PHYTOCHEMICAL STUDIES OF *SESAMUM RADIATUM* SEEDS

by

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DECLARATION

I declare that this report entitled "Phytochemical studies of *Sesamum radiatum* 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 other degree.

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Date : 10th December 2018

APPROVAL

"I hereby declare that I have read this report and in our opinion this thesis is sufficient in terms of scope and quality for the award of the degree of Bachelor of Applied Science (Natural Resources Science) with Honors"

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Phytochemical studies of *Sesamum radiatum* seeds

ABSTRACT

Plant-based products widely growth in food and pharmaceutical industry in order to reduce prevalence ailment as it is become resilience to existing medicines. *Sesamum radiatum* is one of the traditional medicinal plant used in Africa and can be found Malaysia. Qualitative analysis of *S.radiatum* seeds revealed the presence of flavonoids, saponins, terpenoids and steroids in ethanolic extract. The aqueous extract simultaneously inhibits the presence of saponins and steroids. The results on quantitative analysis show *S.radiatum* seeds contain alkaloids and saponins with percentage content 36.70 % and 12.04 % respectively. However, UV Visible spectrophotometer was used to obtain a standard calibration curve to determine the concentration of tannins content (4.475 mg GAE/g), ascorbic acid (15.171 mg AAE/g), total phenolic content (9.187 mg GAE/g) and total flavonoid content (4.585 mg QE/g). For DPPH scavenging activity test, ascorbic acid act as positive control to compare with the *S.radiatum* seeds. The sample extracts inhibit 32 % of scavenging activity with IC_{50} value is 11.47 mg/L. *S.radiatum* seeds are suggested as a good sources of supplement based on phytochemicals presence in it.

Kajian Fitokimia Terhadap Benih *Sesamum radiatum*

ABSTRAK

Produk berasaskan tumbuhan semakin meluas digunakan dalam industri makanan dan farmaseutikal untuk mengawal penyakit kronik kerana ia menjadi kurang berkesan terhadap ubat-ubatan yang sedia ada. *Sesamum radiatum* adalah salah satu tanaman ubat tradisional yang digunakan di Afrika dan boleh ditemui di Malaysia. Kualitatif analisis benih *Sesamum radiatum* menunjukkan kehadiran flavonoid, saponin, terpenoid dan steroid dalam ekstrak etanol. Sementara itu, ekstrak daripada air didapati tidak menunjukkan kehadiran saponin dan steroid. Hasil kuantitatif analisis menunjukkan benih *Sesamum radiatum* mengandungi alkaloid dan saponin dengan kandungan peratusan masing-masing 36.70% dan 12.04%. Walau bagaimanapun, UV spektrofotometer digunakan untuk mendapatkan lengkuk penentuan piawai untuk menentukan kepekatan kandungan tanin (4.475 mg GAE / g), asid askorbik (15.171 mg AAE / g), jumlah kandungan fenolik (9.187 mg GAE / g) dan jumlah kandungan flavonoid (4.585 mg QE / g). Untuk ujian aktiviti penangkapan DPPH, asid askorbik bertindak sebagai kawalan positif untuk dibandingkan dengan biji *S.radiatum*. Ekstrak sampel menghalang 32% radikal bebas dengan nilai IC_{50} ialah 11.47 mg / L. Benih *Sesamum radiatum* dicadangkan sebagai sumber makanan yang baik sebagai supplemen berdasarkan kehadiran bahan fitokimia di dalam benih tersebut.

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LIST OF ABBREVIATIONS AND SYMBOLS

H_2SO_4	Sulphuric acid
NH_4OH	Ammonium hydroxide
CH_3COOH	Glacial Acetic acid
HCl	Hydrochloric acid
C_2H_5OH	Ethanol
DCIP	2,6-dichloroindophenol sodium salt hydrate
$C_6H_8O_6$	Ascorbic acid
$C_{10}H_5NaO_5S$	Follin-Ciocalteu' reagent
$C_{15}H_{10}O_7$	Quercetin
$C_6N_6FeK_4$	Potassium ferrocyanide
HO_3P	Metamosphoric acid
$FeCl_3$	Ferric chloride
$AlCl_3$	Aluminium Trichloride
$CHCL_3$	Chloroform
$C_4H_{10}O$	Diethyl ether
n-($C_4H_{10}O$)	n-butanol
NaCl	Sodium chloride
NaOH	Sodium hydroxide
$C_7H_6O_5H_2O$	Gallic acid
DPPH	1, 1-diphenyl-2-picryl-hydrazyl
g	gram
µ	Micro 10^{-6}
mM	millimoles
^oC	Degree Celcius
nm	Wavelength
%	Percentage
x	Multiply
Mins	Minutes
ml	millilitre
mg	milligram
mg/L	Milligram per litre

CHAPTER 1

INTRODUCTION

1.1 Background of study

Malaysia with tropical climate and abundant rainfall contribute over 30 000 plants species in 150 millions years history of Malaysia's rainforest. Malaysia also known as one of the mega diversity centre of the world (Hassan *et al*., 2013). Most of develop the countries use plant based medicines as the treatment curing illness. However, there are several countries that still used traditional or herbal plant to cure illness. Traditional medicines and dietary supplement continue to be used in the search for bioactive agents that maintain health and prevent disease.

As part of their adaption to the environment, plant accumulate large number of phytochemical. Phytochemical are simply bioactive plant substances that provide a health benefit (Delgoda *et al*., 2017). It is also known as naturally occur chemical compound in plants. Phytochemical analysis become popular among the scientist due to human health benefit. Somehow, many of these compound at one time considered antinutrient (Martínez & Paredes, 2014). The benefits of the therapeutic uses of medicinal plants in various ailment are their safety side effect caused by natural properties plus being economical, effective and the abundant availability.

Secondary metabolism are derived from primary metabolism through biosynthesis and basically have lower amount than primary metabolism in different plants (Delgoda *et al*., 2017). In addition, secondary metabolism are produced in specialized cell and at different development phases. Furthermore, secondary

metabolism in plants are essential for biological and pharmacological responses. According to Stanković (2011), there are several diseases for example cancer and Alzheimer disease severed by human due to higher content of free radicals in the body.

1.2 Problem statement

Nowadays, more researches have been done conducted for beneficial or non-beneficial plants as well, because it is crucial to have new and enhanced formulation to act protection for body systems. So, it is necessary to investigate new drugs to cure disease as it is become more resistant to the existing medicines. Therefore, it is possible to find chemical constituents in *Sesamum radiatum* seeds as natural products from plants. However, there are no data reported on phytochemical studies of *Sesamum radiatum* seeds. There is a need to explore the potential chemical elements of *Sesamum radiatum* seeds that have high medicinal values.

1.3 Objectives

i. To analyse the presence of flavonoids, saponins, tannins, glycosides, phlobatannins, terpenoids and steroids in *Sesamum radiatum* seeds.
ii. To determine the alkaloids, tannins, total flavonoid content (TFC), total phenolic content (TPC) and Ascorbic acid in *Sesamum radiatum* seeds.

1.4 Scope of Study

This research focused on identifying the phytochemicals of *Sesamum radiatum* seeds. The seeds was extracted with two solvents which are distilled water and ethanol. Seeds of *Sesamum radiatum* was analysed to test the presence of chemical compounds for qualitative analysis that observed the colour change. The seeds extracts also was

determined using chemical test and UV-visible spectrophotometer for quantitative analysis. Therefore, 1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity conducted to show the potential antioxidants in seeds.

1.5 Significant of Study

Previous research showed phytochemicals that found in any seeds have a lot of benefits for human consumption. So, the phytochemical studies of *Sesamum radiatum* seeds was tested. The significance of the study is to provide empirical information about *Sesamum radiatum* seeds. In the future research, phytochemical properties of this seeds can be developed in the sector of pharmaceutical, cosmetic, food and nutraceutical products for commercial purposes

CHAPTER 2

LITERATURE REVIEW

2.1 Health Benefit of Sesame Seeds

Sesame seeds (*Sesamum indicum*) either black or white was found in rich assortment of minerals and essential oil. One of the mineral is copper that provides relief for Rheumatoid Arthritis. Copper is known for its use in reducing some of the pain and swelling of rheumatoid arthritis (Das *et al*., 2017). In addition, copper plays an important role in the activity of glycosidases, an enzyme needed for cross-liking of collagen and elastic the substances that provide structure, strength and elasticity to blood vessels, bones and joints (Langley *et al*., 2013).

Phytosterols of sesame seeds can lower cholesterol in human body. Phytosterols are compound found in plants that have a chemical structure very similar to cholesterols (Leone *et al*., 2016). The present of phytosterols in the diet in sufficient amount, are believed to reduce blood level of cholesterol, enhance the immune response and decrease the risk of certain cancers. According to Langley *et al*., 2013, phytosterols also act as an antioxidant that have a special element called sesame-lignin to helps rid of free radicals that cause aging and cancer, including fatty acid production.

Sesame oil is made from sesame seeds and proves to be a rich source of polyunsaturated fats, monounsaturated fats, antioxidant and several vitamin and minerals (Qadir *et al*., 2017). Sesame seeds and sesame oil rich in antioxidant. Antioxidants counter the effects of molecules in the body that damage cells and accelerate the aging process, including microbes.

2.2 Phytochemicals

Phytochemicals are a powerful group of chemicals that are derived from natural resource, especially with plants origin either from roots, stems, leave, flowers and seeds (Faridi, 2015). In present day, phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism (Saxena *et al*., 2013). Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignin, plant steroids, curcumins, saponins, phenolics, flavonoids and glucosides.

Flavonoids are polyphenol exacerbates that are pervasive in nature. Flavonoids have been perceived, a considerable lot of which happen in vegetables, leafy foods like tea, espresso and natural product drinks. The flavonoids seem to have assumed a noteworthy job in fruitful therapeutic medicines of old occasions, and their utilization has held on up to now. Flavonoids that gather in most plant seeds and are engaged with physiological capacities, for example, lethargy or practicality (Blasa *et al*., 2010).

In addition, vitamin C and E, β-carotene and α-tocopherol presence in plants shows in sign of antioxidant properties. Previous researchers had found that phenolic content has strong relationship with antioxidant activities (Rahman *et al*., 2016; Ivanova *et al*., 2005). Polyphenolic compound such as flavonoids are safe and toxic free. High dietary intake of natural phenolics is reduced risk of developing some chronic diseases, various types of cancer, diabetes, obesity, improved endothelial function and reduced blood pressure (Farasat et al., 2013).

2.3 Pedaliaceae Family

The Pedaliaceae is a small family of around 12 or 13 genera and 100 species of dicotyledonous flowering herbs and shrubs from sub-tropical to tropical desert and

coastal habitats. Flowers often have a tubular form with prominent lips. Stems and leaves often carry mucilaginous hairs and often bear fruit capsules or nuts with hooks, spikes or wings which are designed to catch on and attach to passing animals as a method of distributing the seed over substantial distances (Kiew., 2010).

One genus, *Sesamum*, with two naturalised in Peninsular Malaysia. According to Kiew (2010), Pedaliaceae family can be found mainly in dry region such as wasteland or on sandy beaches. Most of plant from this family used to cure disease such as *Sesamum indicum*. *Sesamum indicum* widely used in food industry in order to extract essential oil for diet supplement. Every plant have their own active compounds that can be harmful or beneficial to human health (Shamsizadeh *et al*., 2017) . Previous research found that fruit of *Pedalium murex* one of the member family Pedaliaceae contain high polyphenolics (flavonoids and phenolics) that act as antioxidant as it is important for human health (Patel *et al*., 2011).

2.3.1 *Sesamum radiatum*

Sesamum radiatum is a flowering plant that belongs to the family Pedaliaceae and is the same genus as sesame (*Sesamum indicum*), known in Malay as bijan. *Sesamum* is derived from the Greek word sesamon while radiatum is coined from the Latin word radiate meaning radiating which refers to the straight ribs that radiate from the centre of the seed. The species has its origins in Africa. In Malaysia, this species was first recorded in 1885 from Melaka. It appears to have been naturalized, as has occurred with *Sesamum indicum* throughout Peninsular Malaysia (Kiew, 2010). Commonly known as Black sesame, Black benniseed and English wild benniseed. *Sesamum radiatum* plant as shown as Figure 2.1.

Figure 2.1: The *Sesamum radiatum* with it matured fruit

Based on previous study, *Sesamum radiatum* have chemically characterising the gum to provide relevant structural information. In addition, the physical properties of the hydrated gum were also investigated at concentrations comparable to those encountered with other similar polysaccharides in food and pharmaceutical applications (Nep *et al*., 2016). Furthermore, *Sesamum radiatum* leaves can healing for some infection disease from bacteria and yeast. The antimicrobial effectiveness of locally consumed *Sesame* leaves extracts (Bankole *et al*,. 2007). This showed that, there were have active compounds either in *Sesamum radiatum* leaves, stems or seeds. The sample seeds of *Sesamum radiatum* as show as Figure 2.2.

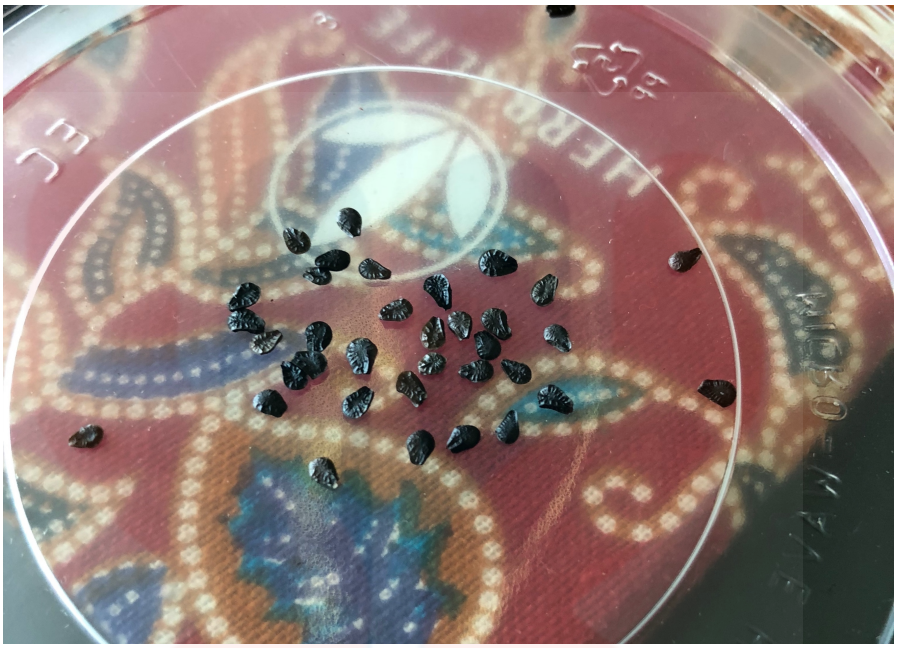

Figure 2.2: *Sesamum radiatum* seeds

2.4 Solvent Extraction

The extraction of secondary metabolite compounds such as phytochemicals from plant materials is the initial step in the utilization of phytochemicals in the preparation of supplements or nutraceuticals, food ingredients, pharmaceutical and cosmetic products (Oshadie *et al*., 2017). Before extraction, plant sample are treated by drying, milling, grind and homogenization, may be preceded by air-drying or freeze-drying.

According to Oshadie *et al*., 2017, freeze-drying retains higher levels of phenolics content in plant samples compare to air-drying. However, drying processes can cause undesirable effects on the constituent profiles of plant sample (Dai *et al*., 2010). Therefore, caution should be taken when planning and analysing research studies on the medicinal properties of plant.

The most commonly used extraction for the procedure to prepare extracts from plant materials is solvent extraction due to their ease of use, efficiency and wide applicability. The solubility of phytochemicals is governed by the chemical nature of

the plant sample, as well as the polarity of the solvent used (Altemimi *et al*., 2017). A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield.

The polarity, from least polar to most polar. Moreover, phytochemicals also be associated with other plant components such as carbohydrates and proteins. A mixture of phytochemicals soluble in the solvent will be extracted from plant materials. It is also contain some non-phytochemicals compounds such as sugar, organic acids and fats during the extraction. Solvent such as methanol, ethanol, acetone, ethyl acetate and their combination have been used for the extraction of phytochemicals from plant materials, often with different proportions of water because of differences in polarity the solvent had.

Selecting the right solvent affects the amount and rate of phenols extracted (Butsat & Siriamornpun, 2016). In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavonoids are better extracted with aqueous acetone. Ethanol is another good solvent for flavonoids extraction and is safe for human consumption (Do *et al*., 2014). In preparing plant extraction, most commonly methanol or ethanol, is used.

CHAPTER 3

MATERIALS AND METHODS

3.1 Plant Collection

Sesamum radiatum seeds were collected around Universiti Malaysia Kelantan Jeli Campus. The matured fruits were collected from its plant and dried to collect the seeds. The dried seeds kept in desiccator silica gel for further use.

3.2 Chemicals

Sulphuric acid (H_2SO_4), Ammonium hydroxide (NH_4OH), Glacial Acetic acid (CH_3COOH), Hydrochloric acid (HCl), Ethanol (C_2H_5OH), 2,6-dichloroindophenol sodium salt hydrate (DCIP) and Ascorbic acid ($C_6H_8O_6$) were obtained from HmbG Chemicals (Malaysia). Follin-Ciocalteu' reagent ($C_{10}H_5NaO_5S$), Quercetin ($C_{15}H_{10}O_7$), Potassium ferrocyanide ($C_6N_6FeK_4$), and Metamosphoric acid (HO_3P) were purchased from Merck Germany. Ferric chloride ($FeCl_3$) and Aluminium Trichloride ($AlCl_3$) were purchased from Bendosen (Malaysia). Chloroform ($CHCL_3$), Diethyl ether ($C_4H_{10}O$), n-butanol n-($C_4H_{10}O$), Sodium chloride (NaCl), Sodium hydroxide (NaOH), and Gallic acid ($C_7H_6O_5H_2O$) were purchased from R&M Chemicals (Malaysia). The stable free radical 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Sigma Aldrich Corporation. All other chemicals used were of analytical grade.

3.3 Equipment and Apparatus

Hach DR 6000 UV Visible Spectrophotometer, mechanical shaker, mortar and pestle, magnetic stirrer and electronic balance.

3.4 Preparation of Seeds Powder

The seeds were washed carefully by using tap water and rinsed. The seeds were dried in an oven at a temperature of 60 ^{o}C for one day. After drying process, the dried seeds were crushed by using mortar and pestle. The seeds crushed until fine in powder form. The powder was stored in desiccator silica gel to remove extra moisture and for further use.

3.5 Preparation of Plant Extract

20 g of powdered sample was soaked in 100 ml of distilled water and ethanol separately for 24 hours at 40 ^{o}C. The extracts were filtered with filter paper and stored at room temperature (21 ^{o}C).

3.6 Qualitative Analysis of *Sesamum radiatum* Seeds

Qualitative analysis was carried out to identify the chemical compounds that followed the procedure by Khanam *et al*., (2015) that used aqueous and ethanolic extracts.

3.6.1 Test for Flavonoids

5 ml of each extracts were added with few drops of sodium hydroxide. The yellow colour was appeared indicated the presence of flavonoids.

3.6.2 Test for Saponins

5 g of powdered sample was added into 50 ml of ethanol and distilled water separately and boiled both samples in water bath at 60 ^{o}C. 10 ml of ethanolic extract and aqueous extract were added 5 ml of distilled water each. The both mixtures were shaken to form a stable froth. The formation of froth indicated the presence of saponins.

3.6.3 Test for Tannins

2 g of powdered sample was added into 20 ml of ethanol and distilled water separately and boiled the both samples. 5 ml of each filtrate extracts were added with 0.1 % of ferric chloride drop wisely. The colour changes to blue black coloration show the presence of tannins.

3.6.4 Test for Cardiac Glycosides

5 ml of ethanolic and aqueous extract each were added with 2 ml of glacial acetic acid. Then, few drops 0.1 % of ferric chloride was added into each solution. The appearance of brown ring while the acetic layer forms a greenish ring that indicted the presence of cardiac glycoside.

3.6.5 Test for Phlobatannins

5 ml of ethanolic and aqueous extract separately boiled with 2.5 ml of 1 % hydrochloric acid each. The deposit of red precipitate that indicated the presence of phlobatannins.

3.6.6 Test for Terpenoids

5 ml of each ethanolic and aqueous extract were mixed with 2 ml of chloroform and carefully added 2 ml concentrated sulphuric acid to form a layer. The appearance of a reddish brown coloration show the presence of terpenoids.

3.6.7 Test for Steroids

5 ml of each ethanolic and aqueous extract were added with 2 ml of acetic anhydride and 2 ml concentrated sulphuric acid carefully added. The formation of dark brown ring indicated the presence of steroids.

3.7 Quantitative Analysis of *Sesamum radiatum* Seeds

3.7.1 Determination of Alkaloids Content

Determination of alkaloids content followed the procedure by Ezeonu *et al*., (2016). 5 g of powdered sample was weighted and transfer into 500 ml beaker that contain 200 ml of 10% acetic acid in ethanol will be added and allowed to stand for several hours. The extract was filtered and then concentrated on a water bath. The concentrated ammonium hydroxide was added drop wise to the extract until the precipitation form immediately. The supernatant was discarded, and then the precipitate was washed with dilute ammonium hydroxide and filtered. The residue was dried and weighted. The alkaloids content was calculated in percentage by using Equation 3.1.

$$\text{Percentage of Alkaloids (\%)} = \frac{\text{Weight of Alkaloid (g)}}{\text{Weight of Sample(g)}} \text{ X } 100\ \% \qquad (3.1)$$

3.7.2 Determination of Saponins Content

Quantitative analysis for determination of saponins will be carried out using the method reported by Ejikeme *et al*., (2014). 20 g of powdered sample was weighted into 500 ml of conical flask and 100 ml of 20 % aqueous ethanol was added. The mixture was heated over a hot water bath for several hours with continuous stirring at a temperature of 55°C. The mixture was filtered, and the residue was re-extracted with 200 ml of 20 % aqueous ethanol until reduced to 40 ml and then transfer the mixture into 200 ml separator tunnel. 20 ml of diethyl ether was mixed into separatory tunnel and shaken. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated. To this purified, 60 ml of aqueous n-butanol was added. The extract was washed twice with 55 % of aqueous sodium chloride. The remaining solution will be heated in a water bath for 30 minutes. Then, the solution was transferred into a crucible and was dried in an oven to obtain a constant weight. The saponins content was calculated in percentage using Equation 3.2.

$$\text{Percentage of Saponin (\%)} = \frac{\text{Weight of Saponin (g)}}{\text{Weight of Sample(g)}} \text{ X } 100\ \% \qquad (3.2)$$

3.7.3 Determination of Tannins Content

Determination of tannins content was carried out following method by (Agoreyo *et al*., 2017). 0.5 g of powdered sample was weighted into 50 ml of beaker and 20 ml of distilled water was added. Then, shaken for 1 hour in a mechanical shaker. Then, the extract was filtered into a 50 ml of volumetric flask and made up to the mark. 100 µl of sample extract was pipette into a falcon tube and mixed with 100 µl of 0.1 M ferric chloride, 0.1 N hydrochloride acid and 100 µl of 8 mM potassium ferrocyanide. 2.5 ml of distilled water was added into the mixture. The absorbance was measured at 720 nm within 10 minutes. The sample was compared with standard

calibration curve using gallic acid at different concentration (10,20,30,40,50 mg/L) concentration. The result was expressed in term of mg GAE per gram of dry weight of sample. The tannins content was determined from Equation 3.3.

$$\text{Tannin Content (mg/L)} = \frac{C\left(\frac{mg}{L}\right) x V(L)}{M(g)} \quad (3.3)$$

C: Calculated concentration of gallic acid (mg/L)

V: Volume of solvent used (L)

M: Mass of powdered sample

3.7.4 Determination of Total Phenolic Content (TPC)

The total phenolic content in seed extracts was determined using spectrophotometric method that followed the procedure Rebaya *et al*., (2014) with slight modification. 100 µl of extract solution was added into 100 µl of 10 % Follin-Ciocalteau (FC) reagent. After 8 minute, 300 µl of 7.5% sodium hydrogen carbonate ($NaHCO_3$) was added. The mixture was shaken and allowed to stand in the dark at 45 ^{o}C for 1 hour. The absorbance was measured at 765 nm. A calibration curve was prepared with gallic acid as a standard at various concentrations (10, 20, 30,40 and 50 mg/L). The total phenolic content was expressed in terms of mg of GAE/g of dry sample. The total phenolic content was obtained from the given Equation 3.4.

$$\text{Total Phenolic Content (mg/L)} = \frac{C\left(\frac{mg}{L}\right) x V(L)}{M(g)} \quad (3.4)$$

3.7.5 Determination of Total Flavonoid Content (TFC)

The total flavonoid content will be determined by aluminium trichloride method using catechin as reference compound (Rebaya *et al*., 2014) with slight modification. 100 µl seed extract was mixed with 300 µl of 5 % sodium nitrite

($NaNO_2$) solution. After 5 minutes, 300 µl of 10 % aluminium trichloride ($AlCl_3$) solution was added. After 5 min, 2 ml of 1M sodium hydroxide (NaOH) solution. The mixture was shaken for 10 seconds. The absorbance was measured at 510 nm by using UV-visible spectrophotometer. A calibration curve was prepared with quercetin as a standard at various concentrations (10, 20, 30, 40 and 50 mg/L). The total flavonoids content was expressed as mg QE/g of dry sample. The total flavonoid content was obtained from the given Equation 3.5.

$$\text{Total Flavonoid Content (mg/L)} = \frac{\text{C}\left(\frac{\text{mg}}{\text{L}}\right)\text{xV(L)}}{\text{M(g)}} \quad (3.5)$$

3.7.6 Determination of Ascorbic Acid Content (Vitamin C)

Determination of ascorbic acid content was followed the procedure (Okeri & Alonge., 2006). 50 mg of powdered sample was weighted and extracted with 10 ml of distilled water. Then, undergoes re-extraction with 300 µl of 3% metamosphoric acid and allowed to stand for 45 minutes at room temperature. The mixture was filtered. 100 µl of filtered solution was mixed with 900 µl of 50µmol/L 2,6 dichloroindophenol sodium salt hydrate. The absorbance was measured with 30 minutes at 515 nm. Ascorbic acid (vitamin C) content was calculated based on the calibration curve of L-ascorbic acid. The result was expressed as mg AAE/g of dry sample. The formula as shown Equation 3.6.

$$\text{Ascorbic Content (mg/L)} = \frac{\text{C}\left(\frac{\text{mg}}{\text{L}}\right)\text{xV(L)}}{\text{M(g)}} \quad (3.6)$$

3.7.7 1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity

The ability of extract scavengers the DPPH radicals was followed the method by Aksoy *et al*., (2013). 200 µM of DPPH solution in methanol was prepared and

added 500 µl of mixture solution into the seeds extract with different concentration (0.001, 0.01, 0.1,1,10,100 mg/L). The mixture was stored in the dark for 30 min at room temperature. Control sample was prepared without the extracts. The absorbance was measured at 517 nm. The result was compared with the standard antioxidants which is ascorbic acid. All the measurements were taken three times to calculate the means. The IC_{50} value was calculated using the linear regression analysis. Decreasing in IC_{50} value show the strong antioxidant capacity. The ability of DPPH radical scavenging activity was determined using the formula given in Equation 3.7. A_0 is the absorbance of control and A_1 id the absorbance of the extract.

$$\text{DPPH Scavenging Effect (\% of inhibition)} = \frac{(A0-A1)}{A0} \times 100 \quad (3.7)$$

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Qualitative Analysis of *Sesamum radiatum* Seeds

The qualitative analysis of *Sesamum radiatum* was carried out by observing the colour changes but the test of saponin use the froth test to detect the chemical compound in seeds. The chemical compounds present in different solvent used, ethanolic and aqueous extract are shown as given Table 4.1.

Table 4. 1: The qualitative analysis of *Sesamum radiatum* seeds

Chemical Compounds	Ethanolic Extract	Aqueous Extract
Flavonoids	+	+
Saponins	+	-
Tannins	-	-
Cardiac Glycosides	-	-
Phlobatannins	-	-
Terpenoids	+	+
Steroids	+	-

Keys:

(+) indicate presence

(-) indicate absent

The results of qualitative analysis revealed the flavonoids and terpenoids compound in both extract while the presence saponins and steroids were found only in ethanolic extract. This show the ethanolic extract higher reactivity compare to aqueous extract to detect phytochemical compounds in plant. According to Shade *et al*., (2014), ethanolic extract can be allocated to detect high quantities of polyphenols that more efficient in seeds degradation and cell wall of plants. One of characteristic of ethanol was unipolar that released polyphenols from the cells. The less reactivity in aqueous extract can be attributed to the polyphenol oxidase (enzymes) that reduce the amounts of polyphenol in water as ethanol was inactive.

In addition, different concentrations of solvents used, and the time of the extraction can affect the extraction. Based on research conducted by Lapornik *et al*., (2005), the contents in aqueous extract decreased with longer time to extract while the contents in ethanolic extract increased with the time. In this study, distilled water was used to extract that show less effective solvent compare to ethanol in extracting the bioactive compounds in *Sesamum radiatum* seeds. The differences in solubility of the compounds in the selected solvent can affect in the different solvent that has the variations in extractive value (Qadir *et al*., 2015).

Based on Table 4.1, the saponins and steroids compounds were not detected in aqueous extract. Similar studies conducted by Vijay *et al*., (2015), phytochemical content in *Sesamum indicum* seeds (black sesame) show the negative results on saponins, flavonoids and steroids in aqueous extract. However, this divergent results of other experiment where the presence of flavonoids was detected in *Sesamum indicum* (black and white sesame). In addition, most of this study gave positive result on primary metabolites such as carbohydrates, protein, fat or oils and volatile oils (Patil *et al*., 2015).

This experiment used simple extraction method which was soaked with distilled water and ethanol for 24 hours at 40 ° C. The extracts was revealed several chemical constituents in seeds powdered which were flavonoids, terpenoids, saponins and steroids in ethanolic extract while saponins and steroids absent in aqueous extract. Similar experiment conducted by Santhi & Sengottuvel (2016) that used Soxhlet extraction and methanol as a solvent to extract *Moringa concanensis* seeds. The result was detected the presence of flavonoids and other primary metabolites compounds such as protein and carbohydrate.

Dhanani *et al*., (2017) reported that bioactive compounds in plants occurred in low concentrations. Apart of that, the selected extraction methods used gave affect to obtain extracts with high extraction yield and the extracts required with optimal changes. Hence, the suitable extraction technique and solvent selected that based on sample properties and the efficiency of the sample matrix (Oshadie *et al*., 2017). Furthermore, Sani *et al*., (2013) conducted the phytochemicals composition of white *Sesamum indicum* seeds oils. The results show the presence of flavonoid, tannin, saponin, terpenoid and steroids which show the intensity of colour change. Terpenoids and steroid have a high concentration in white sesame seeds oils. This show chemicals compound can be detected especially on secondary metabolites if their oils extracted from sesame seeds for the best result compared to seeds powder itself.

This can justify the report by Shao *et al*., (2012) result indicated that the majority of the oil was extracted as the particle size had a great influence on extraction. Fundamentally, smaller particle size means a shorter mass transfer distance and larger resolve surface area, which ultimately reduces the extraction time and increases the extraction efficiency, because the smaller particle size significantly increased extraction rate.

4.2 Quantitative Analysis of *Sesamum radiatum* Seeds

4.2.1 Determination of Alkaloids and Saponins Content

The results for determination of alkaloids and saponins content were expressed in term of percentage. Based on Table 4.2, the alkaloids and saponins content were 36.7 % and 12.04 % respectively. The alkaloid and saponin content higher in *Sesamum radiatum* compare to Tamarind (*Tamarindus indica*) nut seeds, where the alkaloids content was 3.4 % while saponin content was 1.0 % (Akajiaku *et al*., 2014).

Table 4. 2: Percentage of alkaloids and saponins of *Sesamum radiatum* seeds

Phytochemical Compounds	Sample (g)	Residue (g)	Percentage of Residue (%)
Alkaloids	5	1.835	36.7
Saponins	20	2.407	12.04

The alkaloid content is higher compare to saponin content as shown as Table 4.2. This is due to the solvent that use to extract the bioactive compounds. In detection of saponin, the solvent used to ethanol which less efficient in the extraction. According to Boeing *et al*., (2014), ethanol has low solvation that gave result in a low solvation of antioxidant molecule.

According to Debnath *et al*., (2018), most alkaloids are toxic if the concentration is high, which are used by plants to protect themselves against the aggression from other organisms, and this instinct action is an important ecological function. However, alkaloids one of the effective compound in most medicinal plants, as to cure diseases in traditional herbal medicines.

4.2.2 Determination of Tannin Content

Determination of tannin content in *Sesamum radiatum* was determined by standard calibration curve as shown as Figure 4.1. The graph shows the different concentration of gallic acid again absorbance at 720 nm. The concentration of tannin was calculated by using standard curve that obtained from absorbance of gallic acid solution. The tannin content was expressed in term of mg GAE/g. Based on Table 4.3, show the result of tannin content in *Sesamum radiatum* seeds is 4.475 mg GAE/g. Correspondingly, based on Table 4.1 (qualitative analysis result) show the negative result of tannin in ethanolic extract and aqueous extract.

Table 4. 3: Quantitative analysis of *Sesamum radiatum* seeds

	Standard Unit Equivalent	**Absorbance**	**Concentration (mg/L)**	**mg/g**
Tannin Content	GAE	0.146	4.475	4.475
Total Phenolic Content (TPC)	GAE	0.073	9.187	9.187
Total Flavonoid Content (TFC)	QE	0.007	4.585	4.585
Ascorbic Acid (Vitamin C)	AAE	0.045	15.171	15.171

According to reported by Dhull *et al*., (2016), Marwa (*Origanum majorana*) seeds that extract in acetone contain 6.02 mg GAE/g of tannin which is higher compare to *Sesamum radiatum* seeds.

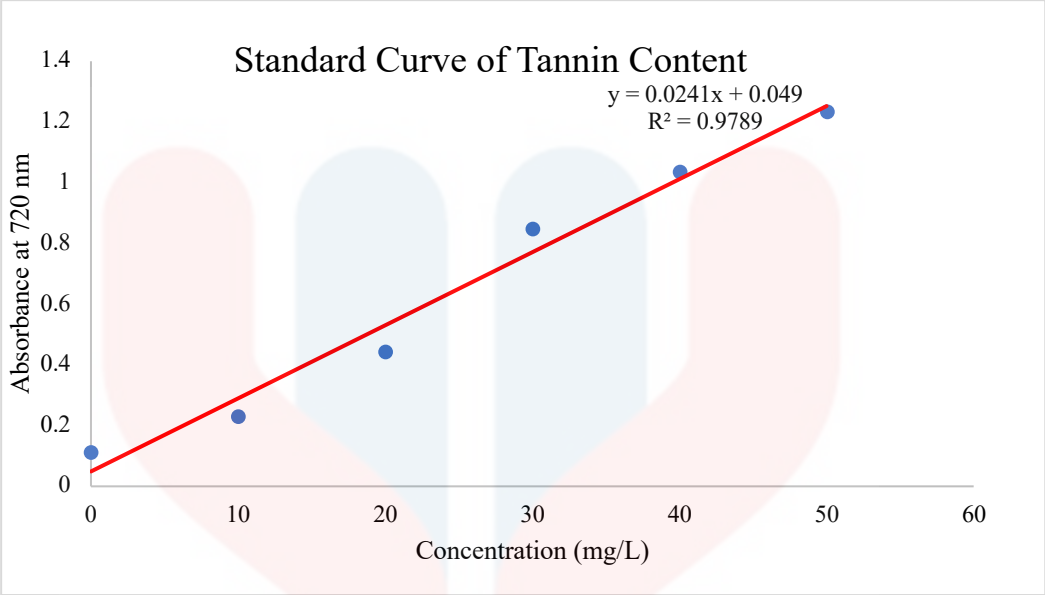

Figure 4.1: Standard Calibration Curve of Gallic Acid

However, *Origanum majorana* seeds also show negative result on the presence of tannin in ethanolic extract Dhull *et al*., (2016). The group of hydroxyl substitution naturally occur in plant caused of the presence of tannin. The dietary supplements containing *Sesamum radiatum* seeds that rich in hydrolysable and condensed tannins, the consumption of which, may provide health benefits.

4.2.3 Determination of Total Phenolic Content (TPC)

Total phenolic content was determined by Follin-Ciocalteu reagent method. The mechanism of Follin-Ciocalteu reagent method based on reduction and oxidation reaction where phenolic group being oxidized and reduced the ion (Singleton *et al*., 1999). In the presence of phenol, the blue colour produced which absorb light 765 nm. This reaction is slow at acidic solution and faster in basic solution, therefore sodium carbonate was added to increase the reaction.

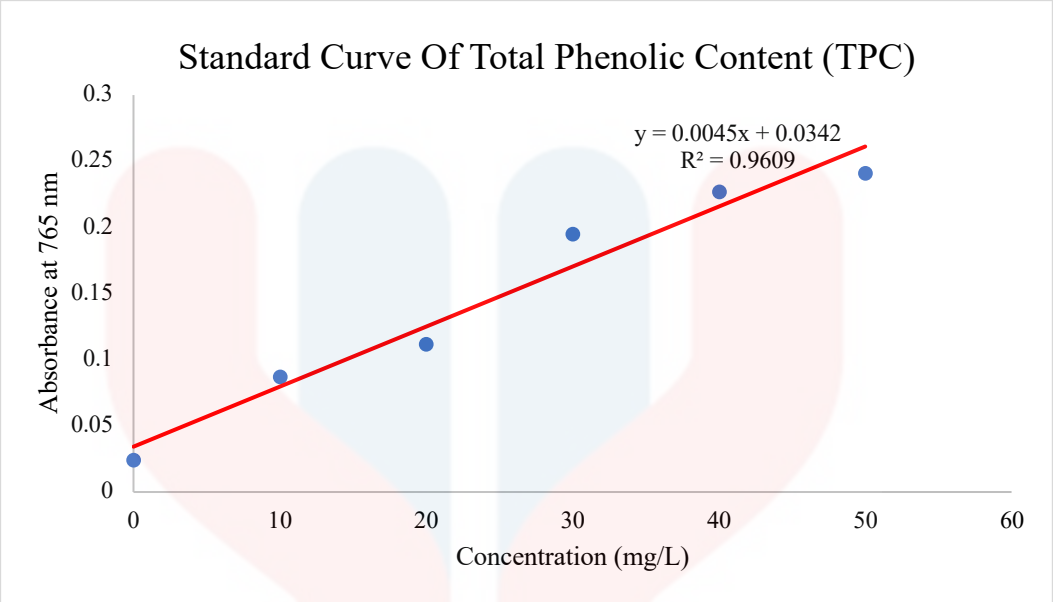

Figure 4.2: Standard Calibration Curve of Gallic Acid

Total phenolic content in *Sesamum radiatum* seeds was determined by using standard calibration curve. The curve was plotted with different concentration gallic acid against absorbance at 765 nm. Based on Table 4.3, total phenolic content in *Sesamum radiatum* seeds is 9.187 mg GAE/g. Compare to *Salvia hispanic*a L (chia seeds), contain 1.6398 mg GAE/g which is lower phenolic content (Martínez & Paredes, 2014). However, the result TPC of watermelon (red pulp) was 10.32 mg GAE/g which is almost similar to *Sesamum radiatum* seeds (Chen *et al*., 2014). Similar studies conducted by Bopitiya & Madhujith, (2013), show the result TPC was 26 mg GAE/g in *Sesamum indicum* seeds oil which is higher compare to *Sesamum radiatum* seeds. However, *Sesamum radiatum* seeds have potential as dietary supplement.

4.2.4 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined with aluminium chloride assay method by using UV visible spectrophotometer. The mechanism of aluminium chloride method is based on the stability of acid formation by aluminium chloride with C4-keto

group or C-5 hydroxyl group of flavanols and flavones. The aluminium chloride with orth-dihydroxyl formed acid labile complexes as the ring of flavonoids (Hassan *et al*., 2013). The standard calibration curve was obtained from the different concentration of quercetin against absorbance at 510 nm (Figure 4.3).

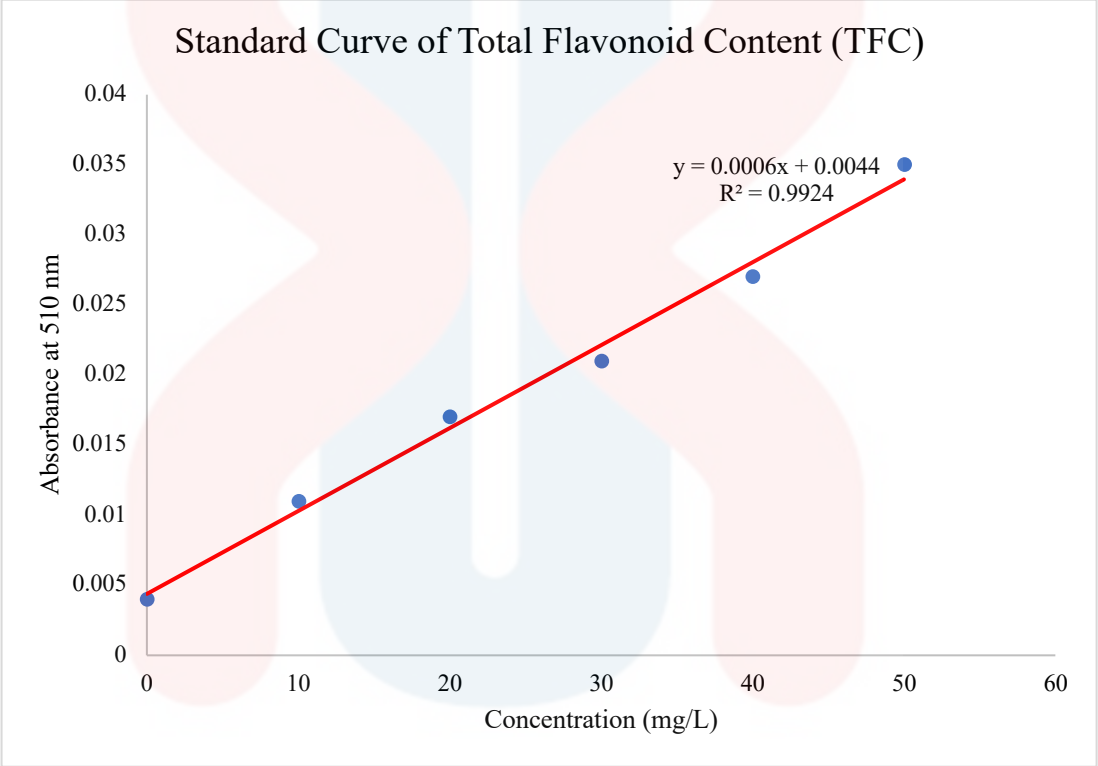

Figure 4.3: Standard Calibration Curve of Quercetin

The total flavonoid content of the *Sesamum radiatum* seeds was calculated by using the standard curve and was expressed in term of mg QE/g. The result of total flavonoid content in *Sesamum radiatum* seeds is 4.585 mg QE/g. The result of this show the similar result with flesh of *Garcinia parvifolia* (5.9 mg QE/g) and peel of *Garcinia parvifolia* (3.7 mg QE/g) in 80% methanol extract. Correspondingly to the flesh of *Garcinia parvifolia* (2.2 mg QE/g) and peel of *Garcinia parvifolia* (2.1 mg QE/g) in aqueous extract which is lower compare to *Sesamum radiatum* seeds (Hassan *et al*., 2013). Based on Table 4.1, the qualitative analysis of *Sesamum radiatum* seeds

show the positive result of the presence of flavonoids in both extract. It is possible to do future study on isolation and characterisation of flavonoids in *Sesamum radiatum* seeds.

According to Patel *et al*., (2011), flavonoids are one class of secondary plant metabolites that are also known as Vitamin P. These metabolites are mostly used in plants to produce yellow and other pigments which play an important role in the colours of plants. In addition, Flavonoids are readily ingested by humans and they seem to display important anti-inflammatory, anti-allergic and anti-cancer activity.

4.2.5 Determination of Ascorbic Acid (Vitamin C) Content

Determination of ascorbic acid also known as vitamin C content was carried out by using spectrophotometric method. The ascorbic acid content was expressed in term of mg AAE/g. The curve was plotted with different concentrations of ascorbic acid against absorbance at 515 nm. The ascorbic acid content in *Sesamum radiatum* seeds was calculated by linear equation (Figure 4.4). Based on Figure 4.4, the standard curve of ascorbic acid decreasing in absorbance as the concentrations of ascorbic acid increasing. This reaction indicated the reduction of pink colour dye to colorless by ascorbic acid. This is due to the reduction of DPPH in methanol solution where, a hydrogen was received antioxidant to form 2,2-diphenyl-1- picrylhydrazine (DPPH-H) (Shrestha *et al*., 2016).

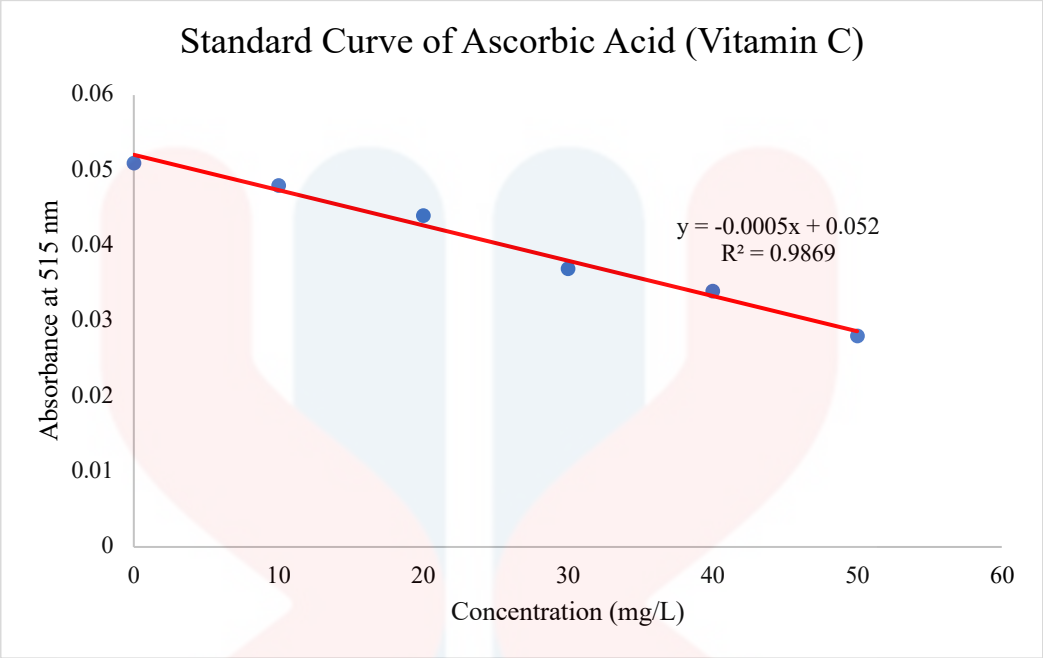

Figure 4.4: Standard Calibration Curve of Ascorbic Acid

Based on Table 4.2, ascorbic acid content in *Sesamum radiatum* seeds show the highest results compare to other bioactive compounds. Ascorbic acid content found in *Sesamum radiatum* seeds is 15.171 mg AAE/g. High ascorbic acid content in *Sesamum radiatum* seeds have ability act as antioxidant that can consume by human as dietary supplement. However, the result of *Sesamum radiatum* seeds lower ascorbic acid content compare to other herbal plant such as *Oldenlandia corymbosa* (49.27 mg AAE/g) and *Dissotis rotundifolia* (41mg AAE/g) (Okeri, & Alonge., 2006). In addition, citron fruit have similar ascorbic acid content which is 20.32 mg AAE/g (Shrestha *et al*., 2016).

This perhaps due to interference of protein in *Sesamum radiatum* seeds extract. By adding meta phosphoric acid act as deproteinizing agent that can stabilized the seeds extract. Cited from Locato *et al*., (2013), high oxidation of ascorbic acid due to catalyst that increase the oxidation process that can lower the ascorbic content of seeds extract.

4.2.6 DPPH Radical Scavenging Activity

Antioxidant activity was determined by DPPH radical scavenging which utilize 1-Diphenyl-2-picryl-hydrazyl (DPPH) that stable the free radical in plant extract. Based on Appendix B, show the result of absorbance decreasing as the concentration increasing. The antioxidant activity of *Sesamum radiatum* seeds was expressed in term of percentage of inhibition (%) and IC_{50} value. Ascorbic acid act as the standard (Figure 4.5).

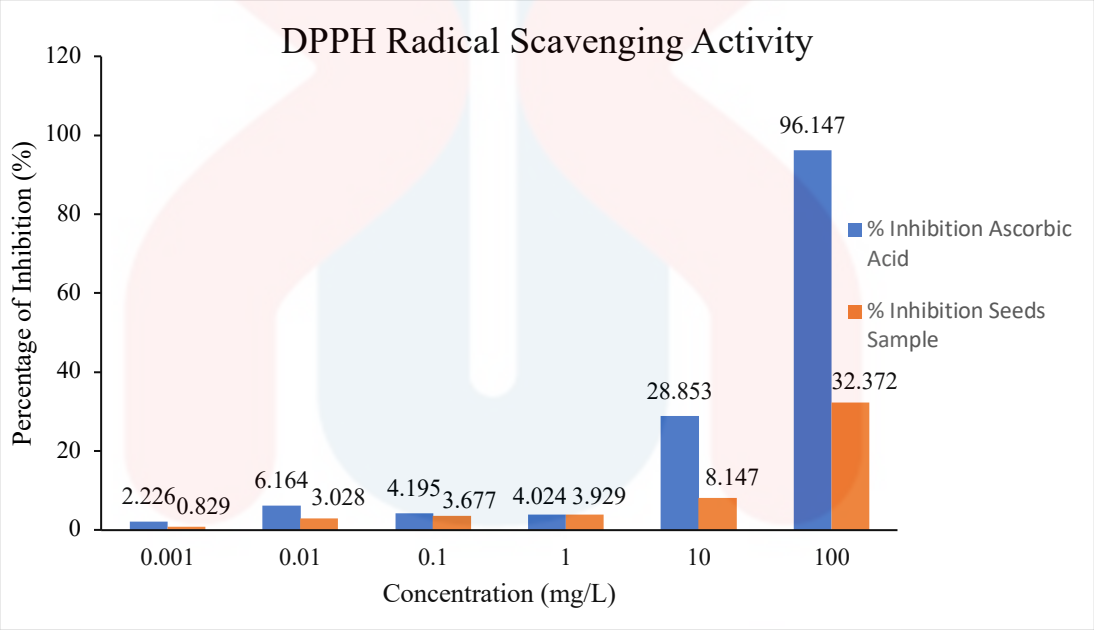

Figure 4.5: The Bar Chart of DPPH Radical Scavenging Activity

Based on Figure 4.5, the *Sesamum radiatum* seeds exhibited low radical scavenging activity compare to ascorbic acid. The minimum percentage of inhibition for seeds extract is at 0.001 mg/L which is 0.83 % while the maximum percentage is at 100 mg/L is 32.37 % and the percentage of inhibition of ascorbic acid is 96 %.

The IC_{50} value of seeds extract is slightly higher compare to standard (Appendix B). The plant required 11.47 mg/L of seed extract to scavenge 50 % of DPPH radical. High antioxidant properties of seeds extract will have low IC_{50} value.

Ascorbic acid required 5.22 mg/L which is half of the seeds extract. This show *S. radiatum* seeds contain low antioxidant potential compare to the ascorbic acid. The result of IC_{50} value slight similar to the studies from Kanta *et al*., (2013) in *Olax psittacorum* (27.56 mg/L). Hence, antioxidant and polyphenols content in seeds might affect from temperature, pH and the condition of soil that caused reducing of nutritional value of the plant.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Phytochemical studies of *Sesamum radiatum* seeds was analyzed the bioactive compounds that naturally occurs. Qualitative analysis shows the presence of flavonoid, terpenoid, saponin and steroids in *Sesamum radiatum* seeds that may give benefitable to living organism in promoting healthy dietary styles. The ethanolic extract more efficient compare to aqueous extract in this study. Besides, quantitative analysis was conducted to know the quantity the compounds such as alkaloid content, saponin content, tannin content, total phenolic content, total flavonoid content and ascorbic acid content that contain nutritionals value which can act new potential sources of antioxidants and safe for human consumption. The IC_{50} value of *Sesamum radiatum* seeds is low that show greater radical scavenging potential. The potential bioactive compound existed in seeds could be improved the existing medicines and also developed in food and cosmetic industries.

5.2 Recommendations

The recommendation for future prospects study in *Sesamum radiatum* seeds is selecting suitable solvent and extraction method in detection of phytochemical compound is highly recommended so that can increase the extraction yield of polyphenol. The particle size can affect extraction yield as smaller size of particle, large surface area that can increase the product yield. Furthermore, the stock solution must be freshly prepared.

Besides, sample preparation during drying process that might denatured the enzyme in high temperature. In addition, using various standard in analyzing antioxidants activity so that it can be comparable and specific. All this step might be help in future research of seeds. For future study, the isolation and characterization of flavonoids must be done as its presence in *Sesamum radiatum* seeds.

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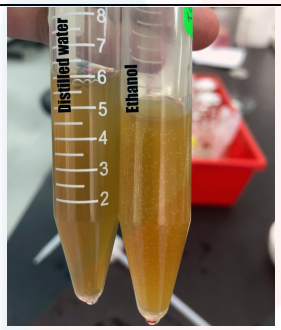
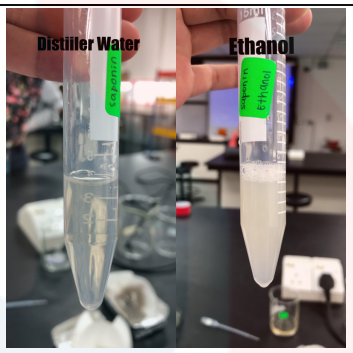
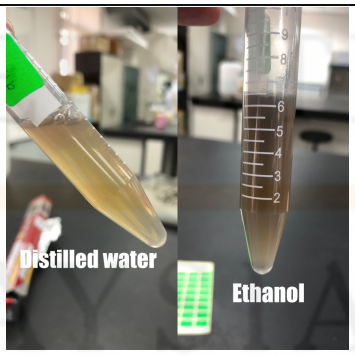
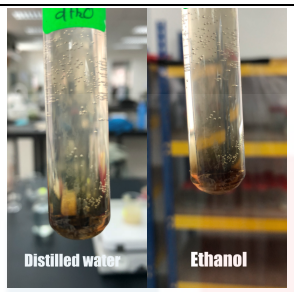
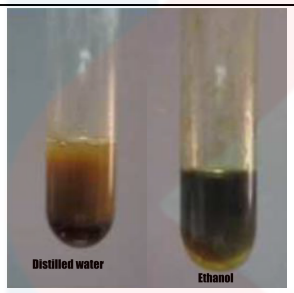
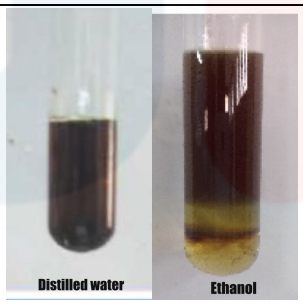
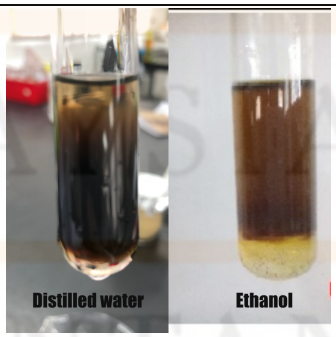
APPENDIX A: QUALITATIVE DATA

Qualitative Analysis	Observation
Test for Flavonoids	 The formation of yellow colour revealed the present of flavonoids in both ethanolic extract (left) and aqueous extract (right).
Test for Saponins	 The formation of stabile froth after 5 minutes revealed the present of saponin in ethanolic extract (right). Meanwhile, the froth in aqueous extract disappeared after 5 minutes.
Test for Tannins	 The blue-black colour does not form in both extraction, ethanolic extract (left) and aqueous extract that show the absent of tannins.

Test for Phlobatannins	 The red precipitate does not formed in both aqueous extract (left) and ethanolic extract (right) that show absent of phlobatannin.
Test for Cardiac Glycosides	 Brown ring does not formed in both aqueous extract (left) and ethanolic extract (right). This indicate the cardiac glycosides was absent.
Test for Terpenoids	 The formation of reddish-brown coloration was formed in both ethanolic extract (right) and aqueous extract (left). This revealed the present of terpenoids in both extract.
Test for Steroids	 The formation of dark brown ring which revealed the present of steroid in ethanolic extract (left). While, dark brown ring does not formed in aqueous extract that show absent of steroid.

APPENDIX B: QUANTITATIVE DATA

Table 4. 4: Standard Calibration Curve of Gallic Acid and Sample Concentration in Determination of Tannins Content

Concentration (mg/L)	Absorbance
0	0.113
10	0.231
20	0.443
30	0.847
40	1.036
50	1.234

Sample Concentration	Absorbance
4.475	0.146

Table 4. 5: Standard Calibration Curve of Gallic Acid and Sample Concentration in Determination of Total Phenolic Content (TPC)

Concentration (mg/L)	Absorbance
0	0.024
10	0.087
20	0.112
30	0.195
40	0.227
50	0.241

Sample Concentration	Absorbance
9.187	0.073

Table 4. 6: Standard Calibration Curve of Quercetin and Sample Concentration in Determination of Total Flavonoids Content (TFC)

Concentration (mg/L)	Absorbance
0	0.004
10	0.011
20	0.017
30	0.021
40	0.027
50	0.035

Sample Concentration	Absorbance
4.585	0.007

Table 4. 7: Standard Calibration Curve of L- Ascorbic Acid and Sample Concentration in Determination of Ascorbic Acid (Vitamin C) Content

Concentration (mg/L)	Absorbance
0	0.051
10	0.048
20	0.044
30	0.037
40	0.034
50	0.028

Sample Concentration	Absorbance
15.171	0.045

Table 4. 8: The reading of Absorbance, percentage of inhibition and IC_{50} value of Ascorbic Acid

Concentration (mg/L)	Absorbance	Percentage of Inhibition Ascorbic Acid (%)	IC_{50} (mg/L)
0	1.168	0	5.22
0.001	1.142	2.226	
0.01	1.096	6.164	
0.1	1.119	4.195	
1	1.121	4.024	
10	0.831	28.853	
100	0.045	96.147	

Table 4. 9: The reading of Absorbance, percentage of inhibition and IC_{50} value of *Sesamum radiatum* Seeds

Concentration (mg/L)	Absorbance	Percentage of Inhibition Seeds Sample (%)	IC_{50} (mg/L)
0	2.774	0	11.470
0.001	2.751	0.829	
0.01	2.690	3.028	
0.1	2.672	3.677	
1	2.665	3.929	
10	2.548	8.147	
100	1.876	32.372	