UNIVERSITI PUTRA MALAYSIA

DETERMINATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITIES BY COLD AQUEOUS, ETHANOL AND METHANOL EXTRACTIONS OF BARNYARD MILLET (Echinochloa frumaentacea) AND LITTLE MILLET (Panicum sumatrense)

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BY

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DETERMINATION OF TOTAL PHENOLIC CONTENT, TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITIES BY COLD AQUEOUS, ETHANOL AND METHANOL EXTRACTIONS OF BARNYARD MILLET (*Echinochloa frumaentacea*) AND LITTLE MILLET (*Panicum sumatrense*)

Izzah Madihah Hannah binti Bostamam

Millet are small-seeded whole grains with various type and genetics of millet also known as drought-resistance crops with unique speciality that grown in tropical and semi-arid region which can withstand high temperature and dry condition. Millet are whole grains contain important source of fibre, vitamins, minerals, and phytochemicals. The aim of this study is to measure antioxidant contents and antioxidant activities of Barnyard millet and Little millet with three different extractions such as cold aqueous, 80% methanol and 70% ethanol. The antioxidant content and antioxidant activities were determined by using Total phenolic content (TPC), Total flavonoid content (TFC), Ferric reducing antioxidant power (FRAP) and DPPH radical scavenging. One-way ANOVA and Pearson correlation was used in the study. Total phenolic content in Barnyard millet and Little millet are the highest in cold aqueous extract which are 1638.976mg GAE/g and 1318.633mg GAE/g compared with other extracts. Meanwhile for total flavonoid content, the results show 675.440mg CE/g and 825.627mg CE/g in cold aqueous extract of Barnyard millet and Little millet, respectively. The antioxidant activity using ferric reducing antioxidant power assay in cold aqueous were the highest value among other extracts in both Barnyard millet and Little millet which are 0.51mmol ferrous/g and 0.42mmol ferrous/g. For radical scavenging activity, the highest value is in 80% methanol extract for both Barnyard millet and Little millet which are 12.627% and 20.47% compared to the other extracts. There is a significantly positive correlations of phenolic content, flavonoid content with ferric reducing antioxidant power assay in both samples but there are no positive significant results in both samples using DPPH radical scavenging activity. Variations of millet used, solvent extract used and different concentration use may affect the study. Different extract in different sample can be additional information on antioxidant content and antioxidant activity in cereal grains. This information could potentially be exploited in the future to extract antioxidant sources especially from millet that can be applied in our daily diet.

ABSTRAK

MENENTUKAN JUMLAH KANDUNGAN FENOLIK, JUMLAH KANDUNGAN FLAVONOID DAN AKTIVITI ANTIOKSIDAN DARI *BARNYARD MILLET* (*Echinochloa frumaentacea*) DAN *LITTLE MILLET* (*Panicum sumatrense*) DENGAN EKSTRAK AIR SEJUK, METANOL DAN ETANOL

Izzah Madihah Hannah binti Bostamam

Milet adalah bijirin berbiji kecil dengan pelbagai jenis dan genetik milet yang juga dikenali sebagai tanaman unik yang tumbuh di kawasan tropika dan separa gersang yang dapat menahan suhu tinggi dan dalam cuaca yang kering. Bijirin penuh millet mengandungi sumber serat, vitamin, mineral, dan fitokimia yang penting. Tujuan kajian ini adalah untuk mengukur kandungan antioksidan dan aktiviti antioksidan *Barnyard millet* dan *Little millet* dengan tiga pengekstrakan yang berbeza iaitu ekstrak air sejuk, 80% metanol dan 70% etanol. Kandungan antioksidan dan aktiviti antioksidan ditentukan dengan menggunakan kaedah iaitu kandungan total fenolik (TPC), kandungan total flavonoid (TFC), dan untuk mengukur aktiviti antioksidan, menggunakan kekuatan antioksidan pengurangan Ferrik (FRAP) dan kekuatan pengambilan radikal (DPPH). *One-way* ANOVA dan *Pearson correlation* telah digunakan didalam kajian ini. Jumlah kandungan fenolik didalam *Barnyard millet* dan *Little millet* dari ekstrak air sejuk menunjukkan nilai yang tertinggi iaitu 1638.976mg GAE/g dan 1318.633mg GAE/g dibandingkan dengan ekstrak lain. Selain itu, untuk jumlah kandungan total flavonoid adalah 675.440mg CE/g dan 825.627mg CE/g yang merupakan nilai tertinggi didalam ekstrak air sejuk bagi *Barnyard millet* dan *Little millet*. Aktiviti antioksidan menggunakan kekuatan antioksidan pengurangan Ferrik di dalam ekstrak air sejuk menunjukkan nilai tertinggi berbanding ekstrak lain didalam *Barnyard millet* dan *Little millet* iaitu 0.51mmol ferrous/g dan 0.42mmol ferrous/g. Untuk pengambilan radikal DPPH, ekstrak 80% methanol menunjukkan nilai tertinggi bagi kedua-dua *Barnyard millet* dan *Little millet*, 12.627% dan 20.47% berbanding dengan ekstrak lain. Terdapat korelasi positif signifikan diantara kandungan total fenolik, kandungan total flavonoid dan kekuatan antioksidan pengurangan Ferrik dan tiada nilai positif signifikan pada kedua sampel menggunakan kekuatan pengambilan radikal DPPH. Penggunaan dari pelbagai jenis millet, perbezaan ekstrak pelarut dan penggunaan kepekatan pelarut yang berbeza boleh mempengaruhi kajian ini. Perbezaan ekstrak dalam sampel yang berbeza dapat menjadikan makumat tambahan untuk kandungan antioksidan dan aktiviti antioksidan dalam bijirin. Maklumat berkaitan ekstrak antioksidan terutama sekali didalam millet ini berpotensi untuk digunakan pada masa akan datang dan dapat diterapkan dalam diet harian kita.

CHAPTER 1

1.1 Study background

Cereals and grains are widely grown around the world. There are many types of cereals and grains such as maize, rice, barley, quinoa, sorghum, millet, wheat, teff and soybeans (Food and Agriculture Organization (FAO), 2011). According to Brown, Walter, and Beathard, (2015), they state that the most major food crops in the world is grain which consists of several types. About 95% of world's production of grains are corn, rice, wheat and barley. The other 5% of grains are oats, sorghum, rye and millets. In Malaysian food pyramid, the main source of energy and a major component at level 1 (base level) consists of rice, noodle, bread, cereal, cereal products and tubers (Malaysian Dietary Guidelines (MDG), 2010). Moreover, it is a great source of vitamins, minerals and also dietary fibre. It is recommended to consume whole grains as it consists more fibre compared to others. Dietary fibre helps in regular bowel movements, good gut health, lowering cholesterol and blood glucose (MDG, 2010). As stated in previous study from (Chandrasekara & Shahidi, 2011), major world cereals include maize, wheat, and rice whereas minor cereals include oat, barley, sorghum, rye, buckwheat, and millet.

In the early human civilization, millets are considered as first cultivated cereal grains. Common millet known as proso millet was domesticated as a staple food in Northern China for 10,000 years ago (Lu et al, 2009). Millet are group in gluten-free cereal grains which mostly found in Africa and Asia (Brown, Walter, & Beathard, 2015). Now, millets becoming common staple foods for populations in Asian and African countries especially among lower income levels and in western countries.

They used millet as feed, forage livestock and also as an important ingredient in the making of multigrain and gluten-free cereals products (Chandrasekara & Shahidi, 2011). Millets are grown in tropical and semi-arid regions of the world (Chandrasekara & Shahidi, 2011). Millet are drought-resistant crops and it is ranked as 6th cereal crop in world agriculture production (Saleh, Zhang, Chen, & Shen, 2013). In addition, it requires warm weather and fast maturity to grow millet in the hot summer months (Anju & Sarita, 2010).

According to Saleh, Zhang, Chen, and Shen, (2013) millets are small-seeded with different varieties and belong to several plant taxonomic groups (Chandrasekara & Shahidi, 2011) such as pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum setaceum*), proso millet (*Penicum miliaceum*), foxtail millet (*Setaria italic*), little millet (*Panicum sumatrense*), and barnyard millet (*Echinochloa utilis*). Other than that, good criteria of millet is pest and disease resistance, it also a short growing season and productivity under drought conditions compared with other cereals (Devi et al., 2011). Due to that, some developing countries use millet as food and use in manufacturing of bioethanol and biofilms (Li et al., 2008).

Other than that, millets is also rich in insoluble fibre that relatively high in antioxidant activity such as phenolic acids, flavonoids, and tocopherols. In addition to their nutritive value, several potential health benefits such as preventing cancer and cardiovascular diseases, reducing tumor incidence, lowering blood pressure, risk of heart disease, cholesterol and rate of fat absorption, delaying gastric emptying, and supplying gastrointestinal bulk have been reported (Saleh et al., 2013). It is reported that millet such as finger millet grains contain high polyphenols as compared to rice, barley, wheat and maize. The phenolic compounds found at the aleurone layer, testa and pericarp which form main components of bran functions as free, soluble conjugated soluble and insoluble bound formed (Viswanath, Urooj, & Malleshi, 2009).

Research showed that millet provide dietary fibers, proteins, energy, minerals, vitamins and antioxidants that required for human health. Millet are rich source of energy and contained 60-70% carbohydrates, 7-11% proteins, 1.5-5% fat, 2-7% crude fibre, minerals and vitamins (Food and Agriculture Organization (FAO), 1995). Some of the millet such as finger millet contain high fat content from 3.5% to 5.2% as compared to other cereals also has high calcium content of 350 mg/100g (FAO, 1995) and rich in iron and phosphorus. Generally, the nutrient content in most millets were higher compared to rice and wheat as they have high amount of proteins, dietary fibers, iron, zinc, calcium, phosphorus, potassium, vitamin B, and essential amino acids (Singh et al., 2018).

As stated by Chandrasekara and Shahidi (2011) various types of millet contain rich sources of *in vitro* antioxidant and anti-proliferative phenolic compounds which linked to the cell wall polysaccharides and constitutes the insoluble bound phenolic in grains. Millets contain mainly free and conjugated forms of phenolic acids, which derivatives of hydroxybenzoic and hydroxycinnamic acids. Severals flavonoids were also found in millet such as anthocynidins, falvanols, flavones, flavonones, chalcones and aminophenolic compounds. Phenolic acids and flavonoid found in millet may vary based on its content of the grain (Shahidi and Chandrasekara, 2013).

1.2 Problem statement

Free radicals that occur in body systems can cause oxidative stress that mostly present in related to chronic diseases. Antioxidants mostly found in fruits and vegetables and some studies mentioned whole grains contain dietary fibre with the presence of antioxidants. However, there is lack of studies in determine antioxidant content and antioxidant activities specifically in Barnard millet and Little millet. In addition, there are few of articles that determine the antioxidant content and activities using different extraction method. Most studies of millet were focused on nutrition compositions and minerals. Not only that, there is insufficient data of millets in Malaysia which create a gap to compare with other study. Besides, it is important to emphasize nutrition knowledge regarding other cereal grains such as millet which is not familiar among Malaysian society.

1.3 Significance of study

The result of this study will give a new information regarding phenolic and flavonoid content also antioxidant activity in Barnyard and Little millet. Furthermore, new findings on antioxidant content and activities in Barnyard and Little millet could be a potential source in reducing oxidative stress that cause by related chronic diseases in Malaysia. However, the content of antioxidant in millets depend on the variety used thus there has been limited findings and research of millet as potential antioxidant by modulating phenolic and flavonoids which are considered important for prevention of non-communicable diseases. This study will increase the knowledge of society regarding the benefits of antioxidants in millet compared to other cereal grains. Besides that, this study will provide information of millet that can be a potential source of antioxidant for additional ingredients in food and pharmaceutical. Lastly, this study will contribute for further analysis to an existing evidence-based study that may benefit local understanding and knowledge.

1.4 Research Objectives

1.4.1 General Objective

To determine the total phenolic content, total flavonoid content and antioxidant activity of Barnyard millet and Little millet with cold aqueous solution, ethanol and methanol extractions.

1.4.2 Specific Objectives

1. To determine total phenolic content and total flavonoid content of Barnyard millet and Little millet in different solvent extracts using Folin-Ciocalteu's method and aluminium chloride colorimetric assay.

2. To determine antioxidant activity of Barnyard millet and Little millet in cold aqueous solution, ethanol and methanol extracts using DPPH assay and FRAP assay.

3. To compare total phenolic content, total flavonoid content and antioxidant activity of Barnyard millet and Little millet in cold aqueous, methanol and ethanol extractions.

4. To determine the correlation between total phenolic content and total flavonoid content and antioxidant activity between Barnyard millet and Little millet.

1.5 Null Hypothesis

1. There are no significance difference in total phenolic content and total flavonoid between Barnyard millet and Little millet in cold aqueous solution, ethanol and methanol extracts.

2. There are no significance difference in antioxidant activities between Barnyard millet and Little millet in cold aqueous solution, ethanol and methanol extracts.

3. There are no significance associations between total phenolic content, total flavonoid and antioxidant activities between Barnyard millet and Little millet.

CHAPTER 2

2.1 Whole grains

Grains are the world's major food crops and grain products which are divided into two subgroups namely whole grains and refined grains. Whole grains are made up of three edible layers- bran, germ and endosperm which contain high amount of nutrients and dietary fibre as stated by Brown, Walter, and Beathard (2015). A review on whole grain conducted by Liu (2007) found that endosperm or starchy endosperm makes up 75-80% of grain weight while remaining total weight is the germ and bran which may vary according to different type of grains and varieties. Whole grains classified into major grains and minor grains. Major grains such as wheat, rice and corn are crucial in human diets. For minor grains like oats, barley, rye, triticale, sorghum, millet and buckwheat are different type of crops and species which not important food (Liu, 2007). As for refined grains, most of them consist of only starchy inner part of the grain, while the bran and germ were removed (Malaysian Dietary Guideline, 2010). This grain-refining process or milling process which remove the bran and germ layer benefits in yielding finer texture and improved shelf life of the grain. However, this process may also stripped of the dietary fibre, iron and B vitamins from the grains.

It plays an important role in decreasing risks of many disorders such as diabetes, cardiovascular diseases, and constipation (Champ & Guillon, 2000). There were findings recommend that diet play an important role in preventing chronic degenerative diseases. Previous study found that the bran or germ of whole wheat are concentrated sources of bioactive compounds and may impart a large health benefits which can be incorporated as a part of meals, thus can reduce risk of chronic diseases (Liu, 2007). A local epidemiological study conducted by Norimah et al. (2015) proves that the consumption of whole grain maybe protective against several chronic diseases such as diabetes, obesity, cardiovascular disease and colorectal cancer among adults. Similarly, other epidemiological evidences also show that whole-grain may protect the body against age-related disease such as diabetes, cardiovascular diseases, and some cancers (Fardet and others, 2008; Saleh, Zhang, Chen, & Shen, 2013). Strong evidence from the Dietary Guidelines Advisory Committee (USDA, 2015a) states that higher consumption of vegetables, fruits, whole grains, regular consumption of nuts and legumes and diets are rich in fibre may reduce the cardiovascular risk (RNI, 2017).

2.2. Millet

Based on Shahidi and Chandrasekara (2013), cereal grains provide a significant amount of energy, protein, selected micronutrients and non-nutrients in the diet of peoples in the world. Based on (Food and Agricultural Organization (FAO), 1997) maize, rice, wheat, barley, sorghum, oat, rye and millets are important cereals in the world. Millet are considered as the oldest food cultivated from the early human civilization and previous study found that millet was domesticated as a staple food 10,000 years ago in China (Lu et al., 2009). Millets are small seeded grains and commonly referred as small seeded grass (Singh et al., 2018). Singh et al. (2018) pointed out that about 90% of millet were utilized as fodder in developing countries as well as source of food for low income populations. According to FAO (1995), millets are warm-season cereals which are served as food, animal feed, planting seed, bird seeds and beer. One of the unique aspects of millet is that it grows on marginal lands in dry areas in temperate, subtropical and tropical regions. In addition, due to its productivity and short growing season under dry, high-temperature conditions, millet has become favoured crop option and received special attention from developing countries in contribution to national food security aspect (Saleh et al., 2013). Therefore, having millet to be also known as drought-resistant crops, it is positioned in the world sixth cereal crop in agriculture production.

Millet is also known as coarse cereals besides maize (Zea mays), sorghum (Sorghum bicolor), oats (Avena sativa), and barley (Hordem vulgare) (Singh et al., 2018). Millet is generic name of grains as it does not belong to a single species or single genus. Various species differ in their physical characteristics, quality attributes, soil and climatic requirements, and growth duration (Singh et al., 2018). The most popular millet type is pearl millet (*Pennesetum glaucum*), followed by foxtail millet (*Setaria italic*), proso millet (*Panicum miliaceum*), and finger millet (*Eleusine coracana*). Some minor millets are kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*), barnyard millet (*Echinochloa utilis*) and teff millets. Various types and species of millet brings about different conditions of production and some countries produce different types of millet which gives a good source of food to animal particularly in developed countries where they produced limited quantity of millets for a high-value specialty market such as bird seed (Singh et al., 2018).

Barnyard millet (*Echinochloa utilis*) is a type of millet which is the fastest in growing as compared to with other millets. Besides, the grains will usually mature in 45 days after sowing under favourable moisture and temperature conditions. Barnyard millet is mostly developed in the Orient and India grown as a forage crop in the USA that produce eight harvests per year in the United States (FAO, 2001). Based on Singh et al. (2018) stated that barnyard millet is the fastest to reproduce as compared to other millets with only 6 weeks of harvesting period and is predominantly cultivated in India, China, Japan, and Korea for food and also as fodder.

Little millet (*Panicum sumatrense*) is grown in India up to altitudes of 2100m. It is generally shorter, with small panicles and seeds. Little millet can appear to thrive under conditions where no other plant will survive despite receiving relatively less attention from plant breeders. It takes 2.5 to 5 months to mature due to that, little millet is generally produced less than 0.5 tonnes per hectare and may reach up to 1 tonnes per hectare under favourable conditions (FAO, 2011). In Eastern Ghats of India, little millet was domesticated and occupied a major portion of the diet of the tribal people and spread to Sri Lanka, Nepal, and Myanmar (Singh et al., 2018).

2.3. Health benefits of millet

Millet was reported to offer potential health benefits to its consumers such as preventing cancer and cardiovascular diseases, reducing tumor incidence, lowering blood pressure, reducing the risk of heart disease, cholesterol and rate of fat absorption and delaying gastric emptying also supply gastrointestinal bulk (Trustwell, 2002; Gupta and others, 2012). Millets are rich in iron and phosphorus, as well as phytochemicals that give health benefits by preventing and delaying the onset of non-communicable diseases (NCDs). In the North American and European, millet was not placed as an important commodity in their usual diet. However, the importance of millet as an ingredient in multigrain and gluten-free cereal products has been focused in these continents.

In addition, epidemiological evidence showed that diets rich in plant foods are protective against several degenerative diseases such as cancer, cardiovascular ailments, diabetes, metabolic syndrome and Parkinson's disease (Saleh, Zhang, Chen and Shen, 2013). Different type of millets possess different benefits (dehulled, debran, defatted, etc) and other benefits from millets may also be contributed from the effect of method and technologies process, and extractions of millet. Diabetic patients may also benefit from millet consumption, for example, finger millet based diets significantly lower plasma glucose levels, mean peak rise, and area under curve which may be due to high fiber content in finger millet as compared to rice and wheat (Kumari and Sumathi, 2002).

To add, it was found that dehulled and heat-treated of barnyard millet give positive impact to type 2 diabetics in which as dehulled millet has low glycemic index of 50.0 while heat-treated barnyard millet has glycemic index of 41.7 (Ugare and others, 2011). Besides, 70% ethanol extracts and aqueous extract of foxtail millet and proso millet have been reported beneficial for diabetic related diseases and shown the milling fractions in millet can be used for future various food products particularly those of diabetics. However, the study was conducted among animal and limited study was performed on living humans.

Moreover, the benefits millet for cardiovascular diseases have been reported based on study conducted on hyperlipidemic rats where the concentrations of serum triglycerides were found to be significantly lower in finger millet and proso millet than white rice and sorghum. Thus, the concentrations of serum total, HDL, and low-density lipoprotein (LDL) in white rice, finger millet and proso millet are lower than in sorghum in which it can be concluded that finger millet and proso millet can overcome cardiovascular disease by reducing plasma triglycerides in hyperlipidemic rat (Lee and others, 2010). There is however limited research conducted among different types of millets in combating cardiovascular disease and limited study was conducted in human trials.

As millet are considered gluten-free cereal grains and have positive impact on those who have celiac diseases where celiac disease is one of the commonest lifelong disorders affecting in human life around the world (Catassi & Fasano, 2008). Previous studies also found that millet can reduce risk of cancer such as colon, breast, oesophageal cancer as millet contain anti-nutrient such as phenolic acids, tannins, and phytate (Graf & Eaton, 1990; Van Rensburg, 1981; Thompson, 1993). There is no specific type and species of millet, however, there are potential of millet and its fractions that may be useful in preventing cancer and producing food products for individuals with celiac disease in the future (Saleh, Zhang, Chen & Shen, 2013).

2.4. Principle of antioxidants

Antioxidants plays a role in delaying or preventing oxidative stress from producing reactive oxygen species (ROS) during metabolism. Antioxidant compounds such as phenolic acids, polyphenols and flavonoids can be very important as these compounds help in preventing oxidative mechanisms that lead to degenerative diseases such as heart disease, cancer, cataracts, brain dysfunction and arthritis. It scavenge free radicals like peroxide, hydroperoxide or lipidperoxyl and reactive oxygen species. According to Yu, Perret, Davy, Wilson, and Melby (2002) wheat and oat based food products may be important sources of dietary antioxidants as the study found that antioxidant properties in oat flour, may contribute to health benefits of grain-based foods in lowering the incidence of aging-related chronic diseases including heart disease and cancers. It may function as free radical scavengers, reducing agents, chelating agents for transition metals and activators of anti-oxidative defense enzyme systems to suppress radical damage in biological systems. However, the antioxidant potential and bioavailability of cereal antioxidants may differ depending on the type, species and varieties, fractions of the grain and processing conditions.

In normal metabolism, reactive oxygen species (ROS) or free radicals are formed as by-products or intermediates. ROS consists of oxygen radicals (hydroxyl radical) and some non-radical reactive derivatives of oxygen (hydrogen peroxide) as stated by Yu et. al (2002). Formation of ROS or free radicals will increase the levels of ROS and free radicals which can cause damage to nucleic acids, proteins, and membrane lipids which are also associated with aging-related health problems such as heart diseases. There are a variety of sources that may lead to the occurrence of high reactive free radicals and oxygen species in the biological system. Therefore, one of the potential stressors in aerobic organisms is the reduced derivatives of oxygen which act as a part of normal physiological and metabolic processes and lead to the formation of reactive oxidative species (ROS). Moreover, with high amount of free radicals production and lipid peroxidation indicates the pathogenesis of diseases such as heart disease, diabetes, cataract and ageing (Dar, 2011). As ROS are toxic and can oxidize biomolecules which eventually leads to cell death and tissue injury. A study conducted by Morrissey and O'Brien (1998) found that oxidative stress and ROS had been associated with assortments of chronic disease such as coronary heart disease (CHD), certain cancers, rheumatoid arthritis, diabetes, retinopathy of prematurity, chronic inflammatory disease such as gastrointestinal tract, disease associated with cartilage, Alzheimer's disease, other neurological disorders and ageing process.

Free radicals are formed through four endogenous sources of ROS which on normal aerobic metabolism, the mitochondria needed oxygen and produce water along with by-products superoxide anion, hydroxyl radical and hydrogen peroxide, H_2O_2. Degradation of fat produces hydrogen peroxide as a by-product which were then degraded by catalase and some the by-products enter to other parts and causes oxidative damage. Free radicals can be formed as the primary defence against xenobiotics and endogenous substances, thus increasing the production of free radicals. In phagocytic activity, a part of superoxide anion may produce and the cells destroy bacteria or virus-infected cells (Morrissey and O'Brien, 1998). However, the content of antioxidant in millets depend on the variety used thus there has been limited findings and research of millet as potential antioxidant by modulating phenolic and flavonoids which are considered important for prevention of non-communicable diseases. Thus, the potential of antioxidant and bioavailability of cereal antioxidants may depend on the species and varieties of grains, fractions of the grain such as bran, flour, or whole grain also processing conditions (Yu, Perret, Davy, Wilson, and Melby, 2002).

2.5. Importance of antioxidant

Other than their function in plants, phenolic compounds in our diet can provide health benefits associated with a reduced risk of chronic diseases. Variety of foods including fruits, vegetables and grains contain phenolic compounds such as polyphenols. However, the concentrations and the types of compounds may differ according to different types of food due to several factors such as genetic, environmental, and processing conditions. For example, there is a study of cereal; rice where several compounds had been found such as phenolic acids and anthocyanins. Several studies have reported that phenolic, tocopherols and fibre in wheat bran have a positive effect on cardiovascular disease also on metabolic activities including reduced plasma cholesterol in laboratory animals and humans. The study stated that rice bran classified as a potential source of high-value antioxidants for additional ingredients in foods, pharmaceuticals, and cosmetics (Yu et al., 2002). Besides, antioxidants are important in preventing oxidative diseases which are on the rise these days. Bound phytochemicals which consist of phenolic and flavonoids can survive inside the stomach and intestinal digestion before reaching the colon thus can prevent the whole digestive tract from any oxidative diseases (Suma & Urooj, 2012).

2.6. Phenolic compounds

In plants, phenolic are the products of secondary metabolism in which they provide essential functions on reproduction and growth of plants, act as security mechanisms which protect from pathogens, parasites and predators, and supply colour to the plant (Liu, 2007). Phenolic compounds are important as a protector for plants from the absorption of harmful short high energy wavelengths at the electron rich parts of sunlight such as double bond of aromatic rings which help to reduce oxidative stress (Shahidi & Yeo, 2016). Shahidi and Yeo (2016) also pointed out that, phenolic can be classified into few groups such as phenolic acids, flavonoids, stilbenes, coumarins, lignins and tannins. Phenolic acids include hydrobenzoic acids such as Gallic, p-hydroxybenzoic, vanillic, syringic, protocatechuic and ellagicacids also hydroxycinnamic acids such as p-coumaric, caffeic, ferulic, sinapic and chlorogenic acids. As for flavonoids, it is a compound which composed of three ring structure based on different substitution patterns of hydroxyl and methoxy groups in which it creates peculiar classes of compounds such as flavones, flavononse, flavonols, flavavonols, isoflavones, flavanols and anthocyanidins (Shahidi & Yeo, 2016).

Phenolic compounds can be formed into soluble and insoluble bound forms based on the location of phenolic at different states and undergo process, mostly in plants. Seeds or legumes comprises few parts and layers. The outer part is the seed coat or hull, while the inner part consists of endosperm, epicotyl, hypocotyl and radicle. Endosperm contains storage cells, which imply a low number of phenolic-containing cells. However, the outer part which are seed coat or hull, consists of epidermis, hypodermis, chlorenchyma, palisade, parenchyma and endothelium cells, in which contain vacuoles and cell walls. The vacuoles and cell walls contain high amounts of phenolics in soluble and insoluble-bound forms (Shahidi & Yeo, 2016).

Millets contain mainly free and conjugated forms of phenolic acids, which are the derivatives of hydroxybenzoic and hydroxycinnamic acids also several flavonoids are found in millet such as anthocynidins, falvanols, flavones, flavonones, chalcones and aminophenolic compounds. Phenolic acids and flavonoids compounds may vary in content at different parts of the grain and composition of millet grain (Shahidi and Chandrasekara, 2013).

The phenolic compounds have the ability to donate hydrogen atoms via hydroxyl groups on benzene rings to electron-deficient free radicals and form a resonance-stabilized and less reactive phenoxyl radical, showing the potency of phenolic compounds as antioxidants. Phenolic compounds, especially phenolic acids, are found in cereals, whole grains, legumes and other seeds (Shahidi & Yeo, 2016). Phenolic acids are antioxidants that have been reported to be found in millet (Yu, Perret, Davy, Wilson, and Melby, 2002). Cereal grains contain an array of free phenolic compounds with glycosides and esters also insoluble-bound that are correlated with polysaccharides in the cell walls (Miller, Rigelhof, Marquat, Prakash & Kanter, 2000).

Previous studies stated that the amount of total free phenolic in corn, wheat, oats, and rice is 15%, 25%, 25% and 38% respectively (Adom and Liu, 2002; Liu, 2007). Meanwhile, the total bound phenolic content in corn was 85%, 76% in wheat, 75% in oats, and 62% in rice which concluded that total phenolic contents of whole grains had been underestimated in literature without including the bound phenolic as most grain phenolic in bound form. Therefore, these studies found that total antioxidant activity of corn was 181.4 ± 0.86 umol of vitamin C equiv/g grain, and the highest ($p<0.01$) compared to wheat, oats, and rice. However, the total antioxidant activity of rice (55.77 ± 1.62 umol/g grain) was lower than wheat (76.70 ± 1.38 umol/g grain) and oats (74.67 ± 1.49 umol/g grain) were similar ($p>0.05$).

As secondary metabolites of plants, flavonoids are found in cereals, legumes and seeds. In cereals, bound flavonoids are found in maize with 15.9 ug/g of kaempferol in pericarp portion and 28.8 ug/g of quercetin in germ parts. Besides, in quinoa seeds, a major flavonoid identified known as catechin with 16.28 ug/g, quercetin with 47.2 ug/g and kaempferol with 30.4ug/g, thus other cereals such as rice, corn, wheat and barley also contain great source of flavonoids (Shahidi & Yeo, 2016). However, flavonoids content in cereal-whole grains and seeded such as millet were not stated in this study. There are limited findings on flavonoids contents in millet based on previous studies.

2.7 Extractions of phenolic compound

Phenolics which are extracted into aqueous or aqueous-organic solvent mixture are soluble phenolics. Phenolic compounds exists in the free, non-conjugated form and in conjugated form where it is conjugated to soluble carbohydrates via ester (esterified) and ether (etherified) bonds (Shahidi & Naczk, 2004). One type of millet such as Japanese barnyard millet grains had been reported containing 3 types of antioxidative phenolic compounds which are 1 serotonin derivative and 2 flavonoids from ethanol extract (Watanabe, 1999). A study was conducted in India in the screening of free radical quenching of 1,1 diphenyl-2-picrylhydrazyl (DPPH) by using electron spin resonance with methanol extract to obtain the phenolic in the seed coat where the phenolic content in the seed coat could be the main indicator for antioxidant activities (Hegde and Chandra, 2005).

Moreover, 80% methanol extractions in determining total phenolic content (TPC) and total flavonoid content (TFC) were present in soluble form which are more than 80.0% than bound form (Pradeep & Sreerama, 2018). Phytochemicals such as terpenoids and tannins were found in all solvent extract such as methanol, ethanol and aqueous water (distilled water). Meanwhile, flavonoids, alkaloids, phenolics and reducing sugars were identified in methanol and aqueous extracts due to wide variation in identifying the presence of phytochemicals from the differences in the polarity of the solvent extractions. Methanol is polar organic solvent compared to other solvents thus, most phytochemicals were easily detected in polar nature (Suma & Urooj, 2012). High concentrations (1.6-2.0mg) methanolic extract present higher radical scavenging activity compared to ethanol and water extracts (Suma & Urooj, 2012). In bran rich fraction of foxtail millet, the highest radical scavenging activity was methanolic extract (51.8%) followed by alcohol (42.9%) and water (33.60%) which indicate that methanolic extracts was the highest followed by ethanol and aqueous extracts that show higher radical scavenging activity (Suma & Urooj, 2012). In bran fraction of foxtail millet for methanol extract, it was showed that the maximum activity at absorbance 700nm was 0.455mmol ferrous/g meanwhile the maximum activity of ethanol and aqueous extracts at the same absorbance was 0.150mmol ferrous/g and 0.092mmol ferrous/g respectively, which can be conclude that methanolic extracts showed higher ferric reducing antioxidant activity compared to ethanol and aqueous extracts (Suma & Urooj, 2012). However, findings on these activities on other types of millet such as barnyard millet and little millet are still at scarcity level in determine antioxidant content and antioxidant activities using different solvent extracts such as ethanol, methanol and distilled water.

CHAPTER 3

3.1 Samples Preparation

There are two types of millet selected which is Barnyard millet and little millet in this study. Both millet were purchased from Uyir Inspired Sdn Bhd. Both millet were cleaned to remove any dirt or any foreign material. The process in making powder of both millet involved drying and grinding the millet into small particles and was sieved through 44 mesh and stored in the refrigerator at 4°C for further analysis.

3.2 Chemical and Reagent

Distilled water (Favorit W4L Genristo, Nottingham, UK), 70% ethanol, 80% methanol (Systerm, Malaysia), Follin-Ciocalteu reagent (Merck, Germany), 7% sodium carbonate (Na_2CO_3), gallic acid , 10% aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), 5% sodium nitrite ($NaNO_2$), catechin, 2,4,6-tripyridyl-s-triazine (TPTZ) solution, ferric chloride ($FeCl_3$), acetic acid, sodium acetate (NaOAc), hydrochloric acid (HCL), ferric sulphate ($FeSO_4$), 1M Sodium Hydroxide (NaOH), DPPH powder, butylated hydroxytoluene (BHT), acetate buffer (Sigma, Germany), and for FRAP reagent, $FeCl_3.6H_2O$ (EMSURE, Germany), and Iron (II) sulphate heptahydrate ($FeSo_4.7H_2O$) (Hamburg, Philippines).

3.3 Extraction methods

3.3.1 Cold aqueous solution

After the sample was grind and turned into powder (whole flour) containing bran rich fraction 15 g was extracted with 200 mL of distilled water in a mechanical shaker for 24 hr, filtered and evaporated to dryness under freeze dryer. The concentrated extracts were re-dissolved with respective solvents, to a concentration of 4 mg/ml and stored in the refrigerator until analyse (Suma & Urooj, 2012).

3.3.2 Ethanol extraction

This method is modified with the same procedure as cold aqueous extraction (Suma & Urooj, 2012), by using 15g of sample powder and extract with 200ml of 70% ethanol in mechanical shaker for 24hr and continued to filtered and evaporated under reduced pressure in a rotary evaporator and complete dryness under freeze dryer. The concentrated extract were re-dissolved with ethanol to a concentration of 4mg/ml and stored in refrigerator until analyse.

3.3.3 Methanol extraction

A sample of 15g of millet flours was mixed with 200ml of 80% methanol in a mechanical shaker for 24hr for extraction. The process was continue with filtration and evaporation of samples under reduced pressure in the rotary evaporator and complete dryness under freeze dryer. The concentrated extract was re-dissolved with methanol to a concentration of 4 mg/mL and stored in the refrigerator until analysis. All the analyses were carried out in triplicates. (Pushparaj & Urooj, 2012)

3.4 Determination of Antioxidant

3.4.1 Total Phenolic Content and Total Flavonoid Content

According to Lee, Mediani, Nur Ashikin, Azliana and Abas (2014) the total phenolic content was determined by using the Folin-Ciocalteu colorimetric method. Sample extracts for each extractions (20*u*l) was reacted with 100*u*l of Folin-Ciocalteu reagent for 5 minutes in 96 well plate. The reaction end with addition of 80*ul* of 7.5% aqueous sodium carbonate solution. The mixture sample is covered in dark on Stovall belly dancer (USA). The absorbance was detected at 765nm using microreader and gallic acid as the standard. The total phenolic content was expressed as mg gallic acid equivalents per 100g dry weight (DW) of sample. The result will be calculated using:

TPC for 1 g of extract=

$$\frac{\text{TPC per ml sample x dilution factor x total sample volume used}}{\text{sample weight}}$$

Next is to determine total flavonoids content by using an aluminium chloride colorimetric assay method (Belguith-Hadriche et al., 2013). The 25*u*l of samples from 3 extractions with 100*u*l of distilled water was pipetted in 96 well-plate. The extract samples were mix for 5 minutes with 7.5*u*l of 5% $NaNo_2$ solution and continue with 7.5*u*l of 10% $AlCl_3.6H_20$ solution for 5 minutes at room temperature. Lastly, 50*ul* of 1M NaOH solution was added to the reaction and 60*ul* of distilled water was added and catechin was used as a standard. Using a microreader, the absorbance was determined at 510nm. Total flavonoid contents were expressed as mg (+) - catechin equivalents per 100g dry weight of sample. Distilled water were added if the absorbance measured was over the linear range of the (+)-catechin standard curve. The result will be calculated using:

TFC for 1 g of extract=

$$\frac{\text{TFC per ml sample x dilution factor x total sample volume used}}{\text{sample weight}}$$

3.4.2 Ferric Reducing Antioxidant Power Assay

According to Benzie & Strain (1996) the FRAP reagents were prepared freshly and warmed 37°C in mechanical shaker. Sodium acetate buffer (300mmol/L, pH 3.6), 10mmol/L TPTZ solution along with 40mmol/L HCl as solvent and 20mmol/L iron (III) chloride solution were used to make FRAP reagent. Next, 20*u*l of samples from above extractions was mixed with 100*u*l of the FRAP reagent in 96 well-plate and continued to incubate for 30 minutes. The mixture absorbance was measured at 593nm and ascorbic acid as a standard. The standard calibration curve was constructed using $FeSO_4$ solution, and the results were expressed as mmol Fe (II)/g dry weight (DW) of sample.

3.4.3 DPPH radical scavenging activity

According to Kong et al (2012) the ability of the extracts such as methanolic, ethanolic and aqueous extracts to scavenge free radicals was determined against a very stable free radical DPPH activity. About 50*u*l samples of 3 extractions at different concentrations (0.98-1000ug/ml) was mixed with 195*u*l of 100uM DPPH solution in 96 well-plate. DPPH solution is made by using 3.9mg DPPH powder combine with 10ml methanol. The mixture is left for 30 minutes at room temperature in the dark. At 515nm the absorbance were measured. The result of activity as percentage DPPH scavenging relative to control using equation below:

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100$$

3.5 Statistical analysis

To determine the result of all variables, one-way ANOVA and Tukey post hoc were used to analyse the mean differences between each extractions of both samples. The correlation of antioxidant contents and activities was determined using the Pearson correlation coefficient. Data are expressed as mean ± standard error mean (n=3). Different letter indicate values are significant different ($p<0.05$). The statistical analysis was run on IBM SPSS Statistics version 22.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Total Phenolic Content

Phenolic compounds were known as a major group of compounds that shows antioxidant activities in grains, vegetables, and other botanical materials (Yu, Perret, Davy, and Wilson, 2002). In this study, the Folin-Ciocalteu method were used to determine the total phenolic content based on the reducing ability of hydroxyl groups attached to phenolic compounds of the extracts (Kumari, Madhujith, and Chandrasekara, 2017). In alkaline conditions, the phenolic groups were deprotonated which lead to a formation of phenolate ions, which reduce the yellow colour of Folin-Ciocalteu reagent to a blue colour that are presence of phenolic compounds in the sample extracts.

The total phenolic content of Little and Barnyard millet in the different extractions were calculated by using a gallic acid standard curve and were expressed in terms of milligram gallic acid equivalent (GAE/g) of the samples. The standard curve equation $y= 0.0014x + 0.0427$ with $R^2 = 0.9793$ were used to determine total phenolic content in both samples with different solvent extract which were70% ethanol, 80% methanol and cold aqueous solution.

4.1.1 Total Phenolic Content in Little millet

The results of total phenolic content of Little millet sample between different extractions which were 70% ethanol, 80% methanol and cold aqueous solution shown in table 1. Little millet extracted with cold aqueous solution showed the highest value of total phenolic content with 1318.633 GAE/mg followed with 80% methanol extracts and 70% ethanol extracts which were 367.083 GAE/mg and 190.953 GAE/mg respectively.

Table 1: Total phenolic content (TPC) of Little millet with different solvent extract.

Solvent extract	**TPC value (mg GAE/g)**
Cold aqueous solution	1318.633 ± 100.274^{a}
80% methanol	367.083 ± 14.776^{b}
70% ethanol	190.953 ± 11.916^{c}

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

Previous study by Zhu, Lian, Guo, Peng, & Zhou (2011) conducted a similar study of antioxidant content and antioxidant activity in various extractions such as water, 30% ethanol, 50% ethanol, 70% ethanol and 100% ethanol on defatted wheat germ. The aqueous extract shows higher value of total phenolic content in comparison to 30% ethanol. The value of total phenolic content in defatted wheat germ from the previous study reported approximately around 13.98mg GAE/g to 16.75mg GAE/g. However, the value of total phenolic content of little millet in current study were higher for all extracts compared to defatted wheat germ.

Meanwhile, a study by Shen et al. (2018) shows that sorghum grains in 85% ethanol extract has the value of total phenolic content within the range from 174.40mg GAE/g to 1238.83mg GAE/g. The total phenolic content of current study for all extracts were comparable and it was within the range of previous study although it was from different grains.

In addition, due to limited study of total phenolic content using Little millet in various extractions, it is impossible to compare with previous study due to different sample used, varieties, different solvents and concentrations in the study.

4.1.2. Total Phenolic Content in Barnyard millet

In table 2 shown the results of total phenolic content in Barnyard millet sample between different extractions solvent which were 70% ethanol, 80% methanol and cold aqueous solution. Based on the value shows, the highest value of total phenolic content in cold aqueous solution was 1638.976 GAE/mg followed with 70% ethanol extract with 195.720 GAE/mg and 80% methanol extract with 176.013 GAE/mg.

Table 2: Total phenolic content (TPC) of Barnyard millet with different solvent extract.

Solvent extract	**TPC value (mg GAE/g)**
Cold aqueous solution	1638.976 ± 36.749^{a}
80% methanol	176.013 ± 3.427^{b}
70% ethanol	195.720 ± 0.000^{c}

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

Based on study results by Bouajila et al. (2020) of total phenolic content of different cultivar pearl millet in 80% methanol as solvent extract, the value of total phenolic content (TPC) in pearl millet in different extractions ranging 72.083mg GAE/g to 136.25mg GAE/g. The TPC value in pearl millet from the previous study showed a lower TPC concentration compared to barnyard millet in present study. However, the slightly different of total phenolic content on both study are due to the type of sample used with different varieties, and may have intrinsic and extrinsic factors which affect phenolic compound for each samples such as plant genetics and cultivars, type of soil used, geographical area for growth, post-harvest conditions and different solvents to be compared with one type of solvent and concentration used in the study.

There is also a study finding in previous study using pearl millet in methanol extraction and it shows TPC ranging from 162.32mg GAE/g to 202.81mg GAE/g (Meena, Khan, Ram, Raiger, & Satyavati, 2018). There were quite similar findings for both previous and present study, however, there were few factors that may affect the phenolic content such as genetics and type of sample used and also different solvents with different concentrations used in the study. As other study by (Ngo, Scarlett, Bowyer, Ngo, & Vuong, 2017) that conducted antioxidant content in plant sample with different solvent used stated that the antioxidant component may differ due to variation in polarities of the solvents, which selectively extract different hydrophilic and hydrophobic compounds in the sample.

4.2 Total Flavonoid Content

Flavonoids are mostly found in fruits, vegetables and grains thus, as the most studied group of phenolic compounds. Total flavonoids content (TFC) were determined on the chelating ability of flavonoids with aluminium (III). It will form pink-coloured complex with aluminium (III) through 4-keto and neighbouring hydroxyl groups or in B ring through adjacent hydroxyl groups (Chandrasekara & Shahidi, 2010).

The total flavonoid content of Little millet and Barnyard millet with different extractions solvent which are 70% ethanol, 80% methanol and cold aqueous solution were determined by standard equation curve , $y = 0.0006x + 0.0424$, $R^2 = 0.9976$ and using catechin as a standard and were expressed as milligram catechin equivalent (CE)/g extract.

4.2.1 Total Flavonoid Content in Little millet

The values of total flavonoid content in Little millet was showed in table 3 with different solvent extracts. The highest value of flavonoid content is cold aqueous solution extract with 825.627 CE/mg followed by 70% ethanol extract with 548.707 CE/mg and the least flavonoid content is 80% methanol with 517.477 CE/mg respectively.

Table 3: Total flavonoid content (TFC) of Little millet with different solvent extracts

Solvent extract	TFC value (mg CE/g)
Cold aqueous solution	825.627 ± 4.512^{a}
80% methanol	517.477 ± 6.083^{b}
70% ethanol	548.707 ± 8.544^{c}

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

According to Ofosu et al. (2020) the total flavonoid content in various millets used in the study were ranged from 101.3mg CE/g to 115.8mg CE/g in 70% ethanol extracts. The study states that finger Italian millet used shows the highest content of total flavonoid compared to other type of millets. The findings in the current study showed higher total flavonoid content value compared with previous study due to genotype and cultivation environment also climate conditions of millet growth also different solvent extract used in the study.

Besides, previous study conducted by Pradeep and Guha (2011) on the effect of antioxidant in little millet with processing method using mixture of methanol extractions found that ranged from 334.9mg catechin/g to 420.2mg catechin/g that were lower than the findings of current study. As there were no processing methods used in the current study, the effects of antioxidant content were compared with raw and processed samples used which were slightly similar in sample preparation in the current study. The previous study shows the highest value of flavonoid content was affected as the processed sample used differently, in which change biochemical of millet that brings to produce plant secondary metabolites and it is quite similar with the process in current study which affects the value of flavonoid content in the sample. However, the sample variation used, sample preparation, process and standard used in previous study were incomparable with current study as using different extractions in the sample to quantify flavonoid content.

4.2.2 Total Flavonoid Content in Barnyard millet

Table 4 shown the values of total flavonoid content in Barnyard millet with different solvent extracts. Cold aqueous solution extract shows the highest value of total flavonoid content with 675.440 CE/mg followed by 512.250 CE/mg and 502.713 CE/mg of 80% methanol extract and 70% ethanol extract respectively.

Table 4: Total flavonoid content (TFC) of Barnyard millet with different solvent extracts

Solvent extract	TFC value (mg CE/g)
Cold aqueous solution	675.440 ± 9.063^a
80% methanol	512.250 ± 7.413^b
70% ethanol	502.713 ± 5.424^c

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

In previous study conducted by Meena, Khan, Ram, Raiger, & Satyavati, (2018), the total flavonoid content in various genetic type of pearl millet in methanol extract, were ranged from 87.39mg QE/g to 147.20mg QE/mg, quercetin as a standard. These values were lower compared to the value of total flavonoid content in current study. This might be due to different genetic and species of sample used along with different procedures and standards used in previous study. Another study by Kumari, Madhujith, & Chandrasekara (2017) on whole grains, de-hulled grains and hull grains of different type of millet with various genetics using 70% aqueous acetone showed a range of total flavonoid content between 0.03 to 0.26, 0.01 to 0.95, and 0.06 to 0.62 micromoles of CE/g. Previous study reported that high flavonoid content were high in phenolic soluble bound form compared with bound millet grains. Thus, different procedures and standard used, various type of sample used, locations and extrinsic factor influenced the total flavonoid content in the study.

4.3 Ferric Reducing Antioxidant Power assay (FRAP)

In this study, FRAP assay were used to determine the ability of the antioxidant with the present of TPTZ at low pH by reducing Fe^{3+} to Fe^{2+}, yellow colour of FRAP reagent will change to blue colour compound as an indicator of presence antioxidant in the sample at wavelength of 593nm (Yang et al., 2014). The results of the FRAP assay of little millet and Barnyard millet with different solvent extracts were determined by a standard calibration curve of ferrous sulphate ($FeSo_4$). The standard ($FeSo_4$) in different concentration were range from 0.1 to 1.0mM. The linear equation obtained from the standard curve, $y = 1.168x + 0.2183$, with $R^2 = 0.9977$ and the results was expressed as mmol ferrous/g extract.

4.3.1 Ferric Reducing Antioxidant Power assay of Little millet

The values of antioxidant activity for ferric reducing antioxidant power assay of Little millet with different solvent extractions shown in table 5. The highest value of ferric reducing antioxidant power is in cold aqueous solution extract with 0.420 mmol ferrous/g, following with 70% ethanol and 80% methanol, 0.330 mmol ferrous/g and 0.257 mmol ferrous/g respectively.

Table 5: Ferric reducing antioxidant power of Little millet with different solvent extracts

Solvent extract	FRAP value (mmol/Fe^{2+} g)
Cold aqueous solution	0.420 ± 0.023^a
80% methanol	0.257 ± 0.034^b
70% ethanol	0.330 ± 0.050

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

Based on previous study the ferric reducing antioxidant power in Little millet with processing method using mixture of 1% HCL-methanol extractions Pradeep S.

R., & Guha M.(2011) ranged from 0.06mmol ferrous/g to 0.10mmol ferrous/g which is in contrast with current study. However, the higher iron reducing power value of processed millet gives higher antioxidant activity and indicates higher total antioxidant content as compared to raw millet and it is beneficial to be used in food formulations or in food industry (Omar et al., 2012). Thus, different type of solvent used also may affect the value of antioxidant activity along with extractions procedures that affect the antioxidant levels.

Other than that, previous study by Sutharut, J. and Sudarat, J. (2012) reported different varieties of germinated rice extracted using methanol has average values of ferric reducing antioxidant power (FRAP) ranged from 4.56mmol ferrous/g to 7.20mmol ferrous/g which showed a big difference to compared with current study. Due to different process and method procedure used in the previous study in which the rice samples were undergo germination process before extracted that could be affect the antioxidant content along with antioxidant activity in the sample. The values of antioxidant activity were high especially in rough rice sample, in which not undergo a lot of process in germination, drying and filtration. However, the study may not be c between previous study and current study due different varieties of sample used, type of solvent extractions and concentrations used may give different result in the study.

4.3.2 Ferric Reducing Antioxidant Power assay of Barnyard millet

Table 6 shows, the results of ferric reducing antioxidant power assay of Barnyard millet with different solvent extractions. Sample with cold aqueous solution contain the highest value of ferric reducing antioxidant power assay, 0.510 mmol ferrous/g. With the extractions of 80% methanol, the ferric reducing antioxidant power value were 0.297 mmol ferrous/g and followed by 70% ethanol with 0.08 mmol ferrous/g respectively.

Table 6: Ferric reducing antioxidant power of Barnyard millet with different solvent extracts

Solvent extract	FRAP value (mmol/Fe2+ g)
Cold aqueous solution	0.510 ± 0.050^{a}
80% methanol	0.297 ± 0.022^{b}
70% ethanol	0.08 ± 0.003^{c}

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

According to Suma & Urooj, (2012) that used different extractions solvent in determining the antioxidant activity in different fraction of foxtail millet, there were significantly higher content of reducing power in 100% methanol extract, followed by 100% ethanol extract and least reducing power content in cold aqueous extract of absorbance 700nm (0.381, 0.341 and 0.120mmol ferrous/g, respectively) in between fractions of foxtail millet. Compared with the current study that used similar solvent extract, it shows that there were different values of reducing power, due to different concentrations of solvent extract used in the previous study and thus it may affect the difference in the results.

Another previous study by Pushparaj & Urooj, (2014) shows that raw form of pearl millet methanol extract between two varieties have FRAP values of 2.24umol ferrous/g and 1.85umol ferrous/g which are incomparable with current study due to the effect of processed method in both varieties used may give changes to the value of ferric reducing antioxidant power content. However, the processed method were not significant for both varieties in the sample of previous study which indicate the antioxidant activity of millet were no changes as compared with the raw millet. The effect of processed method, solvent extract used and sample variations may give impact on antioxidant activity in the study.

4.4 DPPH Radical Scavenging Activity

This method was used to test the ability of antioxidant compounds to act as free radical scavengers or hydrogen donors based on the capacity of stable free radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors, including phenols from deep violet colour to colourless compound. The reduction colour was quantified at the wavelength of 515nm. The percentage of standard using BHT with sample Barnyard millet and Little millet of different solvent extract shown in figure 1.

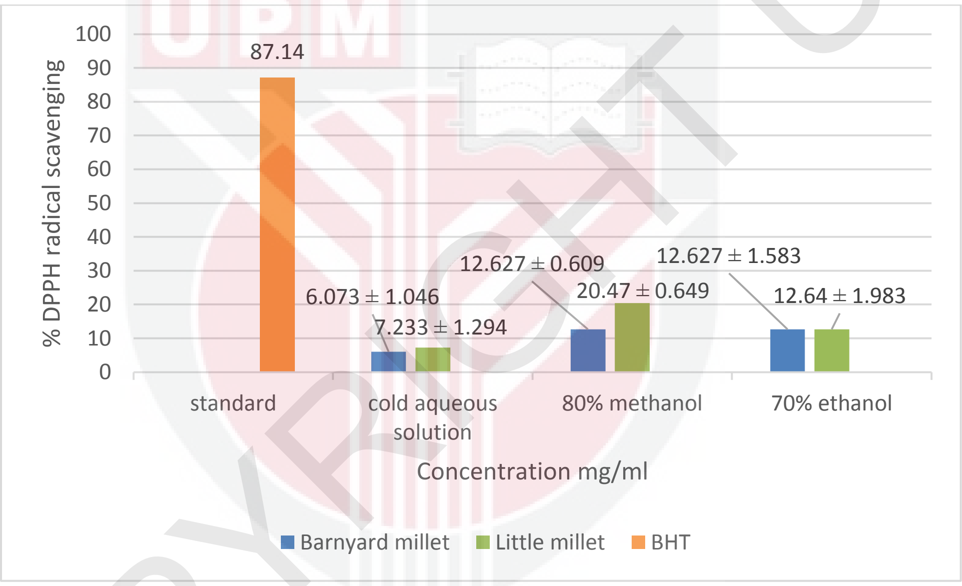

Figure 1: Percentage value of DPPH radical scavenging of Barnyard millet and Little millet with standard

4.4.1 DPPH Radical Scavenging Activity of Little millet

The percentage of DPPH radical scavenging activity of little millet with different solvent extractions is presented in table 7. The highest value for percentage of DPPH radical scavenging activity was 80% methanol extract with 20.470 % DPPH radical scavenging, followed with 70% ethanol extract and cold aqueous solution, 12.640 % DPPH radical scavenging and 7.233 % DPPH radical scavenging, respectively.

Table 7: Percentage (%) DPPH radical scavenging of Little millet with different solvent extractions

Solvent extract	% DPPH radical scavenging
Cold aqueous solution	7.233 ± 1.294^a
80% methanol	20.470 ± 0.649^b
70% ethanol	12.640 ± 1.983^c

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

Based on previous study (Suma & Urooj, 2012) of foxtail millet, the findings shows a quite similar trend but at a higher percentage range with current study in which sample with methanol extract had the highest radical scavenging values besides ethanol extract and cold aqueous extract that were (51.8%) in methanol extract, (42.90%) ethanol extract and (33.60%) in cold aqueous extract. The higher value of radical scavenging in methanol extract was supported by another study in which it determined the potential antioxidant activity. Due to different concentrations of solvent extract used in previous study gives slightly different values with current study. Aqueous alcohol with different levels of water being widely used depend on the purpose, polarity, cost and safety. (Kamath, Chandrashekar, & Rajini, 2004). The process, procedures and standard use in the study may give different impacts of radical scavenging of the sample used.

4.4.2. DPPH Radical Scavenging Activity of Barnyard millet

The value percentage of DPPH radical scavenging of Barnyard millet with different solvent extractions were shown in table 8. The highest values were 12.627 % DPPH radical scavenging in both 80% methanol extract and 70% ethanol extract, meanwhile for cold aqueous solution were 6.073% DPPH radical scavenging respectively.

Table 8: Percentage (%) DPPH radical scavenging of Barnyard millet with different solvent extractions

Solvent extract	% DPPH radical scavenging
Cold aqueous solution	6.073 ± 1.046^a
80% methanol	12.627 ± 0.609^b
70% ethanol	12.627 ± 1.583^c

Data expressed as mean ± SEM (n=3). Different letter indicate values are significant different ($p<0.05$)

Based on previous study conducted by (Suma & Urooj, 2012) on antioxidant activity of foxtail millet showed that the results were influenced by processing extracts. The higher concentration generate high yield of radical scavenging in the sample used. Previous study showed that foxtail millet in methanol extracts was the highest value of radical scavenging percentage with 51.8% compared with ethanol and followed by water extracts with 42.90% and 33.60%, respectively. To compare with current study, the highest radical scavenging percentage in methanol extract and ethanol extract, however, due to different species of sample used may give different values of antioxidant activity (Meena, Khan, Ram, Raiger, & Satyavati, 2018). The processed method were slightly gives effect on the radical scavenging value on the sample as the antioxidant compound reduce due to long duration of exposure to water or heat. Thus, this supported by the study that loss of radical scavenging ability due to loss of some phenolic compound with certain factors such as insufficient dark area, reaction time within the sample also various type of sample used gives different effect in the study.

4.5 Correlation between Antioxidant Content and Antioxidant Activities

The correlation coefficient (r) of the antioxidant content and antioxidant activities of Barnyard millet and Little millet in extractions are shown in table 9. There is a strong positive correlation of total phenolic content and total flavonoid of Barnyard millet with ferric reducing antioxidant power (r = 0.819, r = 0.871). There is a strong significant negative correlation of total phenolic content and total flavonoid content of Barnyard millet with DPPH radical scavenging (r = -0.860, r = -0.903). Meanwhile, there is a strong significant negative correlation of total flavonoid content and DPPH assay of Little millet (r = -0.820). There was no significant correlation between total phenolic content with ferric reducing and DPPH assay of Little millet. Thus, there is a positive correlation of total phenolic content (r = 0.604) and strong significant positive correlations of total flavonoid content with ferric reducing of Little millet (r = 0.712).

Table 9: Pearson correlation analysis of antioxidant content and antioxidant activities in the extracts of Barnyard millet and Little millet

			FRAP	**DPPH**
Barnyard millet	TPC	r-value	0.819**	-0.860**
	TFC	r-value	0.871**	-0.903**
Little millet	TPC	r-value	0.604	-0.639
	TFC	r-value	0.712*	-0.820**

** Correlation is significant at $p<0.01$ level (2-tailed); *correlation is significant at $p<0.05$ level (2-tailed). TPC, total phenolic content; TFC, total flavonoid content; FRAP, ferric reducing antioxidant power assay; DPPH, 2,2-Diphenyl-1-picrylhydrazyl.

The strong positive correlations of total phenolic content and total flavonoid content with ferric reducing antioxidant power were significant in Barnyard millet. As this indicates the high phenolic compound consisting of phenolic and flavonoid in the sample were strongly associated in antioxidant activity. A previous study determine antioxidant content and antioxidant activity in starchy plant tubers (Omar et al., 2012) shows that a strong positive correlation possesses a high antioxidant activity and can be used as a natural source of antioxidant. Meanwhile, the negative correlations of total phenolic content and flavonoid content with percentage of DPPH radical scavenging indicate the higher antioxidant content will lead to lower percentage of DPPH radical scavenging activity.

Besides, the strong positive associated of total phenolic content and significantly positive in total flavonoid content with ferric reducing antioxidant power in Little millet indicate the presence of phenolic compounds especially high value of flavonoid forms give large effect in ferric reducing antioxidant power activity. The negative correlation significantly in total phenolic content and total flavonoid content of Little millet indicates the high value of phenolic and flavonoid content may lead to lower the percentage of DPPH radical scavenging. Previous study from Pushparaj and Urooj (2014) states that there were weak correlation of negative correlation between antioxidant content with antioxidant activity as the antioxidant content such as phenolic nor flavonoids less likely to contribute to the antioxidant activity.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.0 Conclusion

Total phenolic content and total flavonoid content were determined using Folin-ciocalteu assay and aluminium colorimetric assay of Little millet and Barnyard millet in cold aqueous, 70% ethanol and 80% methanol extracts. Meanwhile, antioxidant activities in both Little millet and Barnyard millet were determined using ferric reducing antioxidant power assay and DPPH radical scavenging in cold aqueous, 70% ethanol and 80% methanol extracts. This study found that, cold aqueous extracts shows higher in total phenolic content, total flavonoid content and ferric reducing antioxidant power assay in Little millet and Barnyard millet, followed by 80% methanol and 70% ethanol extract respectively. However, 80% methanol extracts shows the highest percentage of DPPH radical scavenging in Little millet and Barnyard millet. Besides, the findings shows Little millet has the highest value of total flavonoid content and percentage of DPPH radical scavenging, while Barnyard millet has the highest value of total phenolic content and ferric reducing antioxidant power in all extractions.

In correlations between total phenolic content, total flavonoid content and antioxidant activities of Little millet and Barnyard millet, the findings of this study found out that positive correlations shows phenolic content and flavonoid content were high indicate high antioxidant activities in ferric reducing antioxidant power. This study shows that strong association of total phenolic content and total flavonoid content with antioxidant activities of Little millet and Barnyard millet.

Finally yet importantly, Little millet and Barnyard millet are one type of whole and cereal grains that have a lot of benefits to be consumed in our diet. The findings in this study shows that the antioxidant content of both millets can be useful in our future used especially in food industry also a beneficial nutritional information to be comply in future studies and researchers.

5.1 Limitations of study

There are several limitations in current study, which there are limited findings of other type of bioactive components in Little millet and Barnyard millet. Besides, there are limited information on extractions in water or cold aqueous extract by other researchers of antioxidant content and antioxidant activities, specifically in millet. Not only that, in this study, the antioxidant content and antioxidant activities were only determined using three different type of solvents which were cold aqueous, 80% methanol and 70% ethanol, which limits in the findings of the study to be compared with other type of solvent used in other studies. Moreover, the findings of this study were limited as the bioactive compounds only analyze total phenolic content, total flavonoid content and antioxidant activities using Folin-ciocalteu's method, aluminium colorimetric method, ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assay.

5.2 Recommendations

Recommendation from this study for future research, various methods can be used to compare. There are variations in type and genetics of millet, thus with variety type of uncommon millet should be introduced and studied especially on antioxidant content and antioxidant activities. To determine the effective extraction of solvents on antioxidant constituent and antioxidant activities, different types of solvent can be use such as acetone, hexane, ethyl acetate that should be included in the study. Besides, for antioxidant activities analysis, future researchers should include beta-carotene bleaching assay, ABTS, TEAC, ORAC methods to identify the other potential antioxidant capacity of the sample.

REFERENCES

Anju, T., & Sarita, S. (2010). Suitability of foxtail millet (Setaria italica) and barnyard millet (Echinochloa frumentacea) for development of low glycemic index biscuits. *Malaysian Journal of Nutrition*, *16*(3), 361–368.

Barada K, Bitar A, Mokadem MA, Hashash JG, Green P. Celiac disease in Middle Eastern and North African countries: a new burden? *World J Gastroenterol*. 2010;16(12):1449– 1457. pmid:20333784.

Bouajila, A., Lamine, M., Rahali, F. Z., Melki, I., Prakash, G., & Ghorbel, A. (2020). Pearl millet populations characterized by Fusarium prevalence, morphological traits, phenolic content, and antioxidant potential. *Journal of the Science of Food and Agriculture*, *100*(11), 4172–4181. https://doi.org/10.1002/jsfa.10456

Brown, A. C., Walter, J. M., & Beathard, K. (2015). *Understanding food: principles and preparation*. Stamford, CT: Cengage Learning.

Chandrasekara, A., & Shahidi, F. (2010). Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *Journal of Agricultural and Food Chemistry*, 58(11), 6706–6714. https://doi.org/10.1021/jf100868b

Chandrasekara, A., & Shahidi, F. (2011). Antiproliferative potential and DNA scission inhibitory activity of phenolics from whole millet grains. *Journal of Functional Foods*, *3*(3), 159–170. doi: 10.1016/j.jff.2011.03.008

Chandrasekara, A., & Shahidi, F. (2011). Determination of antioxidant activity in free and hydrolyzed fractions of millet grains and characterization of their phenolic profiles by HPLC-DAD-ESI-MSn. *Journal of Functional Foods*, *3*(3), 144–158. https://doi.org/10.1016/j.jff.2011.03.007

Chandra, D., Chandra, S., Pallavi, & Sharma, A. (2016). Review of Finger millet (Eleusine coracana (L.) Gaertn): A power house of health benefiting nutrients. *Food Science and Human Wellness*, *5*(3), 149–155. doi: 10.1016/j.fshw.2016.05.004

FAO. (2001). Millet - Post-harvest Operations. *INPhO-Post-Harvest Compendium.*, 56.

Fasano A, Berti I, Gerarduzzi T, Not T, Colletti RB, Drago S, et al. Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. Arch Intern Med. 2003;163(3):286–392. pmid:12578508

Goh KL. Epidemiology of Helicobacter pylori infection in Malaysia—observations in a multiracial Asian population. *The Medical journal of Malaysia*. 2009;64(3):187–192. pmid:20527265

Health Canada, Celiac Disease—The Gluten Connection 2008. Retrieved from http://www.hc-sc.gc.ca/fn-an/pubs/securit/gluten_conn-lien_gluten-eng.php, (accessed 17.03.16).

Jnawali, P., Kumar, V., & Tanwar, B. (2016). Celiac disease: Overview and considerations for development of gluten-free foods. *Food Science and Human Wellness*, 5(4), 169–176. doi: 10.1016/j.fshw.2016.09.003

Kamath, V. G., Chandrashekar, A., & Rajini, P. S. (2004). Antiradical properties of sorghum (Sorghum bicolor L. Moench) flour extracts. *Journal of Cereal Science*, 40(3), 283–288. https://doi.org/10.1016/j.jcs.2004.08.004

Kumari, D., Madhujith, T., & Chandrasekara, A. (2017). Comparison of phenolic content and antioxidant activities of millet varieties grown in different locations in Sri Lanka. *Food Science and Nutrition*, 5(3), 474–485. https://doi.org/10.1002/fsn3.415

Liu, Q., & Yao, H. (2007). Antioxidant activities of barley seeds extracts. *Food Chemistry*, 102(3), 732–737. https://doi.org/10.1016/j.foodchem.2006.06.051

L.Yu, J.Perret, B.Davy, J.Wilson, A. C. L. M. (2002). Antioxidant Properties of Cereal Products. *Journal of Food Science*, 67(7).

Makharia GK, Verma AK, Amarchand R, Bhatnagar S, Das P, Goswami A, et al. (2011) Prevalence of celiac disease in the northern part of India: a community based study. *Journal Gastroenterology Hepatology*;26(5):894–900. pmid:21182543

Meena, R. C., Khan, I. U., Ram, M., Raiger, P. R., & Satyavati, C. T. (2018). Genetic diversity of total phenolic, flavonoid and antioxidant activity in pearl millet genotypes grown in semi-arid region of Rajasthan, *International Journal of Chemical Studies* 6(3), 1845–1849.

Monroy, Y. M., Rodrigues, R. A. F., Sartoratto, A., & Cabral, F. A. (2016). Extraction of bioactive compounds from cob and pericarp of purple corn (Zea mays L.) by sequential extraction in fixed bed extractor using supercritical CO2, ethanol, and water as solvents. *Journal of Supercritical Fluids*, 107, 250–259. https://doi.org/10.1016/j.supflu.2015.09.020

National Coordinating Committee on Food and Nutrition, Ministry of Health Malaysia. (2010). Malaysian Dietary Guidelines. Kuala Lumpur.

Ngo, T. Van, Scarlett, C. J., Bowyer, M. C., Ngo, P. D., & Vuong, Q. Van. (2017). Impact of different extraction solvents on bioactive compounds and antioxidant capacity from the root of Salacia chinensis L. *Journal of Food Quality*, 2017. https://doi.org/10.1155/2017/9305047

Ofosu, F. K., Elahi, F., Daliri, E. B. M., Chelliah, R., Ham, H. J., Kim, J. H., et al (2020). Phenolic profile, antioxidant, and antidiabetic potential exerted by millet grain varieties. *Journal Antioxidants*, 9(3), 1–14.

Omar, N. F., Hassan, S. A., Yusoff, U. K., Abdullah, N. A. P., Wahab, P. E. M., & Sinniah, U. R. (2012). Phenolics, flavonoids, antioxidant activity and cyanogenic glycosides of organic and mineral-base fertilized cassava tubers. *Journal Molecules*, 17(3), 2378-2387.

Pradeep, S. R., & Guha, M. (2011). Effect of processing methods on the nutraceutical and antioxidant properties of little millet (Panicum sumatrense) extracts. *Food Chemistry*,126(4),1643–1647.https://doi.org/10.1016/j.foodchem.2010.12.047

Pradeep, P. M., & Sreerama, Y. N. (2018). Phenolic antioxidants of foxtail and little millet cultivars and their inhibitory effects on α-amylase and α-glucosidase activities. *Food Chemistry*, *247*(November 2017), 46–55. https://doi.org/10.1016/j.foodchem.2017.11.103

Pushparaj, F. S., & Urooj, A. (2014). Antioxidant activity in two pearl millet (Pennisetum typhoideum) cultivars as influenced by processing. *Journal Antioxidants*, 3(1), 55–66. https://doi.org/10.3390/antiox3010055

Qua CS, Goh KL. (2011) Liver cirrhosis in Malaysia: peculiar epidemiology in a multiracial Asian country. *Journal Gastroenterology Hepatology*.;26(8):1333–1337. pmid:21443669

Rubio-Tapia A, Ludvigsson JF, Brantner TL, Murray JA, Everhart JE. (2012) The prevalence of celiac disease in the United States. *Journal Gastroenterol*.;107(10):1538–1544. pmid:22850429

Saleh, A. S. M., Zhang, Q., Chen, J., & Shen, Q. (2013). Millet grains: Nutritional quality, processing, and potential health benefits. *Comprehensive Reviews in Food Science and Food Safety*, *12*(3), 281–295. https://doi.org/10.1111/1541-4337.12012

Shen, S., Huang, R., Li, C., Wu, W., Chen, H., Shi, J., … Ye, X. (2018). Phenolic Compositions and Antioxidant Activities Differ Significantly among Sorghum Grains with Different Applications. Molecules (Basel, Switzerland), 23(5). https://doi.org/10.3390/molecules23051203

Singh, R. B., Khan, S., Chauhan, A. K., Singh, M., Jaglan, P., Yadav, P., … Juneja, L. R. (2018). Millets as Functional Food, a Gift From Asia to Western World. The Role of Functional Food Security in Global Health. Elsevier Inc. https://doi.org/10.1016/B978-0-12-813148-0.00027-X

S. Picascia, R. Mandile, R. Auricchio, R. Troncone, C. Gianfrani (2015) Gliadin-specific T-cells mobilized in the peripheral blood of coeliac patients by short oral gluten challenge: clinical applications Nutrients, 7,pp. 10020-10031

Suma, P. F., & Urooj, A. (2012). Antioxidant activity of extracts from foxtail millet (Setaria italica). *Journal of Food Science and Technology*, 49(4), 500–504. https://doi.org/10.1007/s13197-011-0300-9

Sutharut, J. and Sudarat, J. (2012). Total anthocyanin content and antioxidant activity of germinated colored rice. *International Food Research Journal 19(1)*, *19*(1), 215–221.

Thompson LV. (1993). Potential health benefits and problems associated with antinutrients in foods. *Food Res Intl* 26:131–49.

Viswanath, V., Urooj, A., & Malleshi, N. (2009). Evaluation of antioxidant and antimicrobial properties of finger millet polyphenols (Eleusine coracana). *Food Chemistry*, *114*(1), 340–346. doi: 10.1016/j.foodchem.2008.09.053

Yang, L., Zhang, H., Cheng, L., Gu, Z., Hua, D., Qi, X., … Wang, L. (2014). Effect of Extrusion on the Hydrophilic Antioxidant Capacity of Four Whole Grains. *Journal of Food and Nutrition Research,* 2(2), 80–87. https://doi.org/10.12691/jfnr-2-2-4

Yap, T. W.-C., Chan, W.-K., Leow, A. H.-R., Azmi, A. N., Loke, M.-F., Vadivelu, J., & Goh, K.-L. (2015). Prevalence of Serum Celiac Antibodies in a Multiracial Asian Population-A First Study in the Young Asian Adult Population of Malaysia. *Plos One*, *10*(3). doi: 10.1371/journal.pone.0121908

Zhu, K. X., Lian, C. X., Guo, X. N., Peng, W., & Zhou, H. M. (2011). Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. *Food Chemistry,* 126(3), 1122–1126. https://doi.org/10.1016/j.foodchem.2010.11.144