 | .002 |
| | Within Groups | 68.950 | 10 | 6.895 | | |
| | Total | 334.211 | 14 | | | |
| Sample | Between Groups | 261.215 | 4 | 65.304 | 6.226 | .009 |
| | Within Groups | 104.893 | 10 | 10.489 | | |
| | Total | 366.108 | 14 | | | |

**Homogeneous Subsets**

**Control**

Duncan

| Concentration | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 4 | 3 | 39.9133 | | |
| 8 | 3 | 44.0467 | 44.0467 | |
| 12 | 3 | | 48.8033 | 48.8033 |
| 16 | 3 | | | 49.7600 |
| 20 | 3 | | | 51.3433 |
| Sig. | | .083 | .051 | .284 |

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Sample

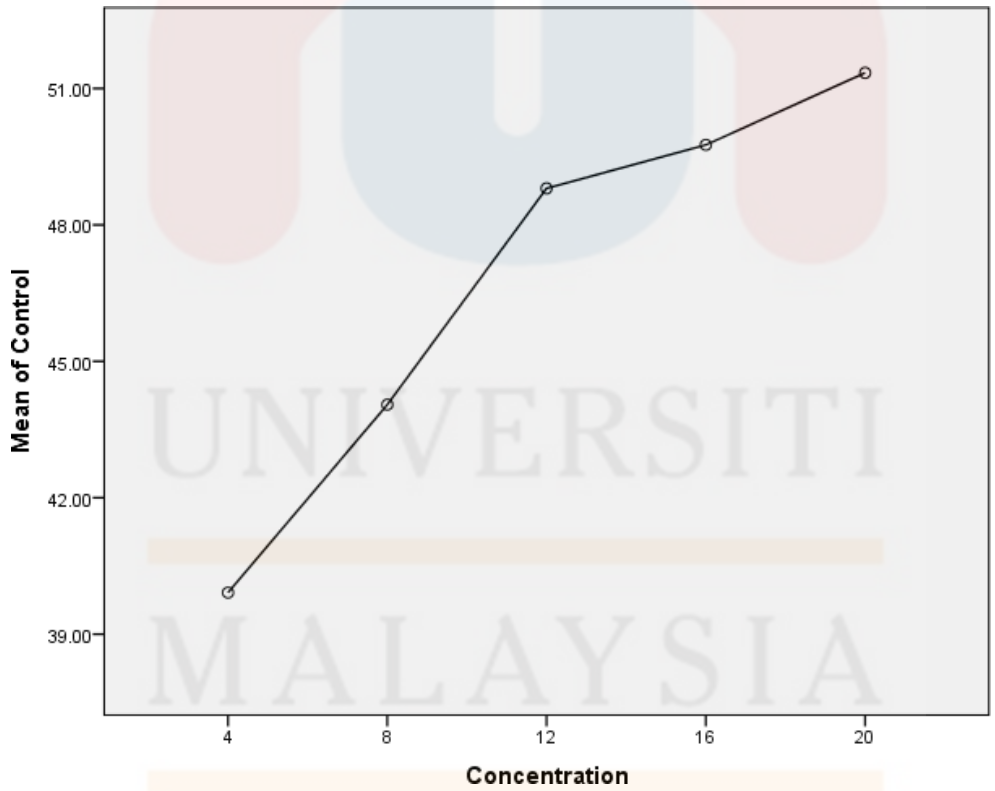
Duncan

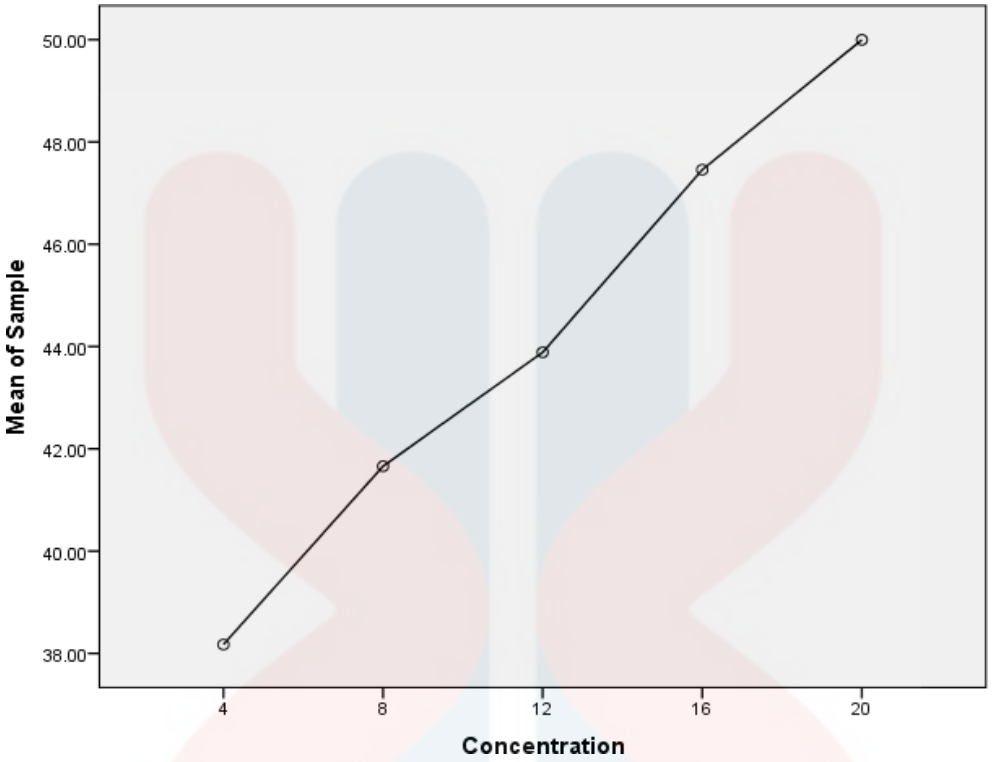
| Concentration | N | Subset for alpha = 0.05 | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| 4 | 3 | 38.1733 | | |
| 8 | 3 | 41.6600 | 41.6600 | |
| 12 | 3 | 43.8867 | 43.8867 | 43.8867 |
| 16 | 3 | | 47.4567 | 47.4567 |
| 20 | 3 | | | 49.9967 |
| Sig. | | .066 | .062 | .051 |

Means for groups in homogeneous subsets are displayed.

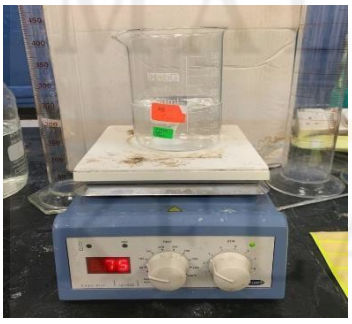
a. Uses Harmonic Mean Sample Size = 3.000.

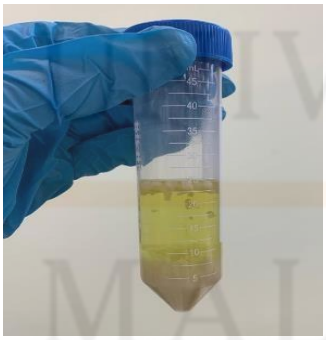
**Means Plots**

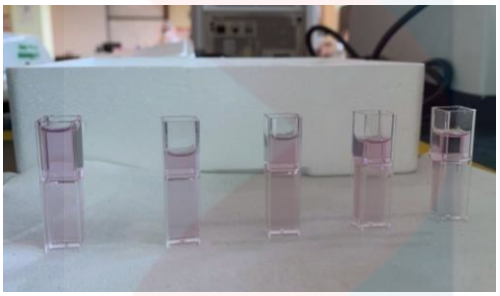




**APPENDIX B**

| FIGURE | DESCRIPTION |
|---|---|
|  | The analysis for ash content using muffle furnace |
|  | The desiccator used to cool the sample |
|  | Crucible used for ash analysis |
|  | Diluted Boric acid using hot plate for protein analysis |

| | |
|---|---|
|  | The analysis of moisture content using oven drying |
|  | The analysis of crude fat using Soxhlet Foss |
|  | Extraction of sample for antioxidant analysis |
|  | Precipitation of watermelon rind after centrifugation process. |

| | |
|---|---|
|  | Filtration process of sample extraction for antioxidant analysis |
|  | The sample in cuvette were placed in spectrophotometer. |