Potential Antioxidant Activity of Ripe *Pandanus tectorius* Fruits Extract in Different Solvents

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A thesis submitted in fulfilment of the requirement for the degree of Bachelor of Applied Science (Product Development Technology) with Honours

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DECLARATION

I hereby declare that the work embodied in this report is the result of the original research and has not been submitted for a higher degree to any universities or institutions.

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I certify that the report of this final year project entitled "**Potential Antioxidant Activity of Ripe *Pandanus tectorius* Fruits Extract in Different Solvents**" by **NUR SYUHADA BINTI MOHD GHAZALI**, matric number **F15A0167** has been examined and all the correction recommended by examiners have been done for the degree of Bachelor of Applied Science (Agriculture Technology) with Honours, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan.

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ABSTRACT

Pandanus *tectorius* is listed as underutilized crops in Malaysia and Indonesia and it fruit part is wasted and still not utilized either as a food source or for research. However, in Micronesia, Pandanus *tectorius* fruits have been used as major source of food but not fully exploited in South East Asia. The aims of the present study were to determined total phenolic content, total flavonoid content and potential of antioxidant from the keys parts of Pandanus *tectorius* fruits extract. The samples were collected from Kuala Nerus, Terengganu, Malaysia. Extracts were obtained by successive extraction using ethanol, acetone, ethyl acetate and petroleum ether as a solvent but petroleum ether could not be proceed as layered and cloudy was form in its mixture for each assay. The TPC and TFC were analysed by Folin-Ciocalteu and Aluminium Chloride Colorimetric assay respectively, meanwhile for antioxidant was analysed by DPPH free radical scavenging assay. The ethanol extract showed the highest TPC and TFC in result than other solvents where the result obtained is; ethanol (ethanol extract (4.52 ± 0.07 µg GAE / g raw material) and ethanol extract (29.42 ± 1.15 µg QE / g raw material), respectively. Result founded that both TPC and TFC of P. *tectorius* fruits were found to be rich in phenolics content and flavonoids content as well as potent as antioxidant agent and the most suitable solvent for extraction of ripe Pandanus *tectorius* fruit is ethanol.

Keywords : *Pandanus tectorius,* Total Phenolic Content, Total Flavonoids Content, Antioxidant

Potensi Aktiviti Antioksidan dari Ekstrak Buah *Pandanus tectorius* yang Masak Dalam Pelarut yang Berbeza

ABSTRAK

Pandanus tectorius disenaraikan sebagai tanaman kurang dimakan di Malaysia dan Indonesia dan buahnya dibazirkan dan tidak digunakan sama ada sebagai sumber makanan atau penyelidikan. Walau bagaimanapun, di Micronesia, buah-buahan Pandanus tectorius telah digunakan sebagai sumber utama makanan tetapi tidak sepenuhnya dieksploitasi di Asia Tenggara. Tujuan kajian ini adalah menentukan jumlah kandungan fenolik, jumlah kandungan flavonoid dan potensi antioksidan dari ekstrak bahagian gugusan buah buah pandanus tectorius. Sampel dikumpul dari Kuala Nerus, Terengganu, Malaysia. Ekstrak diperolehi dengan pengekstrakan berturut-turut menggunakan etanol, aseton, etil asetat dan ether petroleum sebagai pelarut tetapi ether petroleum tidak dapat diteruskan kerana membentuk lapisan dan tidak jernih di dalam campurannya untuk setiap assay. TPC dan TFC dianalisis oleh Folin-Ciocalteu dan Aluminium Chloride Colorimetric assay masing-masing, sementara itu untuk antioksidan, dianalisis oleh ujian pemotongan radikal bebas DPPH. Ekstrak etanol menunjukkan TPC dan TFC tertinggi dalam hasil daripada pelarut lain di mana hasil yang diperolehi adalah; (4.52 ± 0.07 μg GAE / g bahan mentah) dan (29.42 ± 1.15 μg QE / g bahan mentah) masing-masing, and hasil mendapati TPC dan TFC buah tectorius didapati kaya kandungan fenolik dan kandungan flavonoid serta berpotensi sebagai agen antioksidan dan pelarut yang paling sesuai untuk pengekstrakan buah Pandanus tectorius masak adalah etanol.

Kata kunci : *Pandanus tectorius,* jumlah kandungan fenolik, jumlah kandungan flavonoid, antioksidan

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LIST OF ABBREVIATIONS

g	gram
nm	nanometre
µg	microgram
µl	microlitre
ml	millilitre
M	molar
BHA	Butylated hydroxy anisole
BHT	Butylated hydroxy toulene
DPPH	2,2-diphenyl-1-picryhydrazyl
GAE	Gallic acid equivalent
HAT	Hydrogen-atom transfer
QE	Quercetin equivalent
R^2	Correlation coefficient
RM	Raw material
SET	Single electron transfer
SD	Standard deviation
Sig	Significant
TFC	Total flavonoid content
TPC	Total phenolic content
UV-VIS	Ultraviolet visible

LIST OF SYMBOLS

ºC	Degree Celcius
%	Percent
≤	Less than or equal
±	Plus-minus
µ	micro

CHAPTER 1

INTRODUCTION

1.1 Research background

Underutilized plant is the crop that normally use by local and regional, however these species still lack of national recognition and appreciation even these plants mostly were used as traditional medicine, food, oil sources, fodder or even as fibre sources in the past. On the other hand, these species are remain underutilized or unknown as researchers are under estimate their potential use either in food development, cosmetic and also pharmaceutical production. As listed in HockEng et al. (2010), the underutilized fruits are divided into three categories which are species tend to commercial development such as *Ficus* sp., *Artocarpus integer*, *Salacca zallaca* and *Areca catechu*. While the second listed categories are the species may to development for local uses like *Dialum indum*, *Mangifera feotida* and also *Garcinia parvifolia*. The third is the species without current development potential for economic uses. The example for this species is like *Salacca conferta*, *Zizyphus mauritiana* and *Sandoricum koetjape*. Plus, as mentioned by (Andriani et al., 2015), *P. tectorius* also listed as

underutilized crops in Malaysia and Indonesia either use in research or even for food supply. P. *tectorius* fruit was chosen due to make this fruit well known especially for local who live around the beach area then improve the data that available about this fruit.

Nowadays, due to lacking of sources from natural antioxidant, researcher and developer compete to get the latest and the best natural antioxidant sources. Antioxidant, a compound that inhibit oxidation where the reaction that produce free radicals, which may lead to damage the cells of organisms, cause the food spoilage and also chemical materials degradation. Thus, plant species not only give significance for human health, it is also act as contributor for reduce the oxidative damage such as from phenolic compound which is obtain high of antioxidant activity. Plus, antioxidant from plant also rich in vitamin C, vitamin E, carotenes and others that may potential to decrease the risk of diseases to human like cancer Therefore, as mentioned by Prince, L., Prabakaran, (2011) in (Andriani et al., 2015), plants will be the major sources in medicine production in future, as they having therapeutic potentials that being used since ancient times.

1.2 Problem statement

Nowadays, underutilized plants have been recognized for their antioxidant properties. The *P. tectorius* is one of the underutilized plant category commonly consumed by the indigenous people in the rural area or beach area. Previously, native population claimed *P. tectorius* one of the underutilized plants able used in treatments for cold/flu, asthma, hepatitis and even cancer, while the Pandanus oils from their

leaves able to cure headache, laxative and also several skin disease like small pox. However, antioxidant activity of the fruit from *P. tectorius* still in limited as mostly of researcher was more attracted on their leaves and root parts. Plus, the relationship between extraction and the solvent used is need to determined. Thus, to maximize the extract of phytochemical for antioxidant activity in *P. tectorius*, the most suitable solvent need to be choose. So, further research was carried out to examine the potential compound of this plant by concentrating on the different alcoholic extraction and the antioxidant properties of the plant. Hence, from this study, the claim from previous people can be made verification and also particular interest due to lacking information and publication on it previously.

1.3 Hypothesis

H_0 – There is no significant difference in the antioxidant potential in ripe fruit of *P. tectorius* by using different extraction solvent.

H_A – There is significance different in the antioxidant potential in ripe fruit of *P. tectorius* by using different extraction solvent

1.4 Objective

1. To extract ripe fruit of *P. tectorius* by using different extraction solvents.
2. To determine antioxidant potential of ripe fruit of *P. tectorius* with different solvent by using total phenolic, total flavonoids content and DPPH radical assay.
3. To determine the most suitable solvents for extraction of ripe fruit of *P. tectorius*.

1.5 Scope of study

This study conducted to compare the antioxidant activity using various extraction solvent of ripe fruit *P. tectorius*. Sample of *P. tectorius* was collected from location which is around beach area in Kuala Nerus, Terengganu, Peninsular Malaysia. Three analyses were done to identify the antioxidant activities of the plant sample. The methods that were used; 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, total phenolic content (TPC) and total flavonoids content (TFC) assay.

1.6 Significance of study

This particular research was aimed more on the antioxidant properties of *P. tectorius* which can be further explored and add further data on native species of this plant and their antioxidants activities. This study also gave result on the most suitable

solvent of extraction which exhibit the abundant secondary metabolites in fruit of the *P. tectorius* which can low cost and time. Plus, this investigation has data on its antioxidant properties which could be further researched and being used in food, cosmetic and pharmaceutical study. Thus, through the identification obtained from this study, various new researches can be carried out to further. Plus, this further study will enhance the quality of human life in terms the uses of antioxidant phytochemicals like phenolic acids and flavonoids that act as curer in variety diseases such as minimizing the risk of development of cancer.

CHAPTER 2

LITERATURE REVIEW

2.1 *Pandanus tectorius*

Foundation of the genus Pandanus was made since 1679 by Rheede van Draakestein by published its names, descriptions and size and all the detailed illustrations of four species of western India. Continuous in 1743, in his posthumous Herbarium Amboinense, Rumphins named, described, and illustrated 11 species, by putting them in two genera, where one of them he named Pandanus. However, there is a contradiction about first valid publication of the genus Pandanus. But in 1773, Parkinson's report of Pandanus was valid. Then, in 1774, from German edition of Parkinson, the binomial *Pandanus tectorius* was validly published by Parkinson ex Herr Z. where the holotype is still exists in the British Museum of Natural History (Figure A.1) (John, 1980).

Now, the family of Pandanaceae have about 600 species in the same genus which is Pandanus, mainly distributed in subtropical and tropical regions, where around

30 to 40 species are in India (Adkar & Bhaskar, 2014). *Pandanus tectorius*, having different botanical name like *P. odoratissimus* Lam. and *P. fascicularis* Lamk. and locally named as Pandan Laut or Mengkuang Laut (Andriani et al., 2015). This mangrove coastal plant is recognised as Hala in Hawaiian and as screw pine or umbrella tree in English while in Hindi known as kewda (Adkar & Bhaskar, 2014). The distribution of *P. tectorius* mostly found in strandline and near coastal forests in Southeast Asia, where also include Philippines and Indonesia, expending to Papua New Guinea and northern Australia, and throughout the Pacific islands including Hawaii,Fiji and also Micronesia (Thomson, Englberger, Guarino, Thaman, & Elevitch, 2006).

As mentioned by John, (1980) habitat for *P. tectorius* along its distribution area are on sandy beaches, in littoral thickets, on the edges of brackish marshes and mangroves and inland along watercourses at low altitudes where the rainfall should be high. However, this species cannot grow on heavy, poorly drained loams but can grow on wide range of soils. Thus, (Thomson et al., 2006) claim that this Pandanus can be exposed to strong, often salt-laden winds, including buffeting from moderate to severe tropical cyclones, over a large part of its range. Plus, *P. tectorius* usually elevations less than 20 m (66 ft) above sea level, but may cultivated higher like in Hawaii which is up to 600 m (1970 ft) or higher. Besides, either in high temperature or moderately hot through the whole of the year, in primitive habitats show a little variation of *P. tectorius* for both seasonally and diurnally (Thomson et al., 2006). Furthermore, it is more tolerant to insufficiency of water than coconut but it may produce small fruit than usual and will continue to endure (Stone & Robison, 2000).

The propagation of these edible Pandanus varieties is by seedling or stem cutting where any part of branch can be used even the branches already developed. The parts need to trim to fit in a planting hole and leaves need to cut off about two-third to reduce

water loss. If the atolls are dried, the cuttings need to place over pits that consist of organic matter (Stone & Robison, 2000). As mentioned in (Thomson et al., 2006), the growth and development differ with in sex of the plant, variety, and types of planting stock which is either by seedling or branch cutting. Plus, writers also stated that vegetation from branch cutting grow faster than seedling-derived plants like the elongation is about 50- 80 cm per year, and branch from a lower height. *P. Tectorius* start to flower 3 to 4 years after planting in rainy season period (July to October).

John (1980) indicated that Parkinson's published treatment like the leaves are long, like those of sedge, sawed on the edge, the flowers are male and female, growing upon different trees, the fruit is orange colour, and as big as one's head, consisting of congeries of small cones, like those of the Anana, or Pine-apple, which they much resemble.

As in the notes by Solander or in the publication by Parkinson clearly written about one species in the section Pandanus, and if it grow in Tahiti, one could select a new standard specimen, which is the species *P. tectorius* John (1980). Here can clearly state that Parkinson's said in John (1980) is characterizes of *P. tectorius* plant and the description is in Figure A.1. In (Adkar & Bhaskar, 2014), the leaves were details as confluent, bowing; from three to five feet long, narrowing to a long fine triangular point, very smooth and shiny where having fine sharp spines. Plus, the fruits have unique composite where it made up of individual pieces called "keys" (Figure 2.1.1b) attached to a fibrous core (Figure 2.1.1c). The entire composite fruit that include the keys and the core called bunch of *P.* tectoris fruit (Figure 2.1.1a) (Andriani et al., 2015). As stated in (Andriani et al., 2015), by refer to Englberger et al., (2006), the inner parts of the keys are edible and sucked for their sweet pulp.

Figure 2.1.1 *P. tectorius* fruits (a), its keys (b) and core fibrous part (c). Source from Andriani et al., (2015)

Plus, the synonym of *P. tectorius* which is *P.* odoratissimus naturally distribute along Indo-Malaysian coasts from India and Sri Lanka throughout South-ast Asia to Taiwan, the Ryukyu Islands and Micronesia. Besides, as stated by Andriani et al., (2015) *P.* odoratissimus has been introduces into tropical Africa where it is occasionally cultivated. This look-a-like species differ with *P. tectorius* through its main morphological where *P. odoratissimus* has large white or very pale leaf spines, while *P. tectorius* has smaller greenish spines. Both species are thought to interbreed as they are easy to co-occur, then, perhaps consider *P. odoratissimus* as sub-species of *P. tectorius* Thomson et al. (2006). Furthermore, based on St John 24,212 from Ceylon, stated in John, (1980), *P. odoratissimus* has large leaf spines, but does not have fleshy shoulders on the phalanges compared to *P. tectorius*. Plus, in John (1980) also mentioned other species like *P. hueensis* St. John; however this species lacking of fleshy shoulders too than *P. tectorius*.

2.1.1 The uses of others part from *P. tectorius*

P. tectorius fruits are consider as staple food notably in Micronesia as this fruit may provide up to 50% of energy intake, and adult in Micronesia possible consume this fruit at least 20 fresh keys or about 1 kg of fruit per day during the fruiting season (Adkar & Bhaskar, 2014). As this fruit rich in vitamin C, thiamine, riboflavin, and niacin, this staple food also prepared for jam and juices (sweet and slightly acid with a pungent flavour). This fruit not only rich in vitamin C, the flesh of deeper yellow and orange-colored varieties contain high of provitamin A carotenoid levels. Plus, this carotenoid-rich fruit may secure from diabetes, heart disease, and cancer, as consume of Pandanus may ease the problems of the Pacific and also alleviate vitamin A insufficient in Micronesia. Other than use as staple food, Pandanus fruits also used to make fragrant in Polynesia and used as bait for catching lobster in Kiribati. While, the fibrous part, dried, mature drupes normally used as paint brushes, for fuel, for compost, and as fishing line floats.

As stated in Adkar & Bhaskar (2014), kewda oil usually used in earache, headache, arthritis, debility, giddiness, laxative, and rheumatism. Plus, this part also traced that function as aphrodisiac. In Polynesia male flower from uncultivated Pandanus are used to perfume coconut oil either used alone or combine with other flowers. Furthermore, the male flower also used to scent clothes and incorporated in cosmetics production like soaps, hair oils, include incense sticks in Southland Southeast Asia. Otherwise, in Hawaii, this part used to scent tapa.

In past, the usage the other part like trunk and large branches are like as building material, aid in making string, fish traps, or even used as sources of glue or caulking for canoes. Plus, this part used to extract cream from grated coconuts and burnt for fuel wood and to make compost.

The other part; prop or aerial roots are used in fabrication of house walls, supporters (basket handles), and in Kiribati, the production of black dye is derived from this part. Besides, the juice extracted from root of Pandanus are mixed with other ingredients to ease chest pain, while in Palauto used to ease stomach cramps and in Pohnpei use this part as traditional medicine for syphilis. The decoction of Pandanus root use to treat haemorrhoids and also consume for women who had just delivered and still in weak condition. Plus, the aqueous extract of root was tested for its effect on blood glucose levels to verify the activity of antidiabetic in normal and diabetic rats, where the ethanolic extract is reduce increased blood urea and inhibit body weight reduction also leucopenia induced by alloxan administration. This test also proves the ethanolic extract was effectively scavenged the DPPH and lipid peroxide free radicals and the presence of flavonoids and tannis. In the other hand, Pandanus root decoction was found to be hepatocurative but not as hepatoprotective Adkar & Bhaskar (2014).

Different parts of the Pandanus plant have their own role as this plant is useful in medicine (certain varieties) for particular treatment. In past, in Kiribati, the leaves usually help in treatments for flu, hepatitis, dysuria, asthma, boils, and cancer, also alleviate vomiting. While, the young leaves are used for curd and lancing boils. In pharmacology studies, the methanolic extract of Pandanus leaves were reported have antioxidant activity in different assay and anti-inflammatory activity by inhibition paw edema by carrageenan- induced acute and formalin –induced chronic paw edema models with standard drug diclofenac sodium in rats. Besides, petroleum ether,

chloroform and hydroalcoholic extract from this part were efficient for inhibition zones against gram-positive bacteria like *S. aureus* and *B. subtilis*, forthwith prove that Pandanus leaves have antimicrobial activity, but ineffective against gram-negative bacteria such as *E. coli* . Highlighted, the hydroalcoholic extract showed good antimicrobial activity as this extract presence of alkaloids and flavonoids that act as good antimicrobial. Otherwise, methanolic extract of Pandanus leaves possess potential central nervous system depressant action as it produced a reduction in spontaneous motor activity, motor coordination and prolonged Pentobarbital sodium sleeping time Adkar & Bhaskar (2014).

2.2 Solvent of plant extraction

As mentioned in Wu (2018) solvent extraction is the technique of distribute of a solute between two immiscible liquid phase in contact with each other like two-phase distribution of a solute. Plus, as writer stated that scientists and engineers attentive with the extent and flow of the distribution of different solute either organic or inorganic sources and yet its use scientifically and industrially for isolation of solute mixture. Thus, solvent extraction mostly used in chemical industries to produce pure chemical compound either in pharmaceuticals and biomedical or heavy organics and metal or even in analytical chemistry as well as in environmental waste purification. Then, this technique of solvent extract is the most generally used for extraction of plant secondary metabolite compound such as antioxidant.

Nevertheless, the result of extraction yields and antioxidant capacity in plant extract is differ, which is depending on the nature of extracting solvent, cause there are

variety chemical characters and polarities in particular solvent, which may resulting different of existence antioxidants compounds in solute (sample) having, which is , may or may not dissolve in notable solvent. Plus, the parts of plant used also another factors of the yield of extraction as there are different of physical characteristics of the samples used. Other than that, extraction time and temperature as well as the ratio of sample-to-solvent also give different yield of extract (Dai & Mumper, 2010).

Solvents, like methanol, ethanol, acetone, ethyl acetate and their combinations are the mostly solvent used for the extraction of antioxidant compounds like phenolics compound from variety of plant part. Syukriah (2014), stated the extraction of active compounds from plant sample is dependent on the polarity of the solvent due to polar solvent is the best used on the polar compounds. As mentioned by Dai & Mumper, (2010), thus, the right solvent need to be selected as it can affect the yield and rate of antioxidant compound extracted. Thus, as reported by Sultana, Anwar, & Ashraf (2009), ethanol and methanol are the most chosen used to extract antioxidant compounds from various plant, while from other studies by Sultana, Anwar & Ashraf (2009) stated that ethyl acetate is the best solvent used to extract phenolic compound from onion and citrus peel.

2.2.1. Ethanol

Ethanol is a chemical compound and also called as alcohol comes with the chemical formula C_2H_5OH. The others name for ethanol is absolute alcohol, cologne spirit, ethyl alcohol, ethyl hydroxide, hydroxyethane and also known as methylcarbinol; differentiae because of ethanol is systematic name and others is a common name. Plus,

this alcohol is popular in alcohol for drug and for medicine. As this alcohol is safe to consume and applied on human or animal body, it also known as rubbing alcohol as well as spirit of wine.

The formula for ethanol is derived from an ethyl group linked to a hydroxyl group or usually is abbreviated as EtOH. The name of ethanol is for a compound consisting of alkyl group with two carbon atoms, with a single bond between these two atoms, with attachment of functional group of alcohol group.

This alcohol has many uses and function for human and also for animal as well. Usually alcohol was used in hospital and any health care settings act as antiseptic to inhibit or destroy selective biocidesor any microorganisms in or on living tissue, which normally applied as personnel handwashes and on surgical scrubs. However, alcohol not recommended used for sterilization as this reagent have lack of sporicidal activity but still widely used for hard-surface disinfection and skin antisepsis.

2.2.2. Acetone

Acetone is a chemical compound and known as the common name for the simplest of ketone, that come with the formula C_2H_5OH. Acetone also called as dimethyl ketone and it is used in nail polish remover, paints as well as in lacquers. However, acetone is mainly used as a solvent for extraction and it is polar organic solvent, thus it can isolate variety of compounds especially polar compounds. Plus, it has low chemical reactivity; mostly of industry choose it as a solvent as it also low cost.

Hence, about 25% of the acetone was produced and used as solvent directly (Do et al., 2014).

As mentioned by Remler, (1923), the boiling point of acetone may allow it used for extraction at a low cost for fuel and without danger of decomposition of the final product as it not inflammable like ethyl ether, petroleum ether or benzene. Plus, write also stated that acetone is miscible in all proportions with water and other solvent as well as acts like a couple when added to certain immiscible solvents. In the other hand, as non-dangerous chemical, acetone can recovered easily with water by absorption or even with wood-tar oil.

Acetone not only be used as solvent directly, it also uses as technically like; use in connection of smokeless powder and cellulose acetate manufacture, use for extracting wood pulp to remove resins or other impurity, as desiccating agent in leather industry, as precipitating agent in the preparation of soluble starch and act as most suitable solvent for oak tannins (Remler, 1923).

As reported by Garcia-viguera, Zafrilla, & Toma, (1998), acetone is a chemical solvent that suitable use for extract anthocyanins in strawberry. Plus, as mentioned by writers, acetone allows an efficient and more yields of extraction as well as can prevent problems may face with pectins cause acetone have pectin clotting properties. Besides, it permits a much lower temperature for sample concentration which is at $\leq 30°C$. Moreover, the benefit of using acetone as solvent for anthocyanins is that the quantitative determinations obtained were more accurate than using other solvent. Thus, in industry production of strawberry jam, acetone extraction was applied to determine the quantitative and qualitative anthocyanin composition in strawberry.

2.2.3. Ethyl acetate

Ethyl acetate is an organic ester compound with molecular formula of $C_4H_8O_2$ that produced from esterification process of ethanol when the presence of acetic acid which is the combination of ethanol and acetic acid. This derivative of ester also have others name like ethyl ester and acetic ester, where it systematic name is ethyl ethanoate.

This organic ester compound well-known used as organic solvent in chromatography and it is a moderately polar solvent. Plus, as mentioned in Wu, (2018) ethyl acetate is much less applied as reactant in organic synthesis as compared with acetone. However, ethyl acetate can do some typical models of transformation in organic reaction like transesterification, amidation, reduction, and reaction based on weakly acidic α-hydrogen due to existence of ester group in it compound.

Plus, ethyl acetate not only use as extraction solvent singly, but also often mixed with a non-polar solvent like hexanes as a chromatography solvent. Moreover, it widely acts as a solvent for nail varnishes and nail varnish remover. Plus, in food industry, this ester compound used to decaffeinate coffee beans and tea leaves. As mentioned by Bryne & Howell, (2011) in wine, ethyl acetate is the common ester produced, thus, it been associated with wine spoilage for a long period by affected wine with a distinct nail polish remover aroma.

Furthermore, as in grapes and wine, that usually produced by yeast during fermentation, which is creating by-products that react with ethanol to form ester. In additional, ethyl acetate can be produced by acetic acid bacteria with low oxygen conditions and very slow esterification of ethanol and acetic acid over time. On the

other hand, there are two factors affecting formation during fermentation, some species of yeast like 'native' or 'spoilage' yeasts normally lead to increased level of ethyl acetate in finished product which is wine. However, maximum formation of ethyl acetate happens over the temperature range between 10 to 20°C (Bryne & Howell, 2011).

2.2.4. Petroleum ether

Petroleum ether is a mixture of short chain alkanes that derive from pentanes and hexanes, which is come with molecular formula C_6H_6, whereas it is a cyclic, aromatic hydrocarbon. Plus, petroleum ether has others name like benzine, petroleum naphtha and also known as gasoline. Even in it common name have ether, but it not an ether like diethyl ether which is having R-O-R' as functional group, but chemically due to its intermediate between the lighter naphtha and the heavier kerosene.

Moreover, during petroleum distillation process in certain temperature (boiling), give several fraction of petroleum ether, which from 60 to 80°C is resulting fraction that often used as a replacement for hexane. Plus, lipids like triacylglycerides with less or no polar groups are highly soluble in hexanes and also in more polar solvents like diethyl ether. Furthermore, the solubility of lipids increases in alcoholic solvents as increasing the length of carbon chain of alcohol, so they are soluble in ethanol and *n*-butanol, as, the shorter chain fatty acids in the lipids will have greater solubility in the more polar solvent. Thus, hexanes are effectively act as solvent for crude fat for forages than petroleum ether (Moreau & Robert, 2005). Besides, as reported by Buck V. E., (2007)

as hexane is more non polar solvent than petroleum ether, it be more efficient for extracting the oil than petroleum ether.

Petroleum ether is mainly used as a solvent in place of hexanes or pentane and also as glue remover as well as a solvent for cleaning glassware, since it often cheaper than those solvent. Plus, there is good recovery of paracetamol-induced necrosis by petroleum ether extract of *Flacourtia indica* in histopathological examination as the hepatoprotective effects exhibited by petroleum ether extract might be mediated through the inhibition of microsomal drug metabolizing enzymes. Moreover, Iddiqui, Ulzar, Ahmood, Egum, & Han, (2004) claimed that the petroleum ether extract of dried ground whole fruits of *Piper nigrum* L. yield 20 compounds including two new insecticidal amides which are pipnoohine and pipyahyine which these two can exhibited toxicity against fourth instar larvae of *A. aegypti* L. determined by WHO method.

2.3 Antioxidant

According to Halliwell (1999) antioxidant is a substance that is required in small quantities in order to prevent or incredibly retard the oxidation of easily oxidizable materials. Other than that, antioxidant also defined as any substance which is present in low concentrations compared to oxidizable substrate significantly can delays or prevents oxidation of those substances. Furthermore , Youssef, (2015) mentioned that oxidation is a chemical reaction that transfers electron or hydrogen from substances to an oxidizing agent as well as it reaction can produce free radical substances and this will cause damage or death to the cell. However, antioxidants terminate these reactions by removing free radical intermediates and inhibit other oxidative reactions.

Besides, the major cause of various chronic and degenerative diseases like diabetes mellitus, inflammation, stroke or even cancer are cause by free radicals Lien Ai Pham-Huy & Hua He, (2008). Other than that, mentioned by Wsowicz, Gramza, Hêœ, Jeleñ, & Korczak, (2004)free radicals also can cause food deterioration as inducer of lipid peroxidation in food products. Thus, nowadays, mostly on food industries are using antioxidant to preserve the quality and prolong the shelf life of the food products. Thus, reported by Praveen et al., (2015) the free radical scavenging is one of known mechanisms used for screening the radical scavenging activity of specific compounds and the antioxidant activity is governed by several methods like DPPH, ferric reducing antioxidant power, total radical trapping antioxidant parameter, hydroxyl radical scavenging, etc. However, DPPH method is the often used and claimed as the best method.

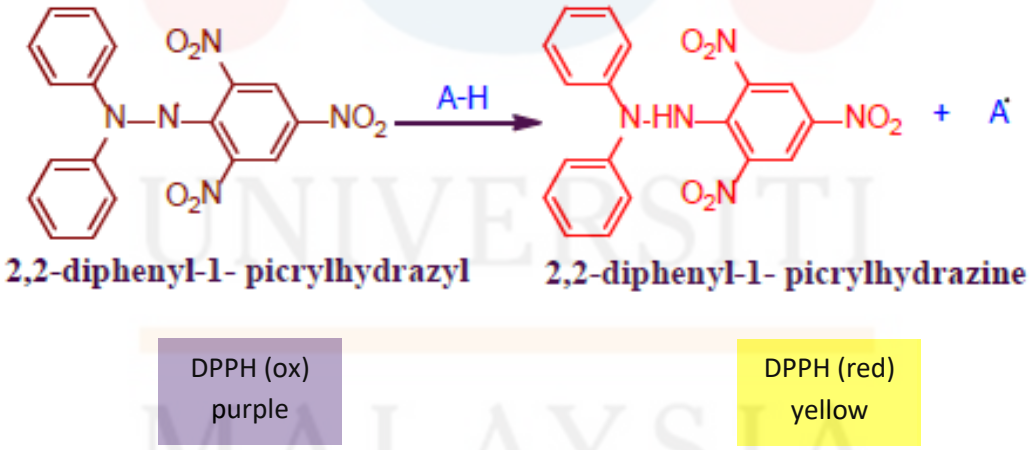

Figure 2.3.1.1 The mechanism of DPPH free radical with antioxidant compound. Source by Praveen Singh, Ranjeet Kumar, Shachi Tiwari, Ranjana S Khanna, (2015)

From the mechanism in Figure 2.3.1.1., the free radical scavenging activity of the synthesized substituted 2,4,5-triaryl imidazole derivatives arise either from phenolic hydroxyl groups or from the imine unit of the imidazole moiety. A reactive free radical can undergo electron transfer or abstract H atom from either of these two sites.

2.3.1 Total phenolic content (TPC)

Phenolic compounds are a natural source of antioxidants and are widely found in plants. They can be divided into four classes which are flavonoids, phenolic acids, hydroxycinnamic acid derivatives and lignans. These simple compounds has been shown to possess the ability to combat or lower the incidence of several pathological conditions caused by oxidative stress such as cardiovascular disease, inflammation, neurodegenerative disease. Phenolic acids which are commonly found in wheat bran and berries are strong antioxidants against free radicals (Kim, 2018). Apart from that, tannins which were either soluble or insoluble in water has been shown to have atheroprotective effects (Zargham, 2008).

Total phenolic assay is the most basic assay to quantify phenolic compounds present in a mixture. Total phenolic content in a sample can be measured through Folin-Ciocalteu method. The Folin-Ciocalteu reagent consists of a mixture of phosphomolybdate and phosphotungstate. This assay depends on the oxidation-reduction reaction of Folin-Ciocalteu reagent with hydroxyl (-OH) groups or reducing agents present in the sample (Folin-Ciocalteu reagent). In the presence of reducing agents, the yellow colored reagent will be reduced to a blue colored solution.

2.3.2 Total flavonoid content (TFC)

Some of the active components of some medicinal products are flavonoids. Flavonoids are sub-group of phenolic compounds and possess various biological bioactivities including antioxidant activity. Flavones possess antioxidant and antimutagenic activity, flavanones and xanthones, exhibit antiviral, antimicrobial, antioxidant and anti-inflammatory activities, and isoflavones and coumestans present important antioxidant action.

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones according to chemical structure. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee, beer, wine and fruit drinks).They occur mostly as glycosylated derivatives, sometimes conjugated with sulphate or organic acids (Panche, Diwan, & Chandra, 2016)

The analysis of the content of main active components in raw materials and phytomedicines is an essential step to evaluate the quality of products and validate the efficacy and safety of their therapeutic use. A widely used technique for the quantification of flavonoids is based on its complexation with aluminum chloride ($AlCl_3$) and the spectrophotometric determination of the formed complex, which provides a bathochromic displacement and the hyperchromic effect. In this method, several factors are crucial for the formation of the flavonoid-$AlCl_3$ complexes and must be optimized to improve the method performance such as the reaction time, the complexant concentration of flavonoid in term of herbal material ratio and the chemical structure of the polyphenol. Thus, a multivariate approach can be used to evaluate the

effect of the critical variables on the selected response. Experimental designs are multivariate tools used for the systematic and effective evaluation of simultaneous modifications of several variables in a specific system.

2.3.3 DPPH assay

The foundation of this method is by Brand-Williams et al., (1995) and was modified by Sanchez-Moreno et al., (1998) and the often used as antioxidant assay. 1, 1- diphenyl-2-picrylhydrazyl (DPPH) is stable organic nitrogen radical, which exhibit a deep purple colour. The measurement of the scavenging ability of antioxidants that present in sample towards DPPH are detected by DPPH assay, which is DPPH radical will be reduced upon reduction by antioxidant and decrease in absorbance at 517 nm (Kedare & Singh, 2011).

Mentioned by Kedare & Singh, (2011) when a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduce form of the radical is generated accompanied by loss of colour. Plus, the loss of this colour (violet) would be required to be a leftover light yellow colour. Moreover, this delocalization process is characterized by an absorption band in ethanol solution (Kedare & Singh, 2011). Plus, in Krishnanand, Himanshu & Nabo (2011) stated that the kinetic reactions of the antiradical measurement depend on the nature and amount of antioxidant present.

CHAPTER 3

METHODOLOGY

3.1 Material, apparatus and equipment

Cuvette, pipette tips, filter paper, aluminium foil, test tube, test tube rack, pipette, medium bottle, knife, chopping board, basin, filter funnel, spatula, weighing scale (Sartorius, Germany), oven, electrical grinder (Panasonic, Japan), UV-VIS spectrophotometer machine (Merek & Co, Germany) and vortex mixer (Smith & Nephew, United State). All equipment were provided by FIAT.

3.2 Chemical and reagents

Ethanol, acetone, ethyl acetate, petroleum ether, distilled water, DMSO, methanol, BHT, Gallic acid, Quercetin, DPPH solution, 7.5% sodium carbonate, 10%

Folin-Ciocalteu reagent, 0.5 M sodium nitrite solution, 0.3 M aluminium chloride hexahydrate solution, 1 M sodium hydroxide solution. All chemical was provided by the FIAT.

3.3 Preparation of samples

The sample of *P. tectorius* fruits was collected from the beach area in Kuala Nerus, Terengganu, Malaysia. The sample (2.1 kg) was collected from end of September 2018. After collection, *P. tectorius* fruits were divided into two parts, keys (1.8 kg) and cores (267 g) and only the keys part were proceed for analysis. The soft part of the keys (857 g) were cut, grinded and then dried using oven in 50°C (dried soft keys = 91 g).

3.4 Preparation of extraction

The dried fruit of *P. tectorius* was extracted using maceration technique for 5 days. 20 g of sample was extracted using 200 mL of ethanol, acetone, ethyl acetate and petroleum ether, respectively. The extracts were filtered with the filter Whatman Qualitative Filter Paper; 18.5 cm diameter, pore size, 0.125 µm to yield 175mL of extract from solvent used.

The concentration of extracts used for analysis is 50% from the 100% extract by using equation $m_1V_1=m_2V_2$. Where; m_1= initial percentage of concentration, %, V_1=

initial volume of solvent, mL, m_2= final percentage of concentration, % and V_2= final volume of solvent, mL. The extracts were analysed on their antioxidant activity by DPPH free radical scavenging assay, total phenolic content (TPC), and total flavonoid content (TFC).

3.5 Determination of total phenolic content (TPC)

The modified Folin-Ciocalteu assay from Chang et al., (2001) & Jothy & Zuraini (2011) was used for the determination of the total phenolic content. 10% Folin-Ciocalteu phenol reagent, 7.5% sodium carbonate and gallic acid were reagent for this method. For the preparation of calibration curve, 0.5 mL of 400, 200, 100, 50, 25, 12.5, 6.25 µg/ mL methanolic gallic acid solution mixed with 1.5 mL 10% Folin-Ciocalteu phenol reagent were put into test tube. The mixture was vortex and incubates for 5 minute at room temperature. Then, 2 mL of 7.5% sodium carbonate were added to the mixture. The mixtures were incubated for 2 hours in the dark place at room temperature. The absorbance was measured by using UV-VIS spectrophotometer at 765 nm. The procedure was repeated for sample with replacing Gallic acid with sample extract. The total phenolic content of the extract was determined from the standard curve of gallic acid and expressed as µg Gallic acid equivalent per gram raw material (µg GAE / g raw material).

3.6 Determination of total flavonoids content (TFC)

The TFC of extract samples determined using Aluminium Chloride Colorimetric method according to method Do et al., (2014) with some modification. Briefly, in a test tube, 0.3 mL extracts sample and 3.4 mL of 30% aqueous methanol, 150 µL of 0.5 M sodium nitrite solution and 150 µL of 0.3 M aluminium chloride solution was mixed. After an interval 5 minutes, 1 mL of 1 M NaOH solution was added. The absorbance reading was recorded using UV-VIS spectrophotometer at 506 nm. Quercetin was used as a standard. Its calibration curve was drawn and total flavonoid content in samples was expressed as µg quercetin equivalent per gram raw material (µg Quecertin/ g raw material).

3.7 DPPH scavenging activity assay

Antioxidant capacity was obtained by DPPH free radical scavenging assay Chang et al., (2001) & Nikhat, Satynarayana & Subhramanyam, (2009) using the butylated hydroxytoluene (BHT) as a positive control and ethanol as negative control. The extracts were prepared in varying concentration by twofold dilution in ethanol with concentration of 200, 100, 50, 25, 12.5, 6.25 µg/mL. 2 mL of DPPH solution was added into all the samples and incubated for 30 minutes (min) at room temperature in the dark place. The absorbance was measured at 517 nm using UV-Vis spectrophotometer. Free radical scavenging activity was determined according to the equation: % of radical

scavenging activity = $\frac{(Ac - As)}{Ac} \times 100$, where; A_c = Absorbance of control and A_s = Absorbance of sample.

3.8 Data analysis

Data were analysed using Microsoft Excel 2010. T-test was used to compare any significant differences between the sample extract in pair. Values were expressed as mean ± standard deviations. All the analyses were carried out in triplicates.

CHAPTER 4

RESULT & DISCUSSION

4.1 Preparation of extract

This study was conducted to determine antioxidant potential and the most suitable solvents for extraction of ripe fruit of *P. tectorius* with different solvent used by using DPPH free radical scavenging assay, total phenolic and total flavonoids content assay. The plant sample was collected at the beach area in Kuala Nerus and the soft part of the keys were cut, grinded for increase the total surface area when dry them. The sample was dried by using oven in 50°C to save the time and to control the temperature instead by using air dry. Then, the dried sample was extract by soaking in four different types of solvents which is ethanol, acetone, ethyl acetate and petroleum ether for 5 days as the best extraction time is 3 days. These solvent were selected due to the difference of their polarity. The observation of the sample extract was tabulate in Table 4.1.1.

Table 4.1.1 The colours of *P. tectorius* fruits extracts, extracted using difference solvents.

Extract	**Observation (colour)**
Ethanol	Red (Figure B.1)
Acetone	Red to orange (Figure B.2)
Ethyl acetate	Yellow to orange (Figure B.3)
Petroleum ether	Light yellow (Figure B.4)

Based on those colour observation due to the pigment that extracted out by respectively solvent; ethanol give red colour of extract that indicate the compound of flavonoid was extract out, acetone give red to orange colour that indicate the compound of carotenoids was extract out, ethyl acetate give yellow to orange colour that indicate the compound of carotenoids was extract out while for petroleum ether give light yellow colour that indicate flavonoids compound was extract out. Plus, based on the study from Andriani et al., (2015), those phytochemical properties had correlation with their bioactivity properties like antioxidant properties.

From this study, petroleum ether extract was stop to proceed, as the absorbance reading for this extract was exceeding all standard curve in this project and the mixture of petroleum ether with DPPH assay reagent were given layered solution. As reported by Zulkefli, (2013) the result for IC_{50} for petroleum ether extract of plants *Ervatamia coronaria* and *Tinospora crispa* cannot be computed as the percentage of inhibition of DPPH radical scavenging activity was less than 50% at all concentrations tested. Thus, petroleum ether extract cannot be proceeding for further result.

4.2 Determination of total phenolic content (TPC)

The gallic acid equivalent curve was showed in Figure 4.2.1, the value obtained were based on the concentration of standard, 400, 200, 100, 50, 25, 12.5, 6.25 mg/ mL methanolic gallic acid solution. The coefficient correlation for this graph is R^2= 0.9466 and the equation for this graph is y = 0.0072x + 0.2908.

Figure 4.2.2 showed the total phenolic content of the samples. The phenolic compound of ethanol, acetone and ethyl acetate extract were expressed in terms of μg GAE / g raw material. The result obtain were ethanol extract (4.52 ± 0.07 μg GAE / g raw material), acetone extract (1.71 ± 0.00 μg GAE / g raw material), and ethyl acetate (0.43 ± 0.01 μg GAE / g raw material). Based on the Figure 4.2.2, analysis of means (T-test) used to find the different of mean in TPC capacity between samples. The TPC capacity between the ethanol, acetone and ethyl acetate extract showed the significant different at the P< 0.05 level.

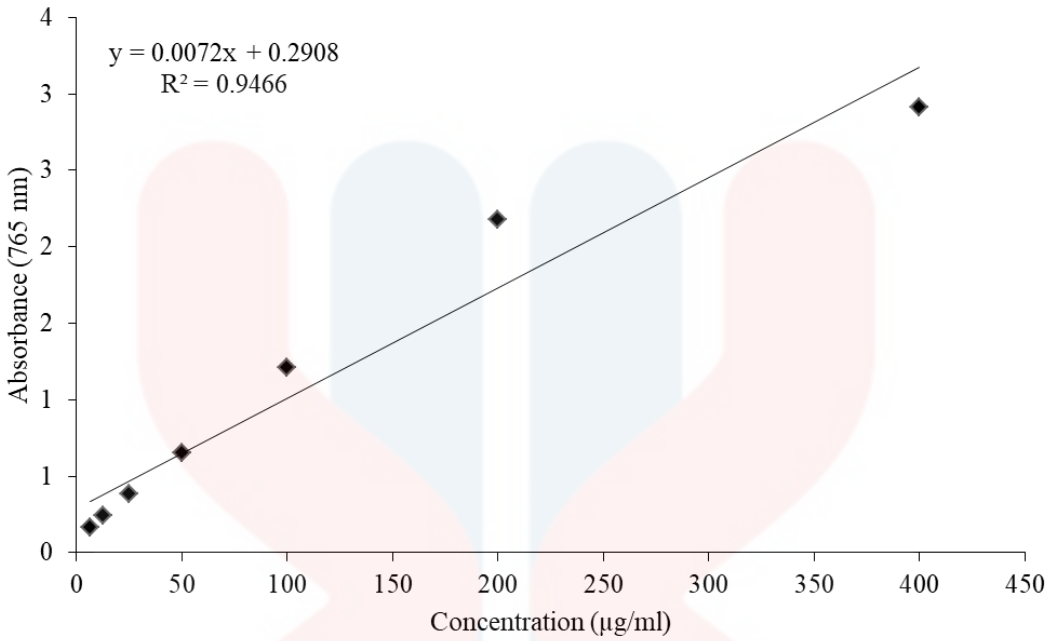

Figure 4.2.1 Gallic acid equivalent curve

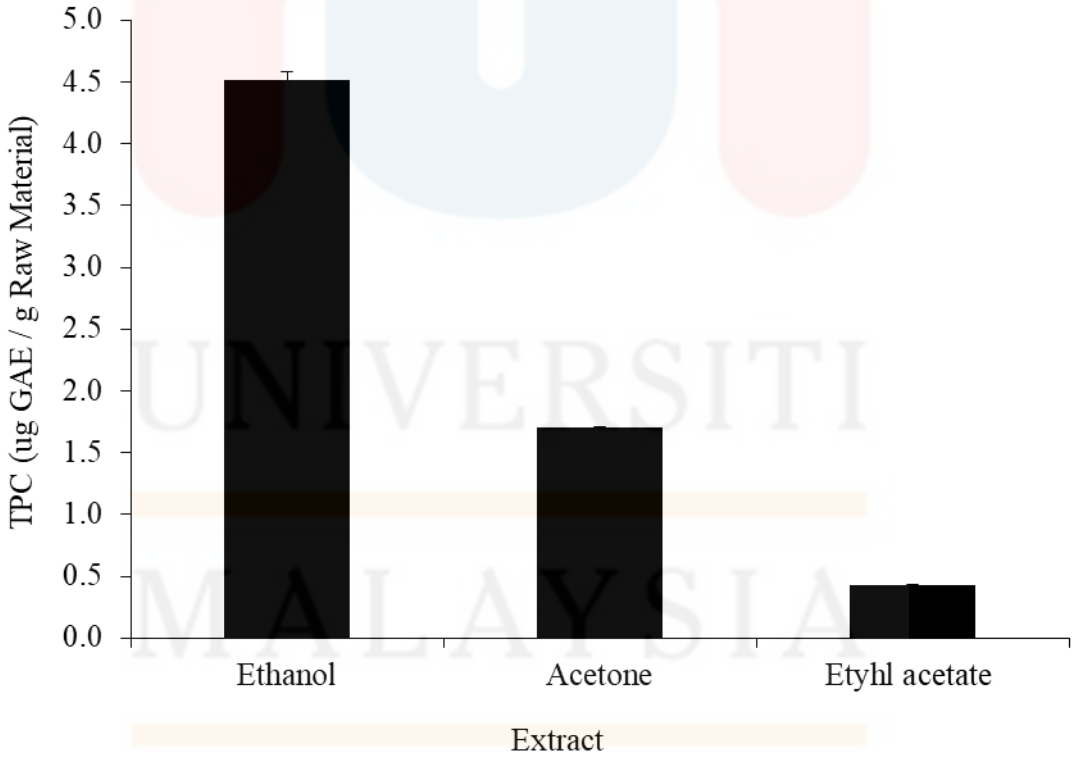

Figure 4.2.2 TPC capacity against ethanol, acetone and ethyl acetate extract of *P. tectorius*

4.3 Determination of total flavonoids content (TFC)

The Quercetin equivalent curve was showed in Figure 4.3.1, the value obtained were based on the concentration of standard, 1000, 400, 200, 100, 50, 25, 12.5, 6.25 mg/ mL methanolic quercetin solution. The coefficient correlation for this graph is R^2= 0.9823 and the equation for this graph is y = 0.0002x + 0.0151

Figure 4.3.2 showed the total flavonoid content of the samples. The flavonoids compound of ethanol, acetone and ethyl acetate extract were expressed in terms of μg QE / g raw material. The result obtain were ethanol extract (29.42 ± 1.15 μg QE / g raw material), acetone extract (8.56 ± 0.75 μg QE / g raw material), and ethyl acetate (12.12 ± 1.86 μg QE / g raw material). Based on the Figure 4.3.2, analysis of means (T-test) used to find the different of mean in TFC capacity between samples. The TFC capacity between the ethanol, acetone and ethyl acetate extract showed the significant different at the P< 0.05 level.

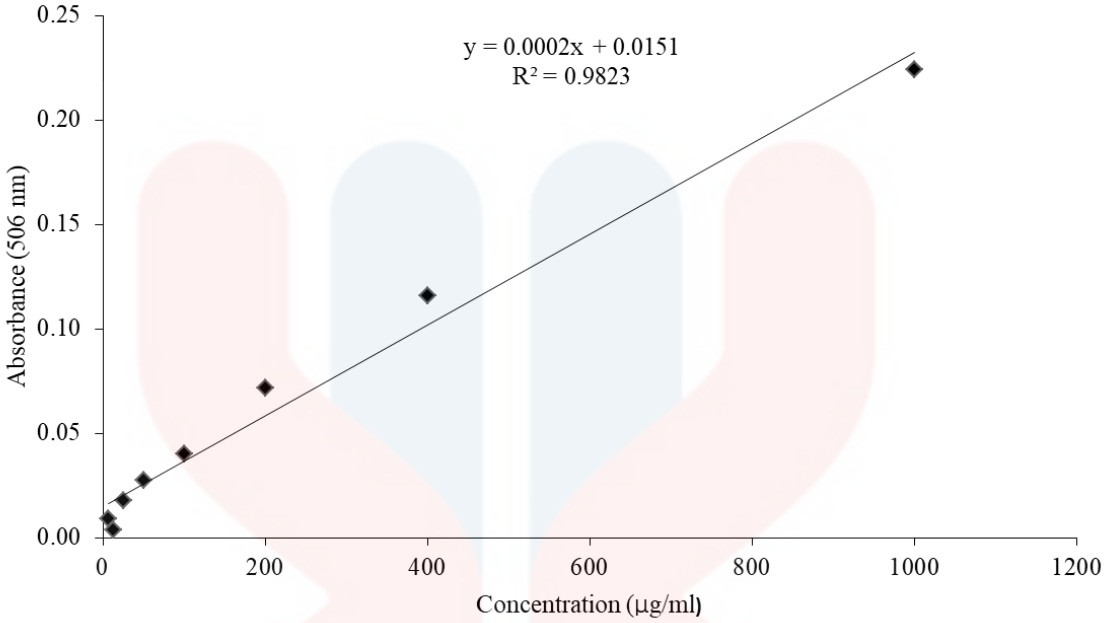

Figure 4.3.1 Quercetin equivalent curve

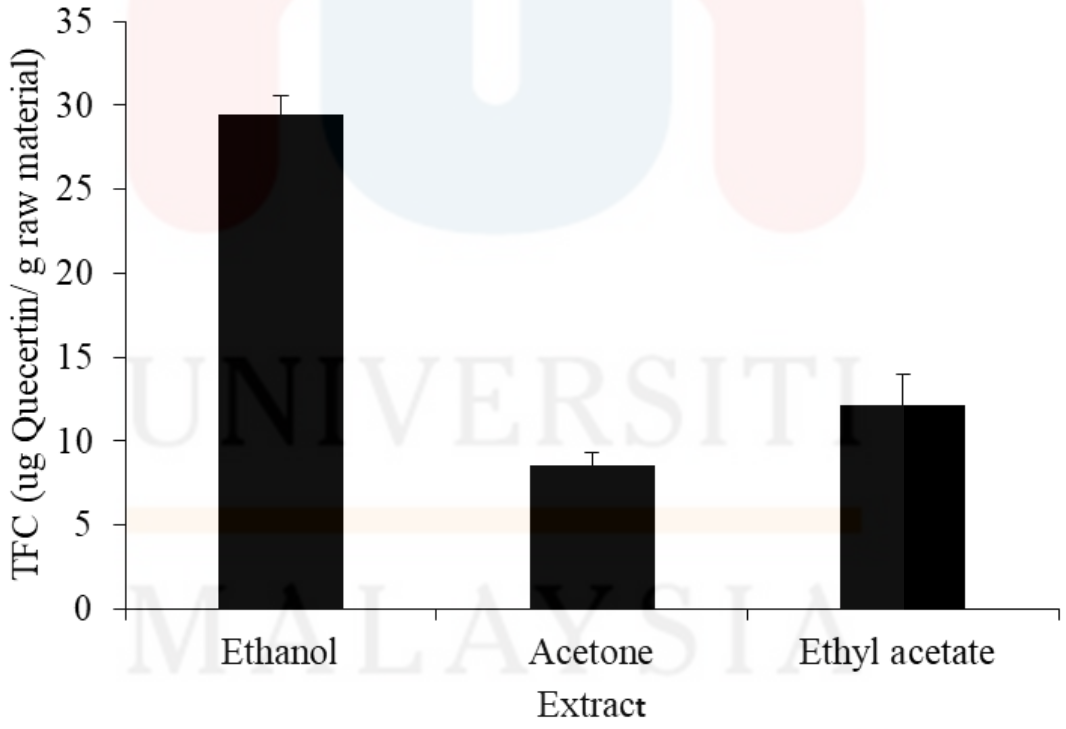

Figure 4.3.2 TFC capacity against ethanol, acetone and ethyl acetate extract of *P. tectorius*

From the result of this study showed that phenolics and flavonoids were chemical constituents in there extracts which could be contributed on their TPC and TFC properties. Plus, the result showed that high TPC and TFC capacity was obtained from sample with the high antioxidant activity which is ethanol extract. Furthermore, from the previous study reported that the extract of *P. tectorius* fruits was isolated the phenolics and flavonoids compounds like ethyl caffeate, dihydroconiferyl alcohol and tangeretin (Zhang et al., 2012) as well as other two new phenolic compounds from *P. tectorius* fruits which is pandanusphenol A and pandanusphenol B (Zhang et al., 2013) means that *P. tectorius* fruit is high of phenolics and flavonoids content.

As mentioned by Andriani et al., (2015), the major phytochemical properties in *P. tectorius* fruit are phenolics, flavonoids and steroids. Plus, as stated by Ghasemzadeh, Jaafar & Rahmat (2010), as increase the phenolic and flavonoids content will increase the antioxidant activity as there was a linear correlation between phenolics and flavonoids content and antioxidant activity. Plus, Andriani et al., (2015) also mentioned that the factor that may affect the antioxidant capacity from total phenolic and total flavonoids content is the coefficient correlation value R^2. Plus, other factors like different phenolic compound will response differently towards Folin-Ciocalteu and also the molecular antioxidant respond of phenolic compounds varied remarkably, depending on their chemical structure. Plus, the factor that may contribute to the correlation is interference of other chemical components that present in extract (Andriani et al., 2015).

Plus, as presented by Anokwuru, Anyasor, & Olusola, (2011), the ethyl acetate extract for *S. scabrum* leaves gave highest flavonoids content than acetone and ethanol extract, thus as suggested by writers, ethyl acetate and methanol are better solvents for the extraction of flavonoids compared to acetone and ethanol in plant sample (*S.*

scabrum). Besides, Ruiz-Larrea, (2002) mentioned that abounded flavonoids usually are found in the woody and external parts of plants. Thus, as the *P. tectorius* fruit is woody-look characteristics (Figure A.5), the flavonoids in it is may high as well as based on the result for TFC showed that ethyl acetate extract is higher than acetone extract but lower than ethanol extract due to ethyl acetate and ethanol is the best solvent to extract flavonoids compound from *P. tectorius* fruit, with agreement from (Anokwuru et al., 2011).

Moreover, in the previous study of red fruit which is scientifically known as *P. conoideus* Lam which is commonly consumed in Papua, Indonesia stated that ethyl acetate extract of this plant sample is the highest antiradical activities and also had a capability to reduce Fe^{3+} to Fe^{2+} which indicate it has high of TPC and TFC as well as indicate that ethyl acetate extract and it fractions is the best solvent to be used to isolate phenol compounds. However, resulted by Chenopodium *album*, the acetone extract is the best solvent for isolation of flavonoids followed by 50% of methanol extract, then ethyl acetate extract and lastly methanol extract. Thus, from this result, the polarity of solvent is not indicating as the best solvent to be used to isolate flavonoids and phenolic compounds.

4.4 Determination of free radical scavenging by using dpph assay

The BHT equivalent curve was showed in Figure 4.4.1 showed the value obtained were based on the concentration of standard, 6.25, 12.5, 25, 50, 100, 200 mg/ mL ethanolic BHT solution. The coefficient correlation for this graph is R^2= 0.9779 and the equation for this graph is y = 0.294x + 7.0564.

The antioxidant capacity for µg BHT equivalent per 1 g of raw material from samples was shown in Figure 4.4.2. The result obtain from samples were ethanol extract (6.89 ±0.06 µg BHTE/ g raw material), acetone extract (4.58 ± 0.07 µg BHTE/ g raw material) and ethyl acetate extract (2.29 ± 0.03 µg BHTE/ g raw material). Based on the Figure 4.1.2, analysis of means (T-test) used to find the different of mean in antioxidant activity between samples. The antioxidant capacity between the ethanol, acetone and ethyl acetate extract showed the significant different at the P< 0.05 level.

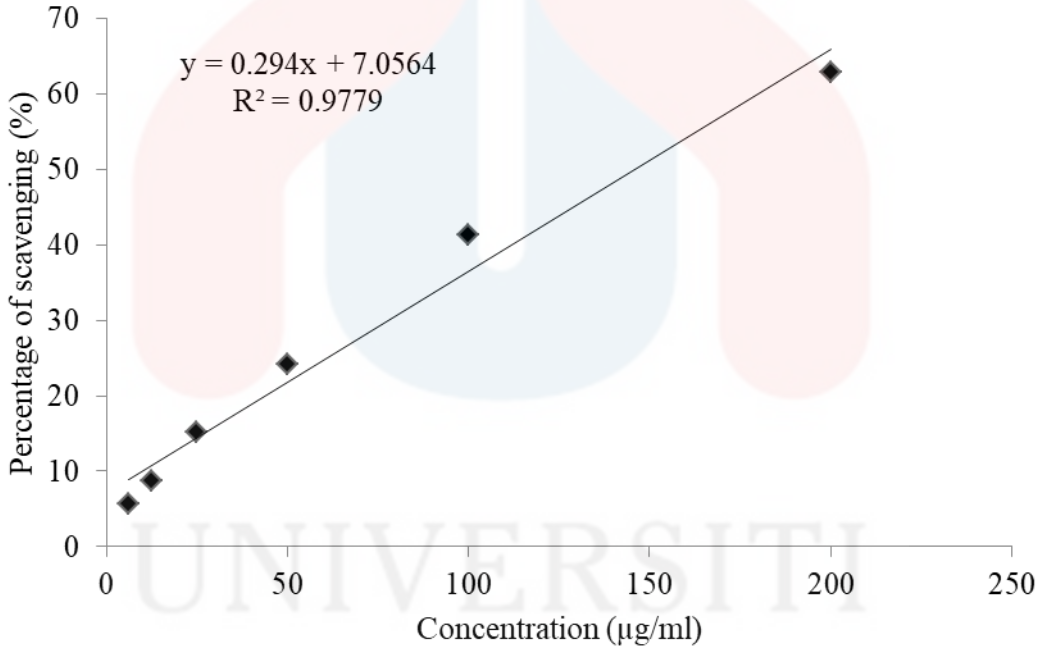

Figure 4.4.1 BHT equivalent curve

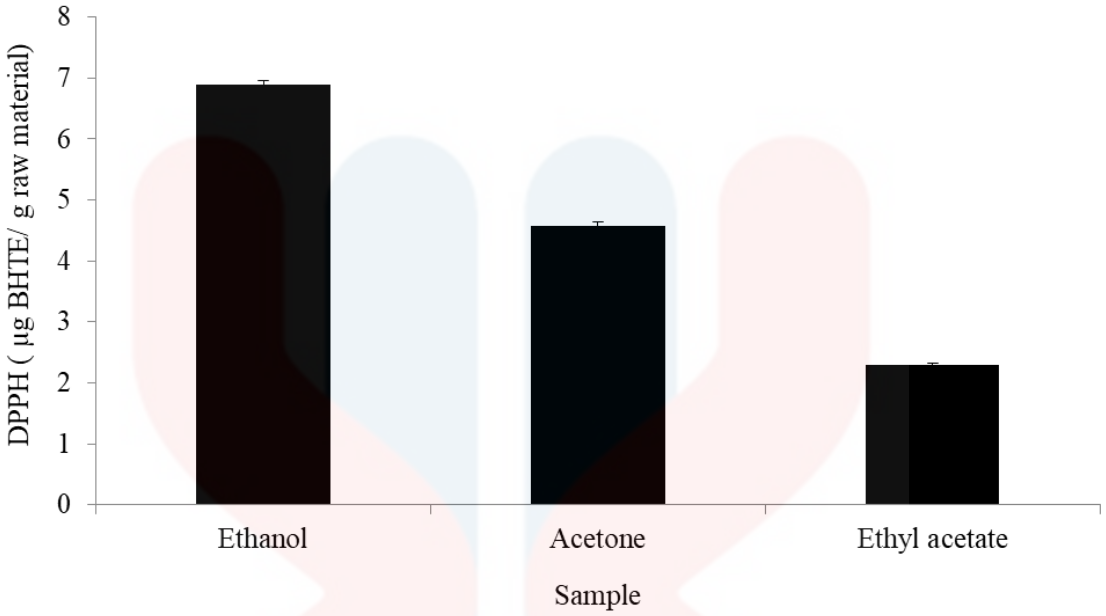

Figure 4.4.2 DPPH capacity against ethanol, acetone and ethyl acetate extract of *P. tectorius*.

Based on the result from this study, the DPPH capacity showed ethanol extract is the higher compared to others solvents thus, ethanol was considered have strong antioxidant activity compare to acetone extract and ethyl acetate extract. This result was in agreement with several researchers that studies on the antioxidant properties from plant extract of *P. tectorius* in fruits and also in different parts.

Based on the previous study reported that methanol extract from *P. tectorius* fruits had antioxidant activity more than 50% and the IC_{50} value of 8 mg/mL, thus writers considered this extract had strong antioxidant activity compare to other solvent like hexane and ethyl acetate, due to the methanol polarity is higher than others (Andriani et al., 2015). Besides, in other studied of *P. amaryllifolius* leaf extracted in propylene glycol and ethanol, the propylene glycol extract had higher DPPH radical scavenging activity than ethanol extract which may indicate that antioxidant active compounds in *P. amaryllifolius* are more soluble in more polar solvent which is

propylene glycol (Jimtaisong & Krisdaphong, 2013). The result of *P. tectorius* fruit ethanol extract from this study had higher DPPH radical scavenging activity than others solvent like acetone and ethyl acetate due to antioxidant active components in *P. tectorius* may are more soluble in more polar solvent like ethanol.

Plus, as studied by Olorunshola Omodamiro, (2016), in DPPH assay, the DPPH scavenging activity of the ethanol extract of *P. tectorius* leaves at different concentration will give different percentage inhibition compared to standard drug use (vitamin C). Thus, as the conclusion from the result showed by him, at increasing concentration, increasing inhibition even the standard vitamin C have more scavenging effect by using DPPH method, then, this system is valid for the primary characterization of the scavenging potential compound and ethanol is the potential solvent to be used to extract *P. tectorius* leaves. Then, this can be conclude that ethanol have potential as a solvent extraction due to its positive result for free radical scavenging activity based on its polarity.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The result concluded that extract of ripe *P. tectorius* fruit from keys part revealed high total phenolic content and total flavonoid content contributing to their antioxidant capacity. The result indicate that ethanol extract of ripe fruit *P. tectorius* showed high antioxidant activity by inhibiting the free radical scavenging activity (DPPH). Therefore, the best extracting solvent of ripe *P. tectorius* fruit in this project is ethanol.

5.2 Recommendation

The objective of this study is achieved successfully since all the assay system showed the positive result. Since *P. tectorius* has positive correlation between

the tests, it can be serve as natural antioxidant by extracting using ethanol and may be able to be considered for the further investigation of the potential antioxidant agent.

The further study of this project, the various assay system should be used like FRAP and ABTS assay system to test the antioxidant activity. By using different assay system, the result of antioxidant potential of sample extract will be more accurate and precise. Plus, since DPPH assay is so sensitive to light, the further study should carry out in dark room to reduce the effect towards the result to get precise result.

Plus, further study on this ripe fruit for bioactive compound like antibacterial and anticancer properties will give new evolution in science. As this fruit already known to its benefit to human consumption and had been used as traditional medicine. Then, the presented data would certainly help to ascertain the potency of the tested ripe *P. tectorius* fruit for medical health functions, functional food and as well as for nutraceutical applications.

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APPENDICES

APPENDIX A

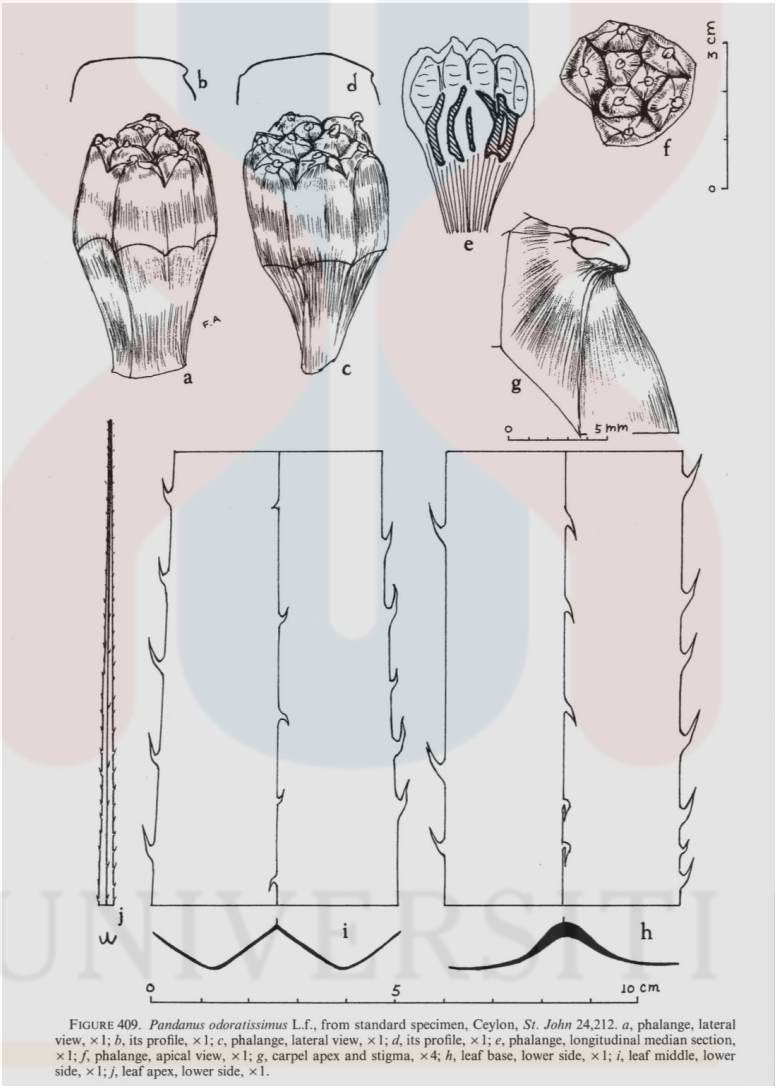

FIGURE 409. *Pandanus odoratissimus* L.f., from standard specimen, Ceylon, *St. John* 24,212. *a*, phalange, lateral view, ×1; *b*, its profile, ×1; *c*, phalange, lateral view, ×1; *d*, its profile, ×1; *e*, phalange, longitudinal median section, ×1; *f*, phalange, apical view, ×1; *g*, carpel apex and stigma, ×4; *h*, leaf base, lower side, ×1; *i*, leaf middle, lower side, ×1; *j*, leaf apex, lower side, ×1.

Figure A.1 Description of the standard specimen by Ceylon, St. John 24,212

Source (John,1980)

IGURE 408. *Pandanus tectorius* Parkins. ex Z, Society Islands, 1769, *Banks & Solander*; illustration of inate plant by Sydney Parkinson (British Museum of Natural History).

Figure A.2 The drawing of a staminate inflorescence and a detail of two staminate fascicles of it by Sydney Parkinson

Source: John, (1980)

Figure A. 3 Tree of *P. tectorius*

Figure A.4 Ripe and premature fruit of *P. tectorius*

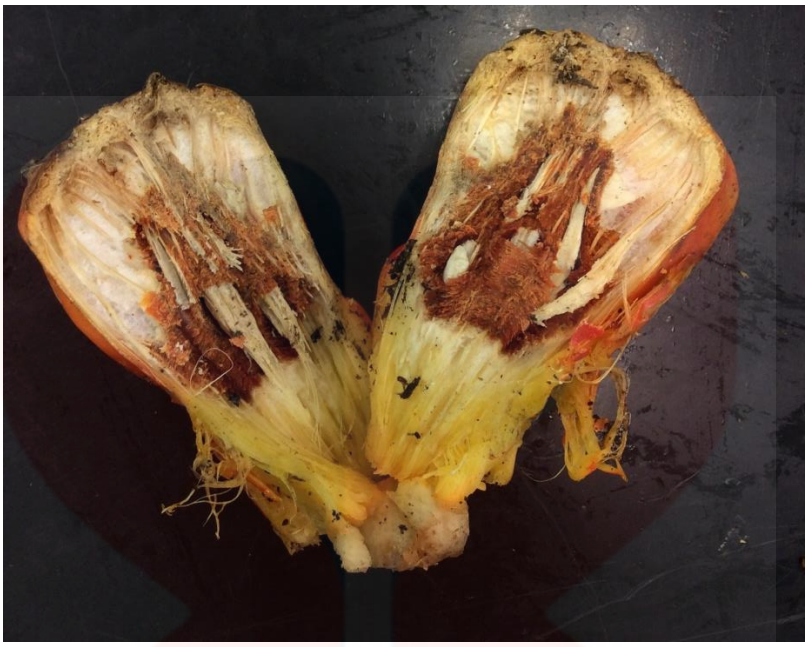

Figure A.5 The cross section (inner part) of *P. tectorius* fruit (key)

APPENDIX B

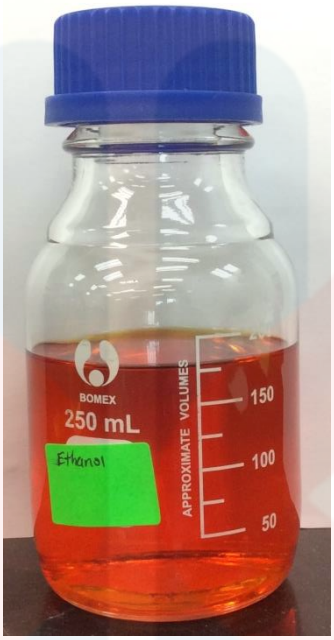

Figure B.1 Ethanol extract of *P. tectorius* fruit

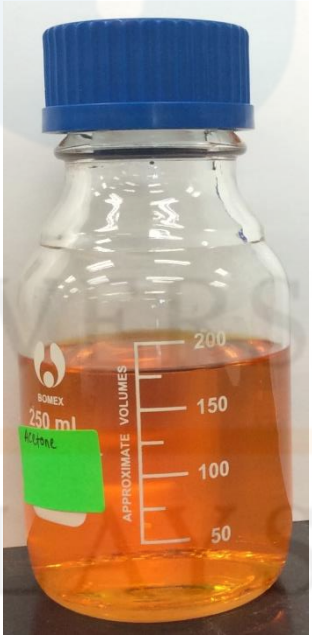

Figure B.2 Acetone extract of *P. tectorius* fruit

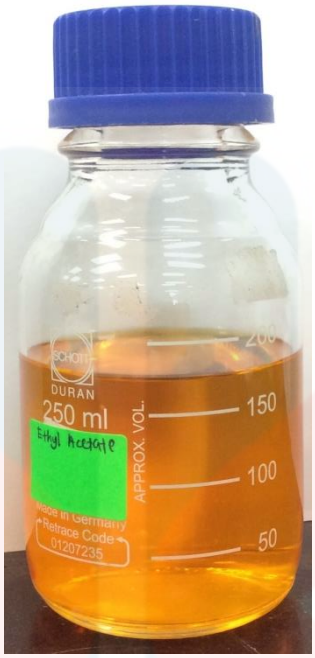

Figure B.3 Ethyl acetate extract of *P. tectorius* fruit

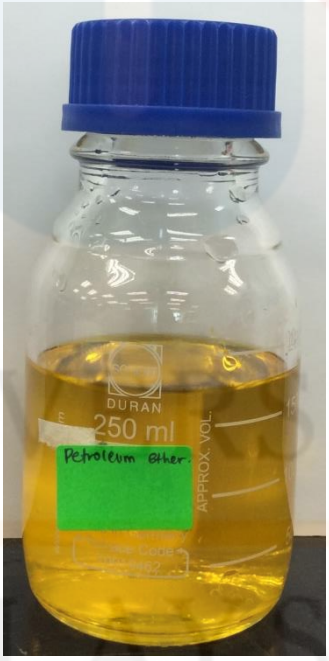

Figure B.4 Petroleum ether extract of *P. tectorius* fruit

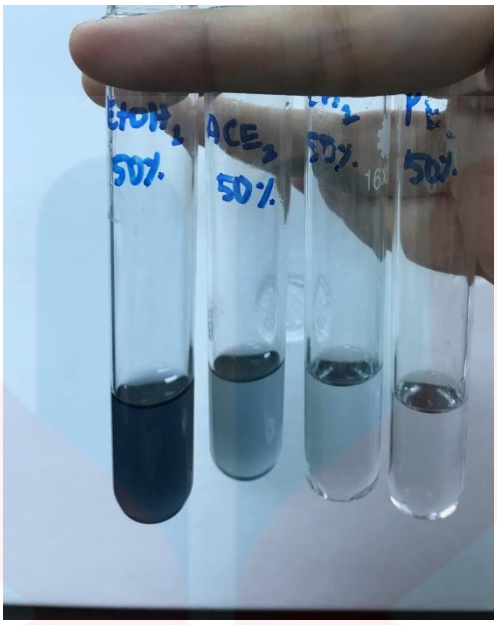

Figure B. 5 TPC analysis of *P. tectorius*

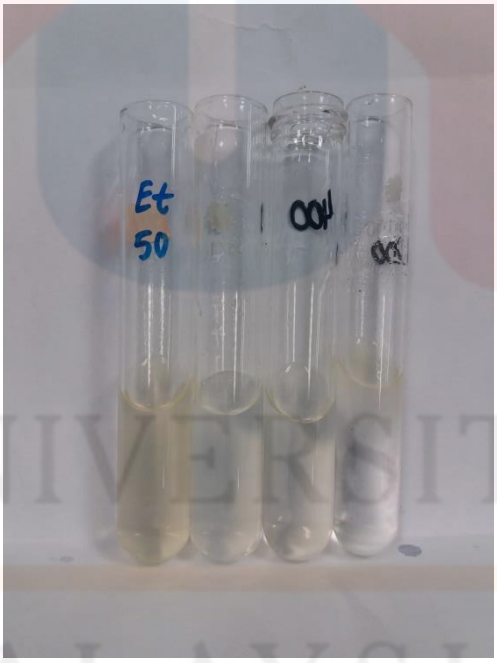

Figure B.6 TFC analysis of *P. tectorius*

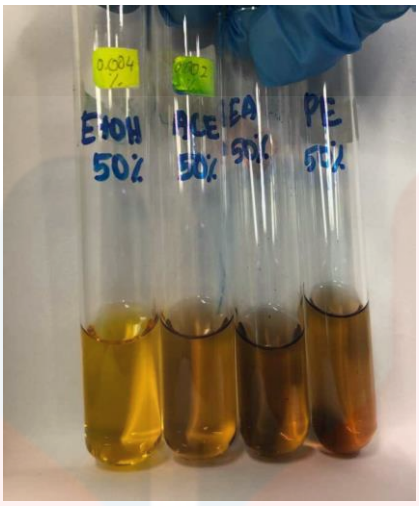

Figure B.7 DPPH analysis of *P. tectorius*

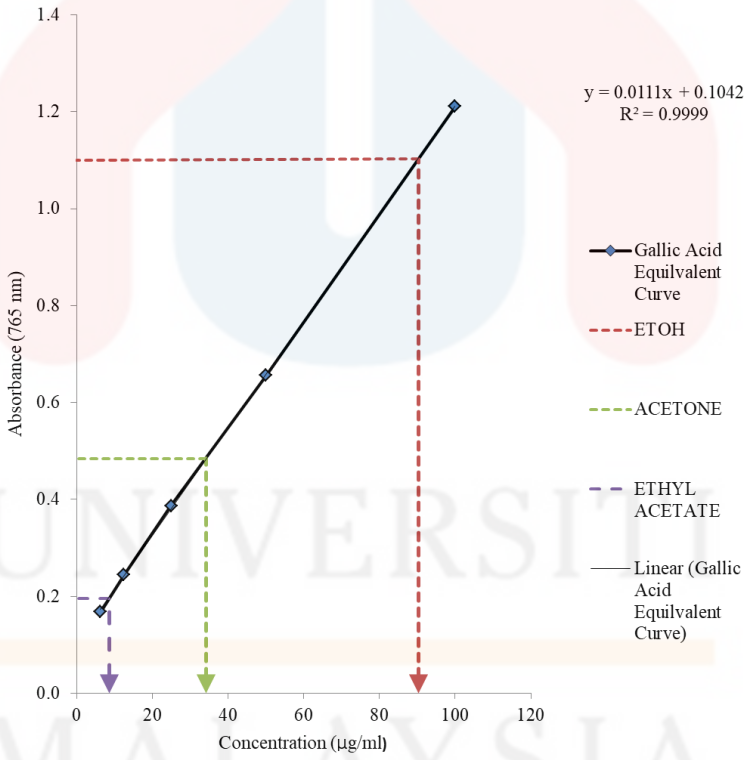

Figure B.8 Gallic acid equivalent curve with sample extract in trend

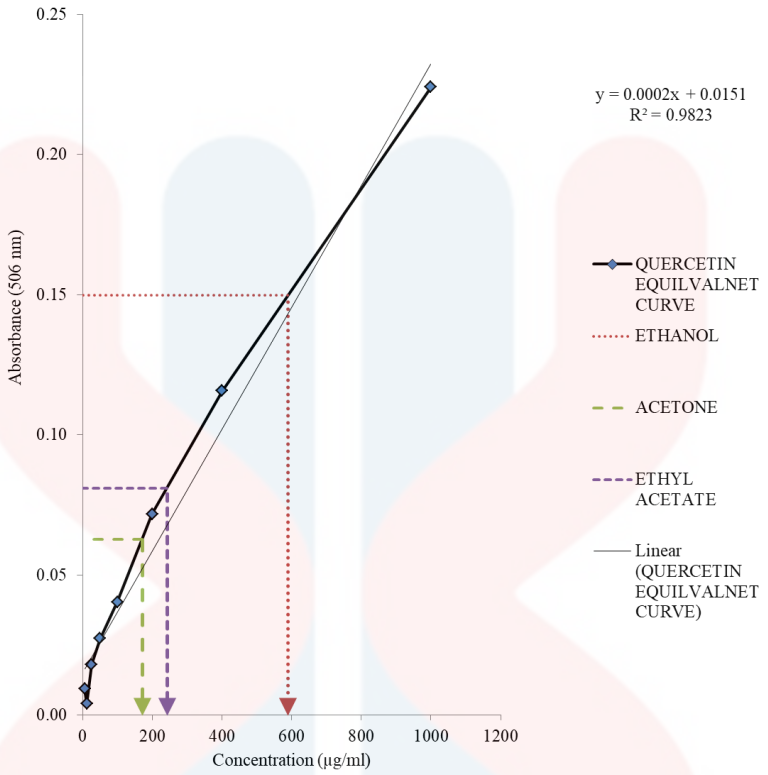

Figure B.9 Quercetin equivalent curve with sample extract in trend

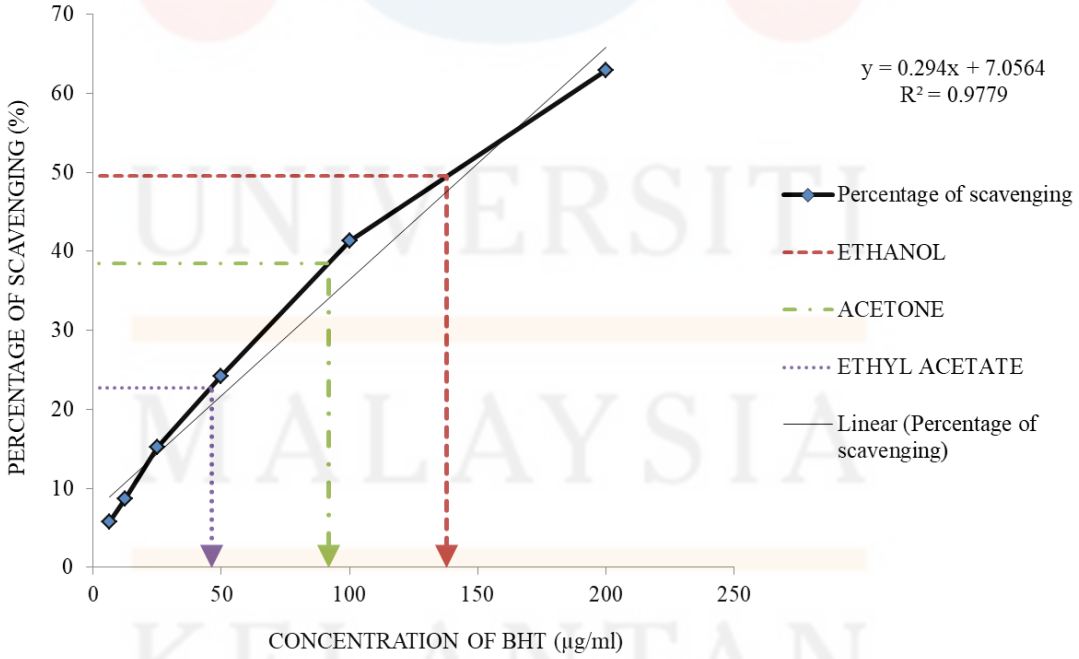

Figure B.10 Percentage of scavenging with sample extract in trend

APPENDIX C

Table C.1 T-test analysis for TPC, TFC, and DPPH assay

Sample	*p*-value		
	TPC	TFC	DPPH
Ethanol vs acetone	1.01×10^{-4}	1.32×10^{-3}	8.51×10^{-7}
Ethanol vs ethyl acetate	3.70×10^{-5}	4.98×10^{-3}	5.28×10^{-5}
Acetone vs ethyl acetate	1.02×10^{-5}	1.98×10^{-2}	2.18×10^{-4}