UNIVERSITI PUTRA MALAYSIA

EFFECT OF SOAKING AND BOILING ON THE PROXIMATE COMPOSITION AND ANTI-NUTRIENT CONTENT (TANNIN) OF LITTLE MILLET (*Panicum Sumantrense*) AND BARNYARD MILLET (Echinochloa Frumaentacea)

ANIS SURAYA BINTI ADNAN

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BY

ANIS SURAYA BINTI ADNAN

A project submitted as a partial fulfillment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia

SUPERVISOR'S SIGNATURE

This project entitled "Effect of soaking and boiling on the proximate composition and anti-nutrient content (tannin) of little millet (*panicum sumantrense*) and barnyard millet (*echinochloa frumaentacea*)" was prepared by Anis Suraya binti Adnan and submitted to the Faculty of Medicine and Health Sciences as a partial fulfilment of the requirement for the degree of Bachelor of Science (Nutrition and Community Health) from the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.

Received and examined by:

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(Dr Siti Raihanah binti Shafie)

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LIST OF ABBREVIATIONS

℃	-	Degree Celcius
%	-	Percentages
Min	-	Minutes
mg	-	Milligram
g	-	Gram
µg	-	Microgram
ml	-	Millilitre
µl	-	Microlitre
UV-Vis	-	Ultraviolet-visible
SD	-	Standard deviation
rpm	-	revolutions per minute
N	-	Normality
dw	-	Dry weight
TAE	-	Tannic Acid Equivalent
SPSS	-	Statistical Package for the Social Sciences
GIS	-	Growth Index Suitability
FAO	-	Food and Agriculture Organization of the United Nations
HDL	-	High density lipoprotein
HCl	-	Hydrochloric acid

ABSTRACT

EFFECT OF SOAKING AND BOILING ON THE PROXIMATE COMPOSITION AND ANTI-NUTRIENT CONTENT (TANNIN) OF LITTLE MILLET (*Panicum Sumantrense*) AND BARNYARD MILLET (*Echinochloa Frumaentacea*)

Anis Suraya binti Adnan

Millets are a group of small seeded grasses that contains significant amount of nutrients with high dietary fibre content. It can be introduced in Malaysian diet for weight management and healthy people to adopt healthy eating. However, the presence of anti-nutrient (tannin) in millet reduces the bioavailability of minerals and protein digestibility. Several preparation methods such as soaking and boiling may affect the nutrient and anti-nutrients in millet. Therefore, this study was aimed to determine the effect of soaking and boiling on the proximate composition and anti-nutrient content (tannin) of little and barnyard millet. Raw, soaked (soaking for 24 hours) and boiled millets (boiling for 25 minutes) were analysed and AOAC method was used to determine proximate composition while tannin was determined using Folin-Ciocalteu assay. The data of study were analysed using one-way ANOVA. The results of both soaked and boiled little and barnyard millets showed that moisture content ranged between 11.24%–72.48%, the ash content ranged between 0.26%-1.63%, the fat content ranged between 4.40%-5.52%, the protein content ranged between 6.32% - 10.73%, the total dietary fibre content ranged between 8.42%-10.91%, available carbohydrate and total tannin content ranged between 2.64%-67.28% and 1.34%-3.91% respectively. From the finding, boiling significantly increased the moisture and total dietary fibre content of both millets and significantly reduced the ash, available carbohydrate and total tannin content of barnyard millet ($p<0.05$). Meanwhile soaking significantly increased the moisture and protein in both millet and increased fat content was seen in little millet ($p<0.05$). However, soaking does not significantly reduced the total tannin content in millet ($p>0.05$). To conclude, boiling is the best method to prepare millet for consumption as it significantly increased total dietary fibre and reduced total tannin content in millet and this could potentially be beneficial in reducing the risk of weight gain and obesity incidence.

ABSTRAK

KESAN RENDAMAN DAN DIDIHAN TERHADAP KOMPOSISI PROKSIMAT DAN KANDUNGAN ANTI-NUTRIEN (TANNIN) DI DALAM MILLET KECIL (*Panicum Sumantrense*) DAN MILLET BARNYARD (*Echinochloa Frumaentacea*)

Anis Suraya binti Adnan

Millet adalah sekumpulan rumput yang berbiji kecil dan ianya mempunyai sejumlah besar kandungan nutrien dan kandungan serat yang tinggi. Ianya boleh diperkenalkan di dalam diet masyarakat Malaysia untuk membantu mengawal berat badan dan pengambilan ini juga dapat membantu individu yang sihat untuk mempraktikkan amalan pemakanan sihat. Walaubagaimanapun, kehadiran anti-nutrien (tannin) di dalam millet dapat mengurangkan ketersediaan mineral dan pencernaan protein hingga seterusnya mengakibatkan kekurangan zat makanan. Beberapa kaedah penyediaan millet seperti rendaman atau pendidihan dapat mempengaruhi kandungan nutrien dan anti-nutrien millet. Oleh itu, tujuan kajian ini adalah untuk menentukan kesan merendam dan mendidih pada komposisi proksimat dan anti-nutrien (tannin) millet kecil dan barnyard. Millet mentah, direndam selama 24 jam dan dididih selama 25 minit untuk dianalisa dan kaedah AOAC digunakan untuk menentukan proksimat manakala kaedah "Folin-Ciocalteu" digunakan untuk menentukan kandungan tannin. Data kajian dianalisa menggunakan "one-way ANOVA". Hasil kajian menunjukkan kandungan kelembapan dalam millet diantara 11.24%-72.48%, kandungan abu diantara 0.26%-1.63%, kandungan lemak diantara 4.40%-5.52%, kandungan protein diantara 6.32%-10.73%, kandungan serat diantara 8.42%-10.91%, kandungan karbohidrat diantara 2.64%-67.28% dan kandungan tannin diantara 1.34%-3.91%. Hasil kajian menunjukkan kedua-dua millet yang dididih mempunyai kelembapan dan kandungan serat yang tinggi manakala kandungan abu, karbohidrat dan tannin di dalam millet barnyard menurun selepas dididihkan ($p<0.05$). Selain itu, rendaman meningkatkan kelembapan dan protein pada kedua-dua millet dengan ketara dan juga meningkatkan kandungan lemak pada millet kecil ($p<0.05$). Kesimpulannya, didihan adalah cara terbaik untuk menyediakan millet untuk dimakan kerana ianya mampu meningkatkan kandungan serat dan mengurangkan jumlah tannin di dalam millet dan seterusnya berpotensi dalam mengurangkan risiko peningkatan berat badan dan obesiti.

CHAPTER 1

INTRODUCTION

1.1 Background

Cereals are staple foods for most of the world's population. There are varieties of grains have been cultivated by mankind and one of them is millet. Millet is a group of small seeded grass and categorized in the grass sub-family *Panicoideae* (Yang et al., 2012). On top of that, millet is one of the oldest grains that are cultivated and consumed by people, especially in Africa and Asia regions such as Nigeria and India (Amadou et al., 2013). Millet contains significant amount of energy, protein, selected macronutrients and micro nutrients. Millet is rank as the world's sixth most important cereal grain after maize, rice, wheat, barley, and sorghum (Changmei et al., 2014). The total annual production of millet grains worldwide in the year 2016 was 28, 459, 020 metric tonnes and India alone produced 10, 777, 887.5 metric tonnes contributing 37.87% (FAO, 2016). Little millet (*Panicum sumatrense*) and barnyard millet (*Echinochloa frumentacea*) are the types of millet that are cultivated by mankind and used as their food sources.

In general, millet kernel consisted of seed coat (testa), germ (embryo) and endosperm (Figure 1). They also have smaller seeds compared to the size of other grains or maize with the height range from one to four feet (Anderson & Martin, 1949). Besides, millet also have hard seed coat to increase their storage life (Kulkarni

DB, 2018). They also can be found varies in colour such as light brown, brown or white. These differences in colour indicate their different cultivar. Brown or light brown cultivar millets are usually used for porridge or beer production while white cultivar is used in the baking industry (Sood et al., 2017).

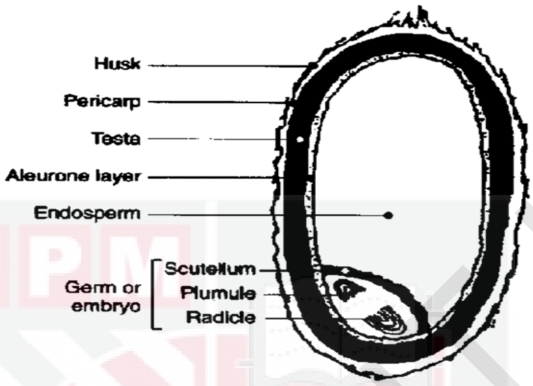

Figure 1 General structure of millet grains

(Source: Ramashia et al., 2019)

Millets are recognized as a healthy choice in a diet because they provide benefits to the consumers. Nutritional content in millets such as energy, protein and carbohydrate are comparable to the other cereals such as rice or wheat (Devi et al., 2014). However, minor millets such as foxtail millet and kodo millet have higher dietary fibre value compared to other cereals (Devi et al., 2014). In accordance to Devi et al., (2014), several studies also reported that little and barnyard millets have a high dietary fibre content which helps in lowering the blood glucose level of the consumers (Ayurvedic et al., 2016; Panwar et al., 2016). Thus, millet can be used as a treatment for the obesity and diabetes mellitus patient to improve their health status (Mounika & Devi, 2019).

Despite its nutritional qualities, there is also presence of anti-nutrient contents in millet. Anti-nutrients are chemical compounds which are synthesized in natural food by the metabolism of an organism and by various mechanisms which have an impact to the bioavailability of nutrients (Soetan & Oyewole, 2009). Anti-nutrient such as tannin is commonly found in legumes or grains. This anti-nutrient will bind to the minerals or protein and reduced the nutrient bioavailability in millet (Hendek Ertop & Bektaş, 2018; Makokha et al., 2002; Singh & Mohanty, 2016). There is no data on the safe limit for tannin consumption, however consume too much tannin in foods such as tea or coffee could result in nutrient deficiencies (Popova & Mihaylova, 2019). Tannin can be reduced through several processing techniques such as soaking, boiling or roasting (Singh et al., 2017). Thus, by reducing the anti-nutrient, absorption and bioavailability of some minerals such as iron and zinc can be improved (Zhang et al., 2017).

Besides, agronomic-friendly characteristics of millet make them become one of the alternatives plant crops planted by the farmers in dry areas in India. Firstly, they have a short growing season and able to grow under unfavourable conditions such as acidic soil and limited rainfalls (Adekunle et al., 2012; Das et al., 2019; Gangaiah, 2006; Habiyaremye et al., 2017; Upadhyaya et al., 2014). These characteristics make millet as a good alternative to replace wheat as they are sensitive to acidic soil and have a poor growth and yielding under this condition. Therefore, millet has been cultivated in many humid areas and underdeveloped countries which have these conditions without obstructing the net productivity.

However, millet is rarely found planted in Malaysia. This is due to the different soil characteristics and climate of Malaysia compared to hot and humid areas such as in African. Growth Index Suitability (GIS) approach can be used to identify soil suitability and suitable area to grow an underutilised crop in Malaysia (Suhairi et al., 2018). However, there is not so much studied conducted on the suitability of millet to be planted in Malaysia.

Besides, millet grains were used largely in food and beverages production in India. They are used for animal feeding and bird seeds while in Africa, East-Asia and India, they have been processed to produce millet flour or malt of the grains (Compendium, n.d.). Other than that, they also can be used in the utilization of food products such as baby foods, dietary foods and snack foods. Other than that, malted millet grains are used in the production of alcoholic and non-alcoholic fermented beverages (Amadou et al., 2013).

In summary, millet is a healthy alternative to other cereal products such as rice, wheat or maize. Their nutritional value allows them to be marketed in food production and consumed by human. The purpose of this study is to determine and compare the proximate composition and anti-nutrient content (tannin) of raw, soaked and boiled little and barnyard millet. The proximate analysis that conducted in this study includes moisture, ash, protein, fat, available carbohydrate and total dietary fibre. The anti-nutrient that will analyse in this study is total tannin content.

1.2 Problem statement

First and foremost, non-communicable disease such as diabetes and obesity are a serious issue as it can impair health and quality of life of an individual. According to the National Health and Morbidity Surveys (NHMS) (2019), the trends of overweight and obesity among Malaysian population showed increment compared to the findings of NHMS 2011 and NHMS 2015. From the survey, it showed that 50.1% of Malaysian adults were overweight or obese with 30.4% were overweight and 19.7% were obese (National Institutes of Health, 2019). As the number of people diagnosed with obesity is increasing, nation is currently experiencing a rise in non-communicable diseases including diabetes and cardiovascular diseases.

Therefore, to help in reducing the prevalence of obesity and other non-communicable diseases, it is important to introduce a suitable dietary management such as consuming high fiber food to increase the satiety and control blood sugar level in the body.

In addition, according to the finding from Malaysian Adults Nutrition Survey (2014), refined grains such as rice are the highest food choices in diet among Malaysian population. Overconsumption of these refined grains will contribute to excessive weight gain and lead to other non-communicable diseases such as diabetes mellitus, cardiovascular diseases, musculoskeletal diseases or cancers. Therefore, by consuming whole grains and cereals that contain high dietary fibre, it will help to enhance satiety and promote satiation, thus reduce the risk of weight gain and incidence of obesity (Maki et al., 2019). However, the consumption of whole grain in Malaysian population was below recommendations (Norimah et al., 2015). Millets are one of the grains apart from rice, wheat or corn. They have high dietary fiber which provides health benefits to the consumers. But, there were limited studies about little and barnyard millet in Malaysia. In order to introduce these millets to the Malaysian, it is important to have a clear database on nutritional composition on commercialized millet. Besides, millet contains anti-nutrient compounds such as tannin which will reduce the bioavailability of minerals and reduce the nutrient qualities of millet. Therefore, it will result in deficient in diet of consumers (Changmei et al., 2014). Deficiencies in micronutrients can adversely affect the growing children due to difficulties in growth, weakened immune system and impaired physical and mental development (Viteri & Gonzalez, 2002). Therefore, it is a serious issue that need to be highlighted, especially among the consumers in which plants are their major source of food.

Millet undergone several preparation methods before consumed such as boiling or soaking. In India, millet grains were soaked overnight and boiled until soft to produce millet rice. Then, it will be served together with vegetables or meat. In addition, in India, fermented millet flours were used to produce pancakes or other fermented breads such as dosa or injera. Other than that, in Nigeria, millet grains that were soaked for 2 days were used to prepare soft porridge (ogi). These preparation methods may cause changes in the proximate composition and anti-nutrient content of millet. Several studies have reported that soaking could reduce the anti-nutrients content in sorghum due to the leaching of anti-nutrients in the soaked water (A. Singh et al., 2017; Gupta et al. 2013). Other than that, heat treatment such as boiling was proven to reduce tannin in finger millet compared to raw finger millet (Folasade, 2011). Finally, it can be concluded that soaking or boiling could help in reducing the anti-nutrient compounds in millet. However, there are limited studies conducted on the effect of soaking and boiling on little and barnyard millet on the proximate composition and its anti-nutrient content (tannin). Thus, it is important to conduct a study to further investigate on this. In conclusion, this study aims to determine and compare the effect of soaking and boiling on the proximate composition and anti-nutrient (tannin) of little and barnyard millet.

1.3 Significance of study

Firstly, the findings of the current study discuss the effect of soaking and boiling on the proximate composition as in moisture, ash, protein, fat, total dietary fibre, available carbohydrate as well as anti-nutrient (tannin) in little and barnyard millet. Therefore, it helps the community whom concerned with healthy food and essential nutrients in the end-products after food undergoing preparation and cooking methods so that they can make a better choice in consuming millets while retaining high nutritional value in it.

Besides, the findings of the study can be used as a baseline data in order to introduce new millet products to the consumers. Other than that, this data can also be added to the recent Malaysian Food Composition Table (2015). These millets have become very popular in Africa, India and other regions but not in Malaysia. Therefore, these millets can be introduced to the Malaysian population as an alternative healthy food choice in the diet because they have a lot of beneficial effects in term of their nutritional values such as high dietary fibres content. Millets also has low glycaemic index which is suitable as a diet of diabetic patients and normal people to adopt healthy eating, thus help in weight management. As a result, the prevalence of obesity and diabetes in Malaysia can be reduced, thus will improve the overall quality of life.

Last but not least, the findings from this study also can be utilized by entrepreneurs or farmers, especially in agriculture or food industry to create a new idea or produce new healthy food products. Since millet has not been widely notable in Malaysia, these groups of peoples can use this opportunity to produce new products on millet-based products. Furthermore, food products such as biscuits, noodles, pasta and breakfast cereal from millet have been suggested by the researchers to be incorporated in the food industries and market due to their health benefits. To conclude, this study was beneficial to the related agencies in the food industry and can also generate more employments to the Malaysian. Since little and barnyard millet are still not established in Malaysia, more study about it needs to be conducted by Malaysian researchers.

1.4 Objectives

1.4.1 General objective

To determine the effect of soaking and boiling on the proximate composition and anti-nutrient content (tannin) of little millet and barnyard millet.

1.4.2 Specific objectives

- To determine the proximate composition (moisture, ash, fat, protein, total dietary fibre and available carbohydrate) of little millet and barnyard millet after soaking and boiling.
- To determine the anti-nutrient content (tannin) of little millet and barnyard millet after soaking and boiling.
- To compare the proximate composition between little and barnyard millet after soaking and boiling.
- To compare the anti-nutrient content (tannin) between little and barnyard millet after soaking and boiling.

1.5 Null Hypothesis

- There is no significant difference in the proximate composition of raw, soaked and boiled little and barnyard millet.
- There is no significant difference in the anti-nutrient content (tannin) of raw, soaked and boiled little and barnyard millet.

CHAPTER 2

LITERATURE REVIEW

2.1 Background of millet

Term millet is derived from the French word 'mille' which means thousand, with a handful of millet containing up to 1000 grains. Millets are considered among oldest cereals because they have been used and consumed at the beginning of human development (Anderson & Martin, 1949). In Northern China, there are some evidences that proso and foxtail millets have been used in the production of noodles 4000 years ago (Lu et al., 2005). Besides, millet also considered as inferior grains by some of the people due to their strong taste. Millet can be categorised as major and minor millets. Pearl millet (*Pennisetum americanum L.*) is categorised as major millet due to its large utilization while finger millet (*Eleusine coracona*), foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumantrense*) and barnyard millet (*Echinochloa Frumentacea*) are classified as minor millets (Padulosi et al., 2015). They are mostly produced in a hot and humid area in Africa and Asia regions such as Nigeria and India. Hence, people who live in these area consumed millets as their major source of energy and protein (Amadou et al., 2013).

2.1.1 Little millet

Little millet (*Panicum sumatrense*) (Figure 2) also known as Sama. They can be found in two races which are nana and robusta. These two races have different in height that varies from 60 to 190 cm. Little millets are commonly grown in India, Myanmar, Nepal and Sri Lanka (Upadhyaya et al., 2014). Besides, little millet has become one of the staple cereal crops in Karnataka and Tamil Nadu. In India, little millet is grown up to 2100 m altitudes. For its physical characteristics, little millet has shorter and smaller panicles and seeds compared to the other millets. Besides, they can be harvested in between 2.5 to 5 months growing period.

Figure 2 Little millet

(Source: Kajuna, 2001)

2.1.2 Barnyard millet

Barnyard millets are mainly grown in India, China, Japan and Korea (Upadhyaya et al., 2014). They are mostly grown in the north to the south Himalayan region. They are the fastest growing crop among other millets because they can reach matures in 6 weeks. Other than that, they can grow in the area with an altitude of 2000 m above sea level. They can grow in an area that has limited rainfalls and acidic soils. In terms of physical characteristics, the height of barnyard varies between 50 and 100cm with up to 15 lateral branches of purple panicles (Kajuna, 2001) (Figure 3). In terms of nutritional composition, millets are rich in nutrients such as proteins, dietary fibre and minerals which make them suitable to be included in a healthy diet.

Figure 3 Barnyard millet

(Source : Kajuna, 2001)

2.2 Nutritional composition

2.2.1 Little millet

Millets are known as a good source of macronutrient and micronutrient grains as millets have a high content of fibre, protein, and other nutrients. Moisture content in food is used by the manufacturers to produce a good quality of products. Low moisture content in the food can help to last longer. Kamatar et al. (2013) reported that moisture content in the little millet was 5.7% which is lower compared to the finding of a report on the Post-harvest Operations of Millet which states that the moisture content in the little millet was 11.1% (Kajuna, 2001). The differences in the moisture content reported between these studies were due to the different analysis method used which were infrared analysis and oven drying method. According to the Indian Food Composition Tables (2017), moisture content of little millet was 14.23±0.45g per 100g.

Total mineral content in food is measured by ash content. According to the Indian Food Composition Tables (2017), the ash content of little millet was 1.72±0.27 g per 100g. Another study conducted by Kamatar et al. (2013) reported that the ash content in little millet was 5%. Other than that, Folasade (2011) states that ash content in boiled samples was lower compared to the soaked samples due to the leaching of nutrients into the water and increasing permeability of testa. For the protein content of little millet, a report from Food and Agriculture Organization of the United Nations on a Post-harvest Operations of Millet (2001) states that protein content of little millet was 13.4% which is higher compared to the finding by Kamatar et al. (2013) which was 7%. According to the Indian Food Composition Tables (2017), protein content in little millet is 8.92±1.09 g per 100g.

Kamatar et al. (2013) further reported that fat content in raw little millet was 4.26% which was slightly higher compared to a study by Kajuna (2001) which reported that fat content in the little millet was only 1.8%. According to the Indian Food Composition Tables (2017), fat content in little millet is 2.55±0.13g per 100g.

In addition, Kamatar et al. (2013) reported that carbohydrate content in raw little millet was 78%. Study by Kajuna (2001) reported that little millet contains 72.3% carbohydrate. Meanwhile, Indian Food Composition Tables (2017) reported that both little and barnyard millet has similar carbohydrate content which was 65.55±1.29 g per 100g and 65.55g per 100g. The 65% -75% of carbohydrate content in millet was comparable with other cereal grains such as maize (62.3%), wheat (64.0%) and rice (77.2%)(Devi et al., 2014). According to the Indian Food Composition Tables (2017), total dietary fiber in little millet was 6.39±0.60 g per 100g.

2.2.2 Barnyard millet

A report from the Food and Agriculture Organization of the United Nations on the Post-harvest Operations of Millet states that moisture content in barnyard millet was 11.9% (Kajuna, 2001). However, a study conducted by Ayurvedic et al. (2016) reported that moisture content of raw barnyard millet was slightly lower (9.4±0.2%). This differences may due to the different cultivar or millet genotypes originated (Shibairo et al., 2014).

According to Ayurvedic et al. (2016), the ash content of soaked barnyard millet was much higher (3.1±0.1%) compared to raw barnyard millet (2.2±0.1%). Indian Food Composition Tables (2017) reported that barnyard millet has 6.20 g per 100 g protein content. Another study conducted by Ayurvedic et al. (2016) reported that the protein content of raw barnyard millet was 5.1±0.1% while soaked barnyard millet was 4.1±0.3%. This result shows that soaking decrease the protein content in millet. Contradict with a study by Kajihausa et al. (2014) that found out protein content of the sesame seed flour found to increase with increases in soaking time.

Ayurvedic et al. (2016) reported raw barnyard millet has higher fat content (2.9±0.1%) compared to soaked barnyard millet (2.4±0.2%). According to the Indian Food Composition Tables (2017), fat content in barnyard millet was 2.20g per 100g. A study by Kajuna (2001) stated that dietary fibre in barnyard millet was 9.8% and another studied by Taylor & Emmambux (2008) showed higher dietary fibre content in barnyard millet which was 14.3%.

2.3 Therapeutic effect of millet

Millet is known for its health benefits due to its high dietary fibre compared to other cereals (Devi et al., 2014). They are also suitable for the prevention and treatment of diabetes mellitus. Diabetes mellitus is a chronic metabolic disorder due to the excessive glucose in the blood, resulting from the insufficient or inefficient of insulin secretion with carbohydrate, protein or lipid disorder. Therefore, controlling the blood glucose level in diabetic patient is needed as a treatment for diabetes, hence reduces other chronic complication (Node & Inoue, 2009). Research had shown that carbohydrates in the millet are broken down slowly compared to other cereals which this characteristic is recommended for diabetic patients (Lakshmi Kumari & Sumathi, 2002; Ugare et al. 2014)

In a study conducted by Ugare et al. (2014), authors reported that subjects with millet intervention in the diet throughout the study shows a reduction in the triglyceride level and increases in the HDL level compared to the control group which is without millet intervention. Hence, it can be concluded that millet could be a possible therapeutic intervention in type 2 diabetes. Other than that, Habiyaremye et al. (2017) explained that protein in barnyard millet can also help to regulate cholesterol metabolism by elevating plasma level of HDL cholesterol.

2.4 Anti-nutrient content in millet

Millet also contains anti-nutrient factors that decrease nutritional bioavailability and quality. These anti-nutrients are allocated throughout the grain. One of the anti-nutrients commonly found in grains is tannin. It can be found largely in the seed coat of grains. Besides, other food sources that contain tannin are coffee, tea or cocoa. Tannin is known as anti-nutrient factors as it shows anti-nutritional properties on the protein and bioavailability of nutrients. They will bind to the protein and formed tannin-protein complexes. Formation of tannin-protein complexes will result in the inactivation of digestive enzymes and reduce the digestibility of protein, thus lowering the nutrient quality of millet (Hendek Ertop & Bektaş, 2018). Millet contains about 218 mg per 100g – 396 mg per 100g tannin content (Panwar et al., 2016). There is no data on the safe limits of tannin for human consumption; however there are few products were given its safe limit for tannin. Joint FAO/WHO Food Standards Program (1995) stated that tannin content in whole sorghum grains products should not more than 0.5% of dry weight basis and 0.3% dry weight basis for hulled sorghum grains (Sharma et al., 2019). The effects of anti-nutritional factors such as tannin in cereal products were reduced to retain the maximum nutrient value (Joint FAO and World Health Organization, 2012).

2.5 Soaking and boiling

Millets usually consumed after they have undergone several common preparation and cooking methods such as soaked, boiled or roasted. Shelf life and bioavailability of nutrients in the foods can be improved by different processing methods. Nutritive value in food can also be affected by the processing technique. According to Ayurvedic et al. (2016), soaking reduces protein contents in raw barnyard millet. The reduction in protein content might be probably due to the loss of soluble protein in soaking water. A study by Karaś et al. (2019) found out that boiling does not affect the protein content of millets due to the presence of heat stable proteins in millets (methionine and cysteine). Other than that, soaking increase ash content compared to the raw barnyard millet. Another study by Afify et al. (2012) found out that ash content in white sorghum decreases after soaking and boiling. Soaking and boiling cause leaching of water soluble minerals into the water. In terms of the anti-nutrient, soaking was reported to reduce the anti-nutrient content in the grains (Saleh et al., 2013). Another study conducted by Folasade (2011) showed that soaking and boiling reduced the tannin content in millet compared to the raw millet. Ayurvedic et al. (2016) reported that soaked barnyard millet has lower tannin content (4.1g per 100gm) compared to raw barnyard millet (5.2g per 100gm).

2.6 Market and production of millet

Traditionally, millets are known as grain food for livestock and birds in the United States. They were also planted as a temporary crop during unfavourable temperature. Nowadays millet was commercialized and known due to their health benefits. An organization of farmers in certain states of Pacific Northwest is working together in planting millets and other warm-season crops such as sorghum and amaranth as their alternative crop. However, the production of millet in India was declined drastically from the year 1995-2014 (Perumal, 2018). The reason for the reduction in the production of millet was due to the decline in the plantation area with increasing in the production of high demand crops such as rice or wheat (Padulosi et al., 2015). As a solution, the government has focused on the development of technology for the efficiency of the production of millet. Besides, they also initiated several programmes for the development of millet such as Initiative for Nutritional Security through Intensive Millets Promotion (INSIMP) and National Food Security Mission (NFSM) (Perumal, 2018).

2.7 Determination of proximate composition

2.7.1 Moisture

Firstly, the moisture content in the samples was analysed in the current study to know the water activity in the samples. It is also important for the food industries to forecast product quality and its longevity (Popping & Diaz-Amigo, 2014). Air-oven method is the most common method used to determine moisture content in the sample.

2.7.2 Ash

Ash determination is important in determining the nutritional composition of the food. Ash content in the sample shows the total mineral content in it (Marshall, 2010). Dry ashing method is the most common method used for proximate composition in sample while wet ashing (oxidation) method is used in analysing certain minerals in the sample such as iron, copper, zinc and phosphorus as these minerals would losses from volatilization during dry ashing method (Marshall, 2010).

2.7.3 Protein

Protein is one of the essential macronutrients that are needed for the human body to perform effectively and efficiently. They consist of hydrogen, carbon, nitrogen, oxygen and sulphur and the most distinguishing element in proteins is nitrogen. Nitrogen content is used to calculate the protein content of the food. The analysis of protein content in food is important as it will be used for nutrition labelling.

The most common method in determining the protein content is using Kjeldahl method which is also a standardized method from AOAC. In the Kjeldahl procedure, sulphuric acid and catalyst will be used to digest proteins and other organic food components. The total organic nitrogen obtained from the digestion process is converted to ammonium sulphate and distilled with boric acid solution. Borate anions formed will be titrated with standardized acid and converted it to nitrogen in the sample. The nitrogen content in the sample will be multiplied with conversion factor to obtain the protein content in the sample.

2.7.4 Fat

Fat is also one of the components of nutritional composition in the food. Fat can be categorised into saturated fatty acid, monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). It is well known that MUFA and PUFA can help in reducing the blood cholesterol level in the body. Analysis of fat content in food is important for nutrition labelling.

Soxhlet method is the most common method in determining the fat (lipid) content in the food sample. Extraction of fat in the Soxhlet method will be using extraction solvents such as petroleum ether, hexane, or ethyl ether due to the solubility of lipid in organic solvent. The principle of Soxhlet method is fat was first extracted with the organic solvent and heated up and let the organic solvent condensed above the sample. The condensed organic solvent will then drip onto the samples and soaks the food samples for the fat extraction (B.Min, 2010).

2.7.5 Available carbohydrate

Carbohydrates are an important source of energy for human. Available carbohydrate or known as digestible carbohydrates are a fraction of carbohydrate which are converted into monosaccharide, absorbed in the body and provide energy to human. Furthermore, carbohydrate account for more than 70% of the diet's calorific value. In this study, available carbohydrate content in the food sample is determined by the difference method which this method exclude dietary fiber (WHO/FAO, 2002).

2.7.6 Total dietary fiber

Dietary fiber is a type of carbohydrates that are indigestible for a human's body. Dietary fiber consists of soluble and insoluble polysaccharides and also non-digestible oligosaccharides. It can be found in edible plant foods such as vegetables, fruits, grains and cereals. Studies have shown that consumption of high-fibre foods may help in reducing blood pressure and blood sugar levels. According to the Recommended Nutrient Intakes for Malaysia (2017), recommended intake of dietary fibre for Malaysian's adult is 20-30g per day. However, a study conducted by Lee & Muda (2019) shows that the intake of dietary fibre among Malaysian adult is below the recommendation suggested by Malaysian Dietary Guidelines 2010. Therefore, the incorporation of millet in the Malaysian diet may help in increasing their dietary intake per day.

In the current study, total dietary fibre in the sample is determined using Sigma-Aldrich Total Dietary Fibre Assay Kit. Combination of enzymatic and gravimetric methods is used in this assay to determine total dietary fibre in the sample. The enzymes that used in this assay are heat stable *α-amylase*, *protease* and *amyloglucosidase*. Next, ethanol is used to precipitate dietary fibre in the sample and further dried and total dietary fibre is calculated from the difference in the weight of protein and ash.

2.8 Determination of anti-nutrient content

2.8.1 Tannin

Tannin is one of the polyphenols found in grains and it is composed of oligomers of flavon-3-ols and flavan-3, 4-diols which can be found highly in the seed's coat (Popova & Mihaylova, 2019). Taylor, Maga, & Yamaguchi (2009) described one of the characteristics of plant tannins used in the several analyses is the ability of tannin to form coloured solutions and precipitation with iron and other metals. Extraction of tannin from the sample usually based on the release of individual bonds from the substrate to compete on the solvent molecules (P. Taylor et al., 2009). Furthermore, P. Taylor et al., (2009) further explained that extraction of tannin using hot water or cold 40% acetone in aqueous solution will prevent the oxidation of labile phenolic compounds in the sample.

In a study conducted by Dwivedi et al., (2015), tannin content in the sample was determined using Folin-Ciocalteu assay. Folin-Ciocalteu assay is an oxidation-reduction method and it is based on the oxidation of phenolic hydroxyl groups. It is also one of the most common methods used in the determination of phenols in plant products or beverages (P. Taylor et al., 2009). The reaction of polyphenol in plant extract with Folin-Ciocalteu reagent will result in forming blue complex which can be measured through UV-spectrophotometer (P. Schofield et al., 2001). Besides, colorimetric reaction used in UV-spectrophotometer method is widely used in the analysis as it is easy to conduct, low cost and rapidly perform. Besides, tannin content in the sample can be extracted using acetone, ethanol, methanol or water (P. Taylor et al., 2009). Adgidzi et al. (2018) perform a study on the determination of tannin in millet using Folin-Ciocalteu assay. However, in this study, the samples were soaked first in different soaking solution and extract using 70% acetone. It is different from the sample extracted in Dwivedi et al. (2015) which is using water. Other than that, the standard solution used in Dwivedi et al. (2015) was tannic acid while Adgidzi et al. (2018) does not mention the standard used in the study. Another study conducted by Ayurvedic et al. (2016b) also determined tannin content in barnyard millet using Folin-Ciocalteu assay.

Another method used in determining tannin in plant samples is vanillin assay which screening the condensed tannin in plants and grains. The principle of this method is vanillin reagent will react with proanthocyanidins in the presence of hydrochloric acid and result in bright red colour. The intensity of the resulted colour is proportional to the amount of tannin in the sample (Sharma et al., 2015).

There are several factors that affect vanillin assay which are extraction time, medium and adequacy of catechin used as a standard (Price et al., 1978). Phenols were easily oxidized during sample preparation and extraction, thus longer extraction time will reduce the tannin content in the sample. Several studies reported that the extraction time carried out for 24 hours will result in decreasing 10% to 15% of tannin content in the sample (Burns, 1971; Price et al., 1978). Several studies further reported that used of catechin as a standard in vanillin assay were not adequate in determining tannin content (Gupta et al., 1980; Price et al., 1978) . Furthermore, standard curve of catechin was deviated from linearity and hence result in overestimation of tannin content in the sample. However, catechin (flavan-3-ol) has been widely used in vanillin assay due to their basic flavan components of tannin. Therefore, tannic acid or catechin is mostly used as a standard in determining tannin content in grains.

Another method that was commonly used in determining tannin content in plant extract is Prussian blue assay. It is a qualitative and quantitative method in determining tannin of sorghums and based on the formation of Prussian blue complex (Price & Butler, 1977). The principle of this method is phenolic compounds will reduce to ferrous ion and formed ferricyanide-ferrous ion complex. The absorbance of blue coloured product will be read at 720 nm using UV-Vis spectrophotometer. Based on Price & Butler (1977), colour changes of the Prussian blue complex was easily distinguished due to the striking changes compared to when vanillin-HCl method is used. Other than that, Prussian blue assay was also similar to other total phenol methods as it does not differentiate between condensed tannin and other phenol compounds.

CHAPTER 3

METHODOLOGY

3.1 Sample selection and sampling method

Both raw little and barnyard millet grains were purchased from Uyir Inspired Sdn. Bhd. Sampling method used in the study was purposive sampling. Millet grains were ground and stored at 4 ℃ for further analysis.

3.2 Reagents and Chemicals

Every analysis in the current study includes reagents except moisture and ash determination. Moisture and ash content in the sample were determined using Air-Oven method and Dry-Ashing method respectively. Firstly, protein content in the sample was determined using Kjeldahl method. Reagents needed in the analysis were sulphuric acid and 0.2N hydrochloric acid from J.t Baker (Centre Valley, Thailand), Kjeldahl tabs from Foss Analytical A/S (Hillereoed, UK), 40% sodium hydroxide from R&M Chemical (Selangor, Malaysia), 4% boric acid, Tashiro's indicator (Chau Sum et al., 2013). Next, Soxhlet method was used to determine fat content and petroleum ether from Fisher Scientific (Loughborough, UK) was used as the reagent in the analysis (Sci et al., 2015). Next, tannin content in the sample was determined using Folin-Ciocalteu assay which was adapted from (Dwivedi et al., 2015). Reagents used in this method were Folin-Ciocalteu reagent from Sigma-

Aldrich (Selangor, Malaysia), 35% sodium carbonate solution from Merck (Germany) and tannic acid from Sigma-Aldrich (China). Sigma-Aldrich dietary fiber kit (USA) was used in the current study to determine the total dietary fiber in the sample. Reagents used in the study were di-Sodium hydrogen phosphate and sodium dihydrogen phosphate monohydrate from Merck (Germany) which were used to make 0.08M phosphate buffer while α-amylase, protease, amyloglucosidase were used from the total dietary fibre kit, 0.325 M hydrochloric acid solution from J.t Baker (Centre Valley, Thailand), 0.275 N sodium hydroxide solution from R&M Chemical (Selangor, Malaysia), 78% ethanol and 95% ethanol from Merck (Germany).

3.3 Preparation of sample

Sample in this study consisted of raw little and barnyard millet grains that were soaked or boiled. Then, they were ground to powder to analyse the proximate composition and anti-nutrient content (tannin) in the sample. The procedures in detailed were explained below.

3.3.1 Raw millet

Raw millet grains were ground and sieved to obtain fine powder. Then, it was stored at 4℃ for further analysis (Ocheme et al., 2010).

3.3.1 Soaking

Millets were soaked in distilled water at room temperature for 24 hours. After soaked, they were thoroughly washed and cleaned using distilled water to remove any foreign materials. The washed grains were then freeze-dried, ground and milled.

Then, the millet powder was sieved and stored at 4 °C for further analysis (Ocheme et al., 2010).

3.3.2 Boiling

Millets were boiled for 30 minutes at 95 - 100°C. After boiling, the water drained off and the millets were freeze-dried. After dried, the millets were ground, sieved and stored at 4°C for further analysis (Ocheme et al., 2010).

3.4 Experimental design

Firstly, homogenised little and barnyard millets were divided into three parts which are for raw, soaked and boiled. Soaked and boiled millets were then dried using freeze-dryer before ground and sieved into powder. Then, the samples were kept in the refrigerator at 4°C. These samples were used to determine proximate composition and anti-nutrient content in millet. Figure 2 below shows the flow diagram of the analysis of proximate composition and anti-nutrient content (tannin) of raw, soaked and boiled little and barnyard millet.

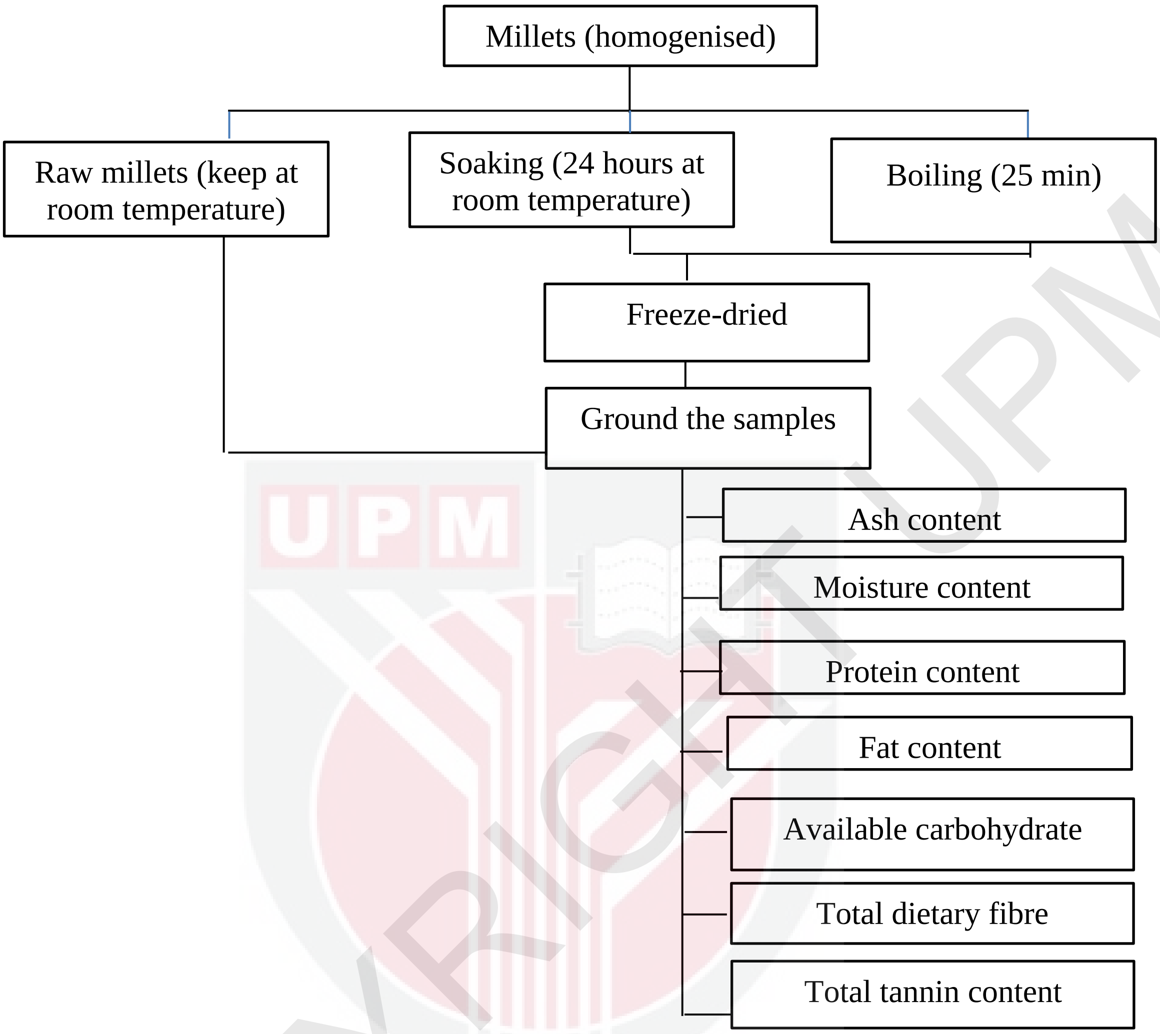

Figure 4 The flow diagram of the analysis of proximate composition and anti-nutrient content (tannin) of raw, soaked and boiled little and barnyard millet.

3.5 Determination of moisture

First and foremost, determination of moisture content in the samples was analysed using the air-oven method using AOAC (2005) adapted from Nassarawa Sanusi (2019). This method was also known as drying method or direct heating. Firstly, the aluminium dish was dried in the air-oven for 30 minute and removed to desiccator to be cool for 20 minutes. Next, the aluminium dish was weighed and 10 g of the sample was added into the dish. It was then placed in the air-oven for overnight at 105°C before allowed to cool in the desiccator. After cooled, the sample was weighed. The process was repeated several times until constant weight of sample was achieved. All samples were analysed in triplicate. The moisture content in the sample was determined using the formula as shown below.

Percentage of moisture in sample by weight, g

$$= \left(\frac{(in\ g, initial\ weight - final\ weight)}{weight\ of\ sample, in\ g} \times 100\%\right)$$

3.6 Determination of ash

Next, ash content in the samples was determined using dry-ashing method using AOAC (2005)(Nassarawa Sanusi, 2019). Ash content in the samples was determined by collecting the inorganic residues that remained after organic matter and water have been removed from heating.

Firstly, the crucible was dried in the oven for 30 minutes at 105℃ and then, cooled in the dessicator and weighed. Then, 3 g of the sample was added into the crucible and then placed in the muffle furnace at 550℃ for overnight until the content became whitish in colour. Lastly, the crucible was cooled and weighed. All samples were analysed in triplicate. The amount of ash content in the sample was calculated using the formula shown below.

Percentage of ash by weight, in g

$$= \left(\frac{(weight\ of\ ash, g)}{weight\ of\ sample, g} \times 100\%\right)$$

3.7 Determination of available carbohydrate

Available carbohydrate in the sample was calculated by difference using the formula as shown below:

Carbohydrate (%) = 100 – (% moisture + %ash + %fat + %protein + %dietary fiber)

3.8 Determination of protein

Protein content in the samples was determined from the amount of nitrogen content in the sample using Kjeldahl method from AOAC (2000) adapted from Chau Sum et al. (2013). Conversion factor was multiplied with the nitrogen content to calculate the protein content in the sample. Three stages involved in Kjeldahl method are digestion, distillation and titration.

One gram of sample was placed in the digestion flask. Next, 15 ml of sulphuric acid was added into the flask with addition of two Kjeldahl tabs as catalyst. The solution was incubated for 1 hour at 420℃. Then, the digested samples were diluted in distillation unit with 80 ml distilled water, 50 ml of 40% sodium hydroxide, 30 ml of 4% boric acid and a few drops of Tashiro's indicator. The distilled sample was titrated with 0.2N hydrochloric acid until light purple colour obtained. All analysis was done in triplicate. The protein content was determined by multiplying the nitrogen content with conversion factor as below:

Percentage of nitrogen content:

$$N\% = \frac{normality\ of\ HCl}{1000} \times \frac{(volume\ of\ corrected\ acid, in\ ml)}{(weight\ of\ sample, in\ g)} \times \frac{14\ g\ of\ nitrogen}{mol} \times 100$$

Where,

Volume of corrected acid = vol of HCl titrated for sample – vol of HCl titrated for blank

% Protein = N% × 5.83

Where,

5.83 = Nitrogen conversion factor of millets (FAO, 2003)

3.9 Determination of fat

Next, fat content in the samples was determined using Soxhlet method from AOAC (2004) adapted from Sci et al. (2015). In this method, the fat content was extracted using petroleum ether as a solvent in the Soxhlet apparatus and the solvent was evaporated to obtain fat.

Firstly, 5 g of sample was weighed and placed into the thimble. The thimble was placed into the extraction tube which was attached to the Soxhlet flask. Next, 200 ml of petroleum ether was poured into the flask. The solvent in the flask was heated to produce condensation to the samples for fat extraction. The extraction was continued for 8 hours. At the end of extraction period, the ether left in the flask on the Soxhlet apparatus was collected and poured into a dry beaker which was previously weighed. Ether was used to rinse and filter the flask. Then, the ether was evaporated using rotary evaporator and flask was dried in oven for 1 hour at 100℃. The beaker containing the residue crude fat was weighed. The difference in the weight of beaker before and after extraction was calculated to identify the amount of crude fat in the sample. All samples were analysed in triplicate. The percentage of crude fat in the samples was calculated using the following formula:

$$\text{Percentage of fat} = \frac{weight\ of\ fat\ in\ the\ sample, in\ g}{weight\ of\ sample} \times 100$$

Where,

Weight of fat in the sample = (weight of beaker +fat) – (weight of beaker)

4.0 Determination of total dietary fiber

Total dietary fiber in the sample was determined using Sigma-Aldrich Total Dietary Fibre Assay Kit. Firstly, the samples were gelatinized with heat stable α-amylase solution and incubated at 95℃ for 15 minutes. Next, proteins in the sample were digested enzymatically with protease and incubate for 30 minutes at 60℃. After that, amyloglucosidase was added to remove starch in the sample. Then, 95% ethanol was used to precipitate soluble dietary fibre. After soluble dietary fibre was precipitated, it was then filtered and dried. Total dietary fibre (TDF) in the content was determined using formula below:

$$\text{Dietary fiber (\%)} = [(Rsample - Psample - Asample - B) / SW] \times 100$$

Where:

R= average residue weight (mg)

P= average protein weight (mg)

A = average ash weight (mg)

B= Rblank –Pblank – Ablank

SW= average sample weight (mg)

4.1 Determination of tannin

Tannin content in the samples was determined using Folin-Ciocalteu assay which was adapted from Dwivedi et al. (2015). Firstly, 1 g of sample was added with 50 ml of water and incubated in boiling water bath for 30 min and centrifuged for 20 min. After centrifuged, 0.1 ml of the supernatant was added with 0.5 ml of Folin-Ciocalteu reagent and 1 ml of 35% of sodium carbonate solution. Final volume was made up to 10 ml with water. Then, the sample was incubated for 30 min and absorbance was read at 700nm using UV-Vis Spectrophotometer. Distilled water was used as blank and the results were expressed as mg Tannic acid (TA)/100 g dry weight. The standard calibration curve of Tannic acid was prepared with the concentration of 0-50 µg TAE/ml (appendix b). Total tannin content in the samples was determined using formula below:

Total tannin content (mg TAE/g sample) = $\frac{C \times dilution\ factor \times volume\ of\ extract}{weight\ of\ sample}$

Where,

C = concentration of calibration from standard curve

4.3 Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics Software Version 22. Determination of moisture, ash, available carbohydrate, total dietary fibre, fat, protein and tannin content in the samples was done in triplicate. For descriptive analysis, the results reported were included mean and standard deviation. For inferential analysis, one-way ANOVA was used in determining the differences in raw, soaked and boiled regarding nutritional composition and anti-nutrient (tannin) content in the sample. Level of significance was set at $p < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

The proximate and anti-nutrient analysis that has been carried out for the current study were moisture, ash, fat, protein, available carbohydrate, total dietary fiber and total tannin content. The samples used in the study were raw, soaked and boiled little and barnyard millet.

4.1 Moisture content

Table 1 shows moisture content of little and barnyard millet samples. Drying method was used in determining moisture content in the samples and results were reported as mean percentage of moisture content and standard deviation. Lower moisture content is important to increase the shelf life of a product (Kajihausa et al., 2014).

Table 1 Comparison of moisture content in little and barnyard millet samples

Samples	Moisture content (% ± SD)
Raw LM	11.37 ± 0.18[d]
Raw BM	11.24 ± 0.19[d]
Soaked LM	32.86 ± 0.65[c]
Soaked BM	32.63 ± 0.12[c]
Boiled LM	69.99 ± 0.07[b]
Boiled BM	72.48 ± 0.36[a]

Results in the table are expressed as mean ± SD, n=3. Values in the same column with different superscripts are significantly different (*p< 0.05*, ANOVA, Tukey-HSD). LM indicates little millet while BM indicates barnyard millet.

In table 1, moisture content of the little and barnyard millet samples in the present study ranged between 11.24 ± 0.19% - 72.48 ± 0.36%. Boiled barnyard millet has the highest moisture content (72.48 ± 0.36%) while raw barnyard millet has the lowest moisture content (11.24 ± 0.19%). There was a significantly difference in moisture content between raw, soaked and boiled samples ($p<0.05$). Moisture content of raw little millet in current study is similar to the finding from Saleh et al. (2013) which reported 12.0% moisture content. Another study by Kajuna (2001) also reported similar finding with 11.1 % of moisture content. Raw barnyard millet in the current study showed similar finding with several study from Ramashia et al. (2019) and Kajuna (2001) which reported that the moisture content in barnyard millet was 11.9%. According to the finding, there were significantly increase in moisture content of little and barnyard millet after soaked and boiled ($p<0.05$).

Soaking significantly increased the moisture content in both little and barnyard millet ($p<0.05$). A study conducted by Vengaiah et al. (2012) showed moisture content of wheat increased after soaked in water at room temperature. Another study by Kajihausa et al. (2014) also found out the moisture content of sesame seed increases with increasing in soaking time. This is due to the water absorption activity of grains during soaking. This reason was supported by a study finding from Agu et al. (2013) which explained that the water absorption of pearl millet increase as soaking time increased and it reach maximal absorption after 24 hours soaking. Boiling also increases the moisture content of both millet significantly in the current study ($p<0.05$). A study by Afify et al. (2012) reported that the moisture content in white sorghum was increased significantly after boiled. This finding was similar with the current study. Higher temperature will result in changing the starch granules and accelerates the rate of water absorption (Wang et al., 2013). Thus, it will result in the increasing of moisture content in the samples.

4.2 Ash content

Table 2 shows ash content of little and barnyard millet samples. Dry-ashing method was used to determine ash content. Ash content was calculated from the remaining of ash weight after dried in muffle furnace for overnight and divide by the weight of sample. Results were expressed as the mean percentage ash content and standard deviation. Ash content was important in food production as it represent the total mineral content present in the food.

Table 2 Comparison of ash content in little and barnyard millet samples

Samples	Ash content (% ± SD)
Raw LM	1.63 ± 0.03[a]
Raw BM	1.42 ± 0.06[b]
Soaked LM	1.15 ± 0.05[c]
Soaked BM	0.94 ± 0.01[d]
Boiled LM	0.35 ± 0.05[e]
Boiled BM	0.26 ± 0.02[e]

Results in the table are expressed as mean ± SD, n=3. Values in the same column with different superscripts are significantly different ($p< 0.05$, ANOVA, Tukey-HSD). LM indicates little millet while BM indicates barnyard millet.

In table 2, ash content of little and barnyard millet samples ranged between 0.26 ± 0.02% - 1.63 ± 0.03%. Raw little millet has the highest ash content (1.63 ± 0.03%) while boiled barnyard millet has the lowest ash content (0.26 ± 0.02%). There were significant difference in the ash content between raw, soaked and boiled little and barnyard millet ($p< 0.05$). Ash content of raw little millet in the current study was similar with ash content reported from Indian Food Composition Table (2017) with a value of 1.72 ± 0.27g per 100 g. Another study by Nassarawa Sanusi (2019) also reported that the ash content in millet ranged from 0.86% - 1.94% which was similar with the current study. Ash content of raw barnyard millet in the current study was similar with the finding from Abdulrahman & Omoniyi (2016) that reported millet contain 1.67% of ash content.

Results from one-way ANOVA showed soaked and boiled causes significantly decreases in ash content of both little and barnyard millet ($p<0.05$). A study by Afify et al. (2012) showed significantly decreases in ash content of white sorghum after soaked and boiled. Besides, a study by Folasade (2011) also reported on the decreasing of ash content of finger millet after soaked for 12 hours. The reduction of ash content was due to the leaching of minerals in soaking and cooking water (EL-Suhaibani et al., 2020). In addition, Shobana Devi (2016) found out that boiling for 12 minutes caused reduction in the sodium and potassium of barnyard millet flour due to its leaching into water.

4.3 Fat content

Table 3 shows the fat content in little and barnyard millet samples. Dry weight sample was used to determine fat content and results of fat content in sample was expressed as percentage of its dry weight and standard deviation. Soxhlet method was used to analyse fat content in the samples.

Table 3 Comparison of fat content in little and barnyard millet samples

Samples	Fat content (% ± SD)
Raw LM	4.41 ± 0.29^{b}
Raw BM	4.68 ± 0.07^{b}
Soaked LM	5.52 ± 0.60^{a}
Soaked BM	5.15 ± 0.10^{ab}
Boiled LM	4.40 ± 0.18^{b}
Boiled BM	4.47 ± 0.25^{b}

Results in the table are expressed as mean ± SD, n=3. Values in the same column with different superscripts are significantly different (*p*< *0.05*, ANOVA, Tukey-HSD). LM indicates little millet while BM indicates barnyard millet.

In table 3, the fat content of little and barnyard millet samples ranged between 4.40 ± 0.18% - 5.52 ± 0.60%. From the table, soaked little millet has the highest fat content (5.52 ± 0.60%) while boiled little millet has the lowest fat content (4.40 ± 0.18%). The results of fat content of raw little millet in the current study was similar with the finding from a study by Kamatar et al. (2013) which reported that fat content in raw little millet was 4.64% - 4.70%. Besides, fat content of raw barnyard millet in the study was similar with a study from Chandra et al. (2016) which reported that fat content in barnyard millet was 4.8%. The result from one-way ANOVA showed that fat content of little millet increased significantly after soaked ($p<0.05$). However, there were no significant difference in the fat content of boiled little millet and both soaked and boiled barnyard millet compared to the raw millet ($p>0.05$).

A previous study by Afify et al. (2012) showed that fat content of sorghum was increased after soaked. This finding was similar with the current study. The increase of the fat content in millet after soaked was probably due to the synthesis of fatty acids occurred during soaking. The conversion of synthetase from inactive form to active form occurred due to the presence of water (Kannangara et al., 1973). Furthermore, water was used as an osmotic medium for synthetase reaction to proceed (Obizoba & Atii, 1991). In the current study, boiling does not affect fat content of both little and barnyard millet. A study by Meera et al. (2011) found out that lipase activity in sorghum was retarded after 15 minutes heat treatment. Thus, retardation of lipase activity results in reducing lipid peroxidation and fat content loss due to heating. Therefore, fat content of millet in current study was not affected by boiling and probably due to the retardation of lipase activity of millets after boiled for 25 minutes. A study by Amadou et al. (2013) showed that millet oil can be a good source of natural oil rich in linoleic acid and tocopherols as millet contain high levels of linoleic, oleic and palmitic acid. Besides, the presence of linolenic and palmitic acid in millet could help in the development of neural tissue and brain (Ramashia et al., 2019). Therefore, fat content in millet could provide health benefits to the consumer.

4.4 Protein content

Table 4 shows the protein content of little and barnyard millet samples. Dry weight samples were used to determine protein content and results of protein content in the samples were expressed as percentage of protein content and standard deviation. Kjeldahl method was used to analyse protein content in the samples.

Table 4 Comparison of protein content in little and barnyard millet samples

Samples	Protein content (% ± SD)
Raw LM	6.32 ± 0.05^{d}
Raw BM	9.38 ± 0.17^{b}
Soaked LM	7.09 ± 0.09^{c}
Soaked BM	10.73 ± 0.47^{a}
Boiled LM	6.74 ± 0.04^{cd}
Boiled BM	9.73 ± 0.13^{b}

Results in the table are expressed as mean ± SD, n=3. Values in the same column with different superscripts are significantly different (*p< 0.05*, ANOVA, Tukey-HSD). LM indicates little millet while BM indicates barnyard millet.

In table 4, protein content of little and barnyard millet samples ranged between 6.32 ± 0.05% - 10.73 ± 0.47%. Soaked barnyard millet has the highest protein content (10.73 ± 0.47%) while raw little millet has the lowest protein content (6.32 ± 0.05%). Based on Recommended Nutrient Intakes for Malaysia (2017), recommended protein intake for Malaysians is 15-20% of total energy intake (TEI). Based on the finding of current study, proteins derived from plant proteins (millet grains) are considered incomplete protein; hence it can be mixed with other animal proteins to produce complete proteins and consumed together to fulfil the protein recommendation (Ministry of Health Malaysia, 2017). Approximately around 56.7g/day protein should be consumed to meet the recommended protein intake.

From the finding, protein content of raw little millet in the current study was slightly lower compared to the finding from Kamatar et al. (2013) which reported that protein content in little millet was 7.0%. Other than that, result of raw barnyard millet in the current study was slightly higher compared to the data from Indian Food Composition Tables (2017) with 6.20g per 100 g protein content of barnyard millet. The difference in the result obtained compared with the previous study could be due to different cultivar or millet genotypes originated (Shibairo et al., 2014).

Soaking caused protein content in both little and barnyard millet increased significantly ($p<0.05$). A previous study by Kajihausa et al. (2014) found out that the protein content of the sesame seed flour increases as soaking time increases from 26.09% to 47.81% after 16 hours soaking and this condition could be seen in the current study as protein content significantly increased with 24 hours soaking. However, a previous study by Ayurvedic et al. (2016) showed contrast finding as they found out that the protein content of barnyard millet slightly reduced after soaked for 4-6 hours. The difference in the finding was probably due to the difference in the duration of soaking. The increase in protein content after soaked in both millet was probably due to the increase of non-protein nitrogen as non-protein nitrogen increases with increase in the soaking time (Narsih et al., 2012). Non-protein nitrogen such as free amino acids, nucleic acids or amino sugars which resulted from the breakdown of protein may contribute to the increase in total nitrogen content and protein content in sample (Popping & Diaz-Amigo, 2014).

According to the one-way ANOVA, there were no significant difference in the protein content of both little and barnyard millet after boiled (*p>0.05*). Millet grains contain high amount of essential amino acids especially the sulphur containing amino acids such as methionine and cysteine. The presence of these heat stable proteins reduces the protein content loss during cooking (Karaś et al., 2019).

4.5 Total dietary fiber

Table 5 shows the total dietary fibre content of little and barnyard millet samples. Dry weight samples were used to determine total dietary fibre (TDF) content and results of total dietary fibre content in the samples were expressed as percentage of total dietary fibre content of its dry weight and standard deviation. TDF assay kit was used to analyse total dietary fibre content in the samples.

Table 5 Comparison of total dietary fibre content in little and barnyard millet samples

Samples	Total dietary fiber (% ± SD)
Raw LM	8.97 ± 0.02^{c}
Raw BM	8.42 ± 0.54^{d}
Soaked LM	6.90 ± 0.03^{e}
Soaked BM	5.19 ± 0.35^{f}
Boiled LM	10.80 ± 0.06^{ab}
Boiled BM	10.91 ± 0.04^{a}

Results in the table are expressed as mean ± SD, n=3. Values in the same column with different superscripts are significantly different ($p< 0.05$, ANOVA, Tukey-HSD). LM indicates little millet while BM indicates barnyard millet.

In table 5, total dietary fibre content of little and barnyard millet samples ranged between 8.42 ± 0.54% - 10.91 ± 0.04%. Boiled barnyard millet has the highest total dietary fibre content (10.91 ± 0.04%) while soaked barnyard millet has the lowest total dietary fibre content (5.19 ± 0.35%). Total dietary fibre content of raw little millet in the current study was higher compared to the data from Indian Food Composition Tables (2017) which contained about 6.39±0.60g per 100 g fibre content. Other than that, total dietary fibre of raw barnyard millet in the current study was slightly lower compared to the finding from Kajuna (2001) which found out that the dietary fibre content of barnyard millet was 9.8%. The difference in the result obtained compared with the previous study could be due to different cultivar or millet genotypes originated (Shibairo et al., 2014).

Soaking caused total dietary fibre content of both little and barnyard millet decreased significantly in the current study ($p<0.05$). A study conducted by Afify et al. (2012) also showed similar finding with the current study and found out that the fibre content of white sorghum decreased after soaked in water for 20 hours. The reduction in total dietary fibre content was due to the partial solubilisation of insoluble dietary fibre from cell wall into the soaking medium and thus reduced the total dietary fibre content (Njoumi et al., 2019). After boiled, total dietary fibre content of both little and barnyard millet have significantly increased in the current study ($p<0.05$). A study by Pushparaj & Urooj (2011) found out that total dietary fibre of pearl millet increased after boiled for 30 minutes. The increased of total dietary fibre content of pearl millet was due to the formation of resistant starch after boiling. The resistant starch in grains was formed due to the retrogradation of starch after gelatinization.

In addition, millet contains higher amount of total dietary fibre (8.42% - 8.97%) compared to other cereal grains such as rice (4.56%) and sorghum (7.40%) (Vasishtha & Srivastava, 2013). High dietary fibre content in millet can help to enhance satiety and promote satiation. Hence, it will reduce the risk of excessive weight gain and obesity.

4.6 Available carbohydrate content

Table 6 shows available carbohydrate content of little and barnyard millet samples. Results of available carbohydrate content in the samples were expressed as percentage of available carbohydrate content and standard deviation. Available carbohydrate content of samples in the study was determined using by-difference method.

Table 6 Comparison of available carbohydrate content in little and barnyard millet samples

Samples	Available carbohydrate (% ± SD)
Raw LM	67.28 ± 0.47^{ab}
Raw BM	65.87 ± 0.73^{a}
Soaked LM	46.49 ± 0.76^{cd}
Soaked BM	46.43 ± 0.47^{c}
Boiled LM	7.63 ± 0.31^{e}
Boiled BM	2.64 ± 0.26^{f}

Results in the table are expressed as mean ± SD, n=3. Values in the same column with different superscripts are significantly different ($p < 0.05$, ANOVA, Tukey-HSD). LM indicates little millet while BM indicates barnyard millet.

In table 6, available carbohydrate content of little and barnyard millet samples ranged between 2.64 ± 0.26% - 67.28 ± 0.47%. Raw little millet has the highest available carbohydrate content (67.28 ± 0.47%) while boiled barnyard millet has the lowest available carbohydrate content (2.64 ± 0.26%). The result of available carbohydrate content of raw little and barnyard millet in the current study was similar to the data from Indian Food Composition Tables (2017) which reported about 65.55% carbohydrate content in both little and barnyard millet. Carbohydrate content of millet in the current study (65.87 %- 67.28%) is comparable with other cereal grains such as wheat (62%) and maize (64.3%) (Devi et al., 2014).

Soaking caused available carbohydrate of both little and barnyard millet decreased significantly in the current study (*$p<0.05$*). A study by Ocheme et al. (2011) showed soaking reduce the carbohydrate content of flour from soaked millet grains compared to raw millet grains. The reduction of carbohydrate content after soaking was due to the solubility of total soluble sugar in water and thus reduces the starch contents (Rehman et al., 2001). In the current study, there was significantly decreased in available carbohydrate content after boiled in both little and barnyard millet (*$p<0.05$*). A study by Emmanuel Chukwuma (2016) also showed significant decrease in the carbohydrate content of quality protein maize after boiled. The reduction in available carbohydrate content may due to the solubility of sugar is much higher in high temperature compared at room temperature (Rehman et al., 2001). Hence, it will cause greater reduction in available carbohydrate content after boiled. Carbohydrate is a source of energy for human. Deficient of carbohydrate may cause the usage of protein and body fat to be used to produce energy and thus it will lead to the depletion in body tissues (Ikanone & Oyekan, 2014). Therefore, other sources of carbohydrate such as legumes or nuts should be incorporated in the diet to ensure adequate carbohydrate intake.

4.7 Anti-nutrient content (tannin)

Table 7 shows the total tannin content of samples based on the Folin-Ciocalteu method by Dwivedi et al. (2015). The dry weights of the samples were used to determine total tannin content and results were expressed as milligram of Tannic Acid Equivalent (TAE) per g of sample and standard deviation.

Table 7 Comparison of total tannin content in little and barnyard millet samples

Samples	mg TAE/g sample (mean ± SD)
Raw LM	2.57 ± 0.43^{b}
Raw BM	3.91 ± 0.75^{a}
Soaked LM	1.79 ± 0.60^{b}
Soaked BM	3.40 ± 0.17^{a}
Boiled LM	1.34 ± 0.15^{b}
Boiled BM	1.88 ± 0.75^{b}

Results in the table are expressed as mean ± SD, n=3. Values in the same column with different superscripts are significantly different ($p < 0.05$, ANOVA, Tukey-HSD). LM indicates little millet while BM indicates barnyard millet.

From table 7, total tannin content in little and barnyard millet samples ranged between 1.34 ± 0.15 - 3.91 ± 0.75 mg TAE/g sample. Raw barnyard millet has the highest total tannin content (3.91 ± 0.75 mg TAE/g sample) while boiled little millet has the lowest total tannin content (1.34 ± 0.15 mg TAE/g sample). A study by Pradeep & Guha (2011) reported that little millet of Samai-4 type has 2.83mg/g tannin content which was similar with the finding from current study. Another study by Panwar et al. (2016) also found out that tannin content in finger millet (2.18 mg/g) showed similar finding with the tannin content of little millet in current study. Total tannin content of raw barnyard millet in current study was similar to a study by Panwar et al. (2016) that reported 3.96 mg/g tannin in barnyard millet.

Tannin had the ability to form tannin-protein complexes and thus reduce the protein digestibility and other mineral bioavailability such as iron or zinc. Several preparation methods may positively influenced the quality of millet for consumption. In the current study, there was a significant decreased in the total tannin content of barnyard little millet ($p<0.05$). However, there were no significant difference in the total tannin content of little millet after boiled and in both little and barnyard millet after soaked ($p>0.05$). In the current study, there was a slight reduction of total tannin content in both soaked little and barnyard millet compared to raw. However, no significant difference observed statistically ($p>0.05$). In comparison with previous study by Singh et al. (2017), they reported on the decreased in tannin content of pearl millet after soaked. This is due to the characteristic of tannin as water-soluble and soaking caused leaching of the water-soluble tannin into water. Nevertheless, slight reduction of tannin in the current study was not affecting the total tannin content. Furthermore, soaking condition such as temperature and its duration might affect the total leaching of anti-nutrient during soaking.

In the current study, boiling significantly reduced the total tannin content of barnyard millet ($p<0.05$). The finding from current study was consistent with a study by Folasade (2011) which found out that the tannin content in finger millet was reduced after boiling. Another study by Mohapatra et al. (2019) also found out the reduction in tannin content of sorghum after undergo boiling process. This is due to the degradation of tannin that occurred when heating as it is heat labile compound and heating result in leaching the tannin into water.

In conclusion, boiling could help in reducing the anti-nutrient in millet through leaching the tannin into water and soaking medium such as hot temperature or longer soaking duration might enhance the loss of tannin in the samples. Therefore, preparation methods can help to improve the bioavailability of minerals and other nutrients in millet, thus reduce the risk of malnutrition.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In conclusion, the finding from the current study shows that soaking and boiling could affect the proximate composition and anti-nutrient content of millet. Soaking increased the moisture, protein and fat content significantly but decreased the ash, available carbohydrate and total dietary fibre content of little millet significantly ($p<0.05$). Meanwhile, as for boiling, it increased the moisture and total dietary fibre content but reduced the ash and available carbohydrate content of little millet significantly ($p<0.05$). However, there were no significant differences in the total tannin content of little millet after soaked ($p>0.05$). Besides, there were no significant differences in the protein, fat and total tannin content of little millet after boiled ($p>0.05$).

On the other hand, soaking significantly increased the moisture and protein content but significantly decreased the ash, available carbohydrate and total dietary fibre content of barnyard millet ($p<0.05$). Boiled barnyard millets have significantly higher moisture and total dietary fibre content but significantly lower ash, available carbohydrate and total tannin content ($p<0.05$). However, there were no significant differences in the fat and total tannin content of barnyard millet after soaked

($p>0.05$). There were also no significant differences in the protein and fat content of barnyard millet after boiled ($p>0.05$).

From the current study, the results showed that boiling is the best method to prepare millet for consumption. This is because boiling significantly increases the dietary fibre content and significantly reduced the total tannin content in millet. High dietary fibre in millet will help to enhance satiety and thus reduce the risk of excessive weight gain and obesity. Reducing anti-nutrient content in millet positively influence the mineral bioavailability and thus provides health benefits to the consumer. Therefore, boiling can be a good method to reduce the anti-nutrient content in millet grains and improved the dietary fibre content of millet.

Besides, from the findings, the effect of soaking and boiling on little millet and barnyard millet can be seen clearly. Therefore, consumers can make a better choice in consuming millets while retaining their high nutritional value in it.

5.2 Limitations and Recommendations

There were some limitations in the current study. Firstly, the current study only determines two preparation methods used for the consumption of millet which were soaking and boiling. There was no analysis on the effect of soaking and further boiling on the millet. As for recommendation, future study can explored on the effect of soaking and continued with boiling as some of the consumers usually soaked the millet first before they cooked. Thus, researchers can understand more on the effect of further processing on the nutrient and anti-nutrient content of millet. Besides, roasting is one of the method used by consumer to prepare millet for consumption. Future study can explore on the effect of roasting to the proximate composition and anti-nutrient content of millet and thus provide more options on the preparation methods that maintain high nutrient content to the consumer.

Other than that, the current study only studied one of anti-nutrient content in millet grains which was tannin. Other anti-nutrient such as phytic acid or oxalate also presents in millet grains and can inhibit the mineral bioavailability. Thus, future study can be explored on these anti-nutrients and its effect to the proximate composition of millet. Last but not least, variety of anti-nutrients methods should be conducted to obtain more reliable and accurate results. As for examples, future study can used vanillin method to determine tannin content as the method specifically measure on the condensed tannin in grains. Besides, only one solvent which is water was used in the current study to extract tannin content in the samples. Therefore, future study can use other solvents such as methanol or ethanol to extract tannin in the samples as tannin is soluble in polar solvents.

In conclusion, these recommendations were important for future study to fill the limitations of the current study. More studies on minor millets should be done in Malaysia to introduce and promote the consumption of millet to Malaysia.

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APPENDICES

Appendix A: Raw millet grains, soaked millet and boiled millet

Raw millet grains

Soaked millet

(soak for 24 hours)

Boiled millet

(boiled for 25 minutes)

Appendix B: Standard curve of tannin determination

Table 8 The absorbance value of the standard curve in different concentration

Concentration of standard curve (μg/ml)	Absorbance at 700 nm
0.19	0.016
0.39	0.020
0.78	0.026
1.56	0.063
3.125	0.053
6.25	0.107
12.5	0.195
25	0.427
50	0.870

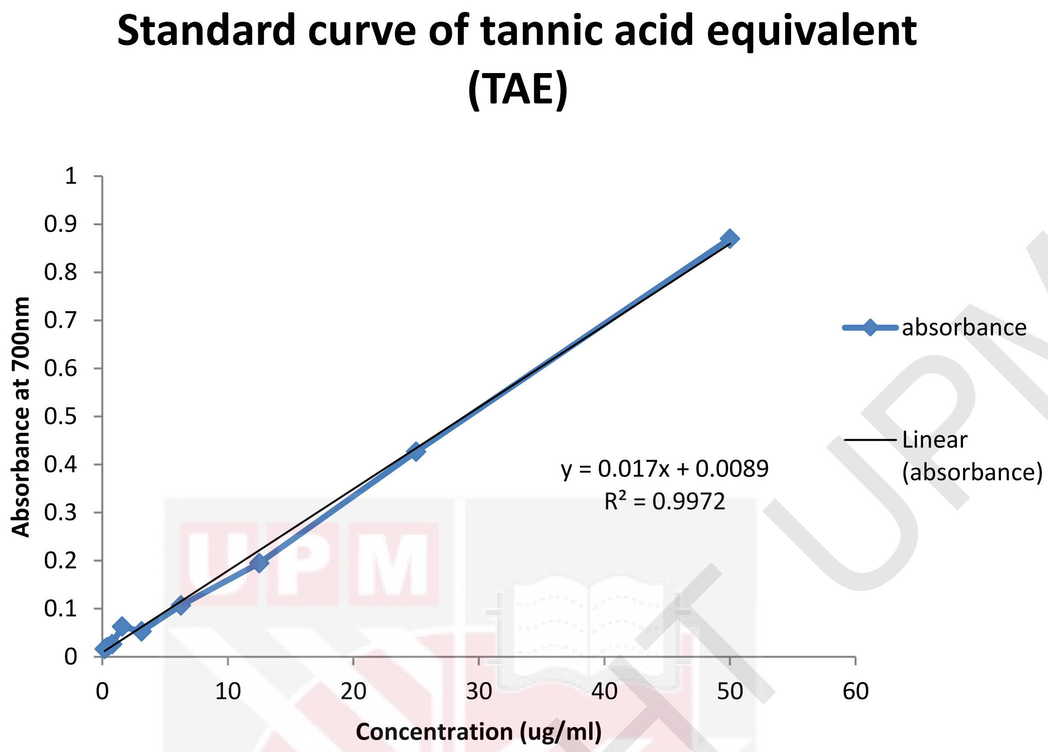

Figure 5 Standard curve of tannic acid equivalent (TAE)