OPTIMIZATION OF PHENOLIC CONTENT FROM TULSI PLANT (OCIMUM SANCTUM) BY ULTRASONIC-ASSISTED EXTRACTION: COMPARISON WITH CONVENTIONAL SOXHLET EXTRACTION METHOD

RAHIMAH BT ABD RAHIM

BACHELOR OF CHEMICAL ENGINEERING
UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

BORANG PENGESAHAN STATUS TESIS♦

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TAMAN SRI SEMANTAN,
28000 TEMERLOH,
PAHANG DARUL MAKMUR

PN. HAMIDAH ABDULLAH
DR REDDY PRASAD
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RAHIMAH BT ABD RAHIM

Report submitted in partial fulfillment of the requirements
for the award of the degree of
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Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

JANUARY 2012

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this project and in my opinion, this project is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

Signature	:
Name of Supervisor	: MDM HAMIDAH BT ABDULLAH
Position	: SUPERVISOR
Date	: 18 JANUARY 2012

Signature	:
Name of Co-supervisor	: DR REDDY PRASAD
Position	: SUPERVISOR
Date	: 18 JANUARY 2012

STUDENT'S DECLARATION

I hereby declare that the work in this project is my own except for quotations and summaries which have been duly acknowledged. The project has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :
Name : RAHIMAH BT ABD RAHIM
ID Number : KA08082
Date : 18 JANUARY 2012

Dedicated to my beloved parents

Mr. Abd Rahim Bin Imam Mohamed

Mrs. Noraini Binti Abdul Manaf

And

All my sisters and brothers

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ABSTRACT

This study is mainly about optimization of the extraction of phenolic compound from one kind of valuable herbs which is Tulsi plant (Ocimum Tenuiflorum) by comparing between ultrasonic assisted and conventional Soxhlet methods. Tulsi plants have variety of function which made it demanding in market due to the application and function of this type of herbs. Thus, this research was conducted as to investigate the total phenolic compound in ethanolic extract of the Tulsi plant leaves by verifying different parameters. The parameters that are considered for this study are the differences size particle of leaves, extraction time, temperature, and volume of ethanol solvent. The total phenolic compound in terms of concentration and mass Gallic acid equivalents was determined using HPLC. From the observation, sonication of ultrasonic assisted extraction method could improve the yield of phenolic compound and shortened the extraction times. Besides, it also can decrease the organic solvent consumption thus may lead to the increasing of pollution prevention. As a conclusion, ultrasonic extraction is one of the potential alternative methods comparing than conventional Soxhlet method.

ABSTRAK

Kajian ini adalah berkisahkan tentang proses pengekstrakan yang dijalankan dari pelbagai aspek terhadap satu jenis pokok herba yang sangat berharga iaitu Pokok Tulsi (Selasih) melalui perbandingan antara dua kaedah iatu kaedah bantuan bunyi (UAE) dan kaedah traditional (Soxhlet).Pokok Selasih ini mempunyai pelbagai fungsi dan keistimewaan untuk diguna pakai malah menjadi permintaan yang tinggi dari kebayakan pasaran disebabkan oleh keistimewaanya.Oleh itu, satu kajian telah dijalankan bagi menguji kandungan fenolik di dalam pengekstrakan daun pokok ini apabila diuji dengan faktor yang berbeza.Aspek yang dijalankan untuk kajian ini ada lah berdasarkan perbezaan saiz daun pokok selasih , masa ,suhu pengekstrakan dijalankan dan jumlah pelarut yang digunakan .Kandungan fenolik di dalam daun tersebut akan dibawa untuk dianalisa melalui alat HPLC bagi menentukan kepekatan dan berat kandungan fenolik didalamnya .Secara jelasnya, dengan kaedah bunyi, boleh meningkatkan hasil sebatian fenolik dan masa pengekstrakan lebih pendek berdasarkan pelbagai kajian .Selain itu juga, ia juga boleh mengurangkan kadar penggunaan pelarut organik seterusnya meningkatkan kadar pencegahan pencemaran alam. Kesimpulannya, pengekstrakan melalui bantuan bunyi adalah salah satu kaedah yang sangat berpotensi jika dibandingkan dengan kaedah dahulu (Soxhlet).

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LIST OF SYMBOLS

˚C	Degree Celsius
%	Percent
min	Minutes
t	Time
g	Gram
µm	Micrometer
mm	Milimeter
nm	Nanometer
mL	Mililiter
µL	Microliter
g/L	Gram/Liter
mg/mL	Miligram/Mililiter
ng/µL	Nanogram/Microliter
mL/min	Milliliter/minutes
Hz	Hertz
kHz	Kilohertz
Rt	Retention time
i.d	internal diameter
v/v	Volume/Volume
v	Volume

LIST OF ABBREVIATIONS

BHT	Butylated Hydroxytoluene
BHA	Butylated Hydroxyanisole
FYP	Final year project
GAE	Gallic acid equivalent
HPLC	High Performance Liquid Chromatography
PG	Propyl Gallate
TBHQ	tert-butylhydroquinone
TPC	Total phenolic content
UAE	Ultrasonic-assisted extraction
UV	UV-Vis Spectrophotometer

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

There is no doubt that the world today has been developing rapidly with many inventions especially in food or medical technology that facilitates our daily lives. However, almost every day, human was shocked by news of the death of many people caused by various factors especially caused by infection of food. As for example, the creation of additional materials based on synthetic materials used in many food industry cause the food looks more varied, interesting and delicious. Grigonis et al. (2005) reported that, since 1949, the synthetic antioxidants is already used as to prevent the oxidations of lipids during processing of food which tendency to decrease the quality of foods besides creates deterioration of large amount of fat containing. It is supported by one research done by Juctachote & Berghofer (2004) that, the synthetic antioxidant such as Butylated Hydroxytoluene (BHT), Butylated Hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and Propyl Gallate (PG) are widely used as an antioxidant in food industry.

Unfortunately, the safety of that synthetic antioxidant has been questioned because it relating to the long terms effects beside it can be toxic (Albu et al., 2004; Boonyuen et al., 2009). Besides that, BHA was shown to be carcinogenic in animal experiments meanwhile BHT may caused internal and external haemorrhaging at high doses which contributes to death in some strain of mice and guinea pigs (Juctachote, T & Berghofer, E., 2004). Due to concern of human health, many researchers have been done with focussing more in natural resources such as herbs, for medical use, especially for traditional remedies that have been used since our ancestors. In fact, antioxidant can

be divided into two types which are synthetic antioxidant and natural antioxidant. Sim, Nurestri & Norhanom (2010) said those natural antioxidants are generally alleged to be safe as they occur in plants besides more desirable than synthetic counterparts.

Moreover, during last decade, large number of plant extracts has been tested for their antioxidant activity and some promising results were obtained (Grigonis et al., 2005).Clearly, the importance of replacing synthetic antioxidant by natural ingredients from oil seeds, herbs and spices has increase due to health implications and increased functionality which improve solubility in both oil and water (Juctachote, T & Berghofer, E, 2004).There are plentiful plants have been investigated and described as potentially natural sources of antioxidant extracted from herbs , spices and fruits due to their biodegradable nature such as Rosemary (Albu et al.,2004) ,Pikul (Boonyuen et al., 2009), Okra (Khomsug et al.,2010), Tulsi leaves (Pandey & Madhuri.,2010) , Chilli & Okra (Singh et al.,2011) , Mangrove trees (Prasad et al.,2011) , oregano, thyme and many more.

Tulsi leaves are one of those wonderful herbs in this world which have variety of functions and believed could be a potential crop in medicinal use. Its Latin name is (*Ocimum tenuiflorum,* Labiates) and it also can be called as *Ocimum sanctum, Holy Basil* or *Tulasi.* Usually, it is known as " The queen of herbs " and it is most closely associated with the use in religious rituals and Ayurvedic medicine especially for Hindus (Maimes,2004).This herbs are originally grows in India and in many Asian countries. It has been commercially in India especially for over five thousand years as a healing balm for many types of affection either body, mind even for spiritual beliefs .In simple words, the herbs are the most sacred herbs for Indian people.

Besides that, the advantages of the crops could not be denied and it is known to bestow an amazing number of health benefits. It is used to treat circulation problems beside for therapeutic uses such as lowering cholesterol and fever, removes toxins, increases stamina, improves digestion , cardiovascular - circulatory, immune and endocrine system and prevents from gastric ulcers (Maimes ,2004) . On top of that, these types of crops also can reduce tension and stress besides provides rich supply of antioxidant and other nutrients. Its herbs and oil are usually used as spice which is

widely used in cooking, additives in food, and other products for its desirable flavour and the pleasant aroma.

On top of that, Tulsi leaves also are believed that contains highly levels of natural of antioxidant such as ethanolic and phenolic content. Obviously Tulsi herb could provide fruitfully antioxidant which was more pure and natural rather than synthetic source which are widely used as additives in food, cosmetics and other products even for medicinal used. However, it is not easy to find a right method that could extract sufficiently high levels of the antioxidants in order to achieve maximum profit returns.

Among of all the process in the chemical engineering, most of it was attributed to extraction process. As we know, extraction process are lately commercially used either in large scale industrial processes or for the used in smaller scale laboratory procedures. It is nevertheless regarded now as an essential unit operation in the sophisticated armoury of modern Chemical Engineering. Several of extraction techniques such as ultrasonic assisted extraction, microwave, and supercritical fluid extraction have been developed for extraction of nutraceuticals from plants in order to shorten the time; decrease the solvent consumption besides could increase the yield and quality of extracts (Wang & Weller, 2006).

1.2 PROBLEM STATEMENT

From previous study, most of the methods chosen while extraction process occurred are usually by using conventional Soxhlet method however there is some limitations considered. Firstly, it is because of multi-step and long flow processes to be done. Moreover, the extraction process is time consuming and require large amount of solvent. As a result, there is a lot of disturbance occurred that affected the process at the same time spoil the precious bio- active compound in the Tulsi leaves.

Current studies have found alternative method to get the bio-active compound in the Tulsi leaves with some advantages which is by using Ultrasonic extraction method. The effect of ultrasound will advance the process occurred and modifying the result. Thus, not only the high extraction yields were getting but also less extraction time and low amount of solvent were used. Finally, the selection of the best method were concluded depend on the advantages and disadvantages along the process occurred.

1.3 RESEARCH OBJECTIVES

The objective of this project is:

(i) To compare the effectiveness of extraction method either using Ultrasonic assisted extraction or conventional Soxhlet method.

(ii) To evaluate the effects of different parameters in extraction process as to obtain the optimum amount of extraction yield.

1.4 SCOPE OF WORKS

During the analysis process, a few items should be scope as a guide of the project flow. Below are a few project scopes for this project as to fulfil the objectives:

(i) The extraction methods that have been used are ultrasound extraction method and conventional Soxhlet method using ethanol as solvent to get total phenolic compound in Tulsi plant.

(ii) The process have run by verifying different particle sizing at the range (315,630,800 and 1000 µm), temperature at the range of (30 - 50˚C), extraction time (20, 40, 60 and 80 min), and also the amount of solvent ratio (50-300 mL) for ultrasonic extraction method while for the Soxhlet method the parameters are verifying extraction time and size particle only.

(iii) The analysis part will evaluate by UV-Vis Spectrometer and HPLC

1.5 RATIONAL & SIGNIFICANT OF STUDY

Ultrasonic assisted extraction is one of the technique recently has been predominantly in many research. It has its own merits make it more favourable techniques in used. Based on that method, extraction time could be decrease. Besides, it only require small amount of solvent to be interact with .Thus, the production cost in terms of electricity, power and as well as utilities could be decrease at the same time we could get at high yield of product. Actually, cost of production is important issue to be considerate as for big scale applying in chemical industry.

In simple word, multi-step procedure, time consuming are remarkably to the increases the price for the final product. This is also one of the negative point taken as less attraction to the users to buy the product as the price is too high .As a conclusion, ultrasonic for extraction from plants in the application of verities aspect are a new powerful processing technology that could not only be applied safely , environmentally, friendly but also efficiently and economically. Besides that, it is more practical than the other method.

CHAPTER 2

LITERATURE REVIEW

2.1 TULSI PLANT

Tulsi plant is commonly known as Holy Basil which it is one type of plant that appears as strongly aroma, and bitter in taste. The plants normally bloom in warm regions and grow wild in the tropics climates especially in Asian countries as figure 2.1. Generally, this type of herbs is commonly can be seen in most of the botanical farm, garden even at the house corner especially for Hindus family. Pandey & Madhuri (2010) noted that Tulsi can be found in most of Indian homes and worshipped because it gives an important symbol of Hindu religious tradition. In fact, Tulsi also have precious ingredients that can be an alternative herbal treatment to treat ailments and problems such as common cold, headache, wound, bronchitis, liver diseases and many more (Pandey & Madhuri,2010). Besides that, it is widely used in varies way such as in cooking, medicine, Hindus worship and a religious tradition for thousands of years.

Beyond this wonderful herbs, there are the secret legend in varies perception by the different places, religions and history. Those plants known as the sacred herbs for Indian people and has traditionally been associated with both weddings and funerals. Besides that, Holy Basil has been revered for thousands years used in Ayurvedic systems of medicine and known as sacred plants for Indian people (Maimes, 2004) Moreover, most of them were associated Basil through their life, significance events and give meaningful symbolic pleasure for their belief. They strongly believe that Holy Basil is symbol of health, wealth and prosperity. Usually, the ladies worship this plant and pray for their husband's long life and family's prosperity.

Apart from all the religious belief, there are scientific researches proving the precious component contain in Tulsi plant. It has an active ingredient which is Phenolic content. One study led by Mukherji (1987), said that the Holy Basil oil has been found to be largely due to eugenol which is a phenolic content (1-hydroxy-2methoxy-4-allybenzene).Basically, Phenolic is one type of organic compound which have one or more hydroxyl group attached to one of the carbon atoms at the aromatic ring. Normally, this organic compound has also found as a part of tannins, which are astringent bitter plant poly phenols. In industrial applications, poly phenols have widely used as natural colorants and preservatives for foods, either in the production of paints, paper and cosmetics.

Recently, Phenolic contents have gained significant interest among various natural antioxidants extract especially in plants due to their benefit effects on health (Boonyuen et al., 2009; Khomsug et al., 2010; Singh et al., 2011). It can give benefits to our health because of the highly complex compound contain in it. In fact, phenolic content are commonly in some herbs, vegetables and fruits that can cure certain disease. There are thousands of beneficial compound which are strong antioxidant, antibacterial, antiviral, adaptogenic (agent that helps the body adapt more efficiently to stress), and immune-enhancing properties that bring positive in health. Moreover, it also can support the body's natural defence against nerves, stress and to sharpen memory (Pandey & Madhuri, 2010). Besides that, this type of herbs is used to treat our body system and enhance our circulatory problem. Tulsi plant also can supporting the heart, blood vessels, liver, and also regulates blood pressure beside blood sugar (Maimes, 2004).

Figure 2.1: Tulsi leaves

2.2 EXTRACTION

As we know, extraction process are lately commercially used either in large scale industrial processes or for the used in smaller scale laboratory procedures. It is nevertheless regarded now as an essential unit operation in the sophisticated armoury of modern Chemical Engineering. Basically, extraction is a process of separation one or more components in a mixture according to physical and chemical properties. As for example, acetic acid can be removed from the water solution by the distillation or extraction process in the presence of organic solvents, isopropyl ether (Geankoplis.C.J, 2003). Besides, herbal plant extracts are quite in high numbers of commercialize recently especially in the food, pharmaceutical and cosmetic industries (Wang & Weller .C.L, 2006). While in the pharmaceutical industry, products such as penicillin occur in fermentation mixture that are quite complex but in liquid extraction it can be done well.

There is a method that quite commercialize nowadays which is doing an ultrasonic extraction as easiest ways to get the pure compound extract naturally Additionally, there are many varieties of extraction techniques that can be done including Soxhlet extraction (Soxtec), ultrasonic assisted extraction, microwave assisted extraction, supercritical fluid extraction, accelerated solvent extraction and many more. The choice of the method would be depend on relatives cost, benefits, the effect to the environment and together the parameter that we used. Besides, a critical review was conducted to introduce and compare the conventional Soxhlet extraction and the new alternatives methods used for extraction of nutraceuticals from plants whereby all practical issues of each extraction techniques were discussed (Wang & Weller .C.L, 2006).

In other example, one study has done by Pal.R et al (2011) shows that one type of natural plants extracts contains higher level of phenol and flavonoids content which is possible to reduce the risk of chronic diseases. Thus, generation sufficiently high levels of antioxidants of phenolic compound from Tulsi leaves by extraction process for this current study are also can be done.

2.2.1 Ultrasound- Assisted Extraction

Ultrasound is a cyclic sound pressure and that term are usually used when the frequency of the sound are above 20 000 Hz. In terms of academic , ultrasound is the process of propagation of the compression (rarefracting) waves with frequencies above the range of human hearing with associated acoustic wavelength in liquid roughly 100-0.15mm (Singh ,Fernando & Hernanendez, 2006). Normally, the upper limit for human hearing is approximately 20 000 Hz and it is due to the limitation of the middle ear. In fact, the limit are varies for a different person even for some animals such as bats ,dolphins, cats, mice and dogs which can stand in range 10-100 KHz. Contrary, those species of animals can stand upper limits frequency than human hear.

On top of that, ultrasound is happen when there is the formation and implosive collapse of bubbles in a liquid irradiated which is called acoustic cavitations .In simple words, when the sound passes through the liquid, it will create negatives pressure (expansion waves) and positives pressure (compression waves).Thus the bubbles will grow and recompress the solvent and solute vapour thus make it well extracted. According to Alupului, Calinescu, & Lavric (2009), the kinetic energy affected the whole volume following the collapse cavitations bubbles near wall or interfaces thus improving the mass transfer across the solid-liquid interfaces. It has been reported by Kadkhodaee & Hemmati-Kakhki (2003) that, the sound waves were affected on the extraction process through the formation and asymmetrical collapse of micro cavities in the vicinity of the cell walls leading to the generation of micro jets rupturing the cells.

The technique is commonly used in industry as to clean the optical parts, jewellery, surgical instrument, lenses and biological cells which bacteria can be disintegrated. In industrial fields, ultrasonic are commonly used to find flaws material in a mixture.One study led by Ishtiag.F (2009) said that ultrasound also has been used in life sciences such as medical imaging, diagnostics ,and fermentation process.

Actually, since in 19th decades, ultrasonic extraction has been prepared for many kind of research unfortunately on 20th was just start to begin. Then, the study in the fields of ultrasound has been widely applied in many kinds of level involving in industrial, food department, laboratory, plant, medicine, science, and many more. Basically, there are two general designs of ultrasonic assisted extractor as figure below which are ultrasonic bath (figure 2.4 & 2.5) or closed extractors fitted with an ultrasonic horn transducer as figure 2.2 and 2.3 (Wang.L & Weller .C.L ,2006).

Figure 2.2: Ultrasonic horn transducer

Figure 2.3: Uses in Industry

Figure 2.4: Ultrasonic Bath

Source: FKKSA laboratory

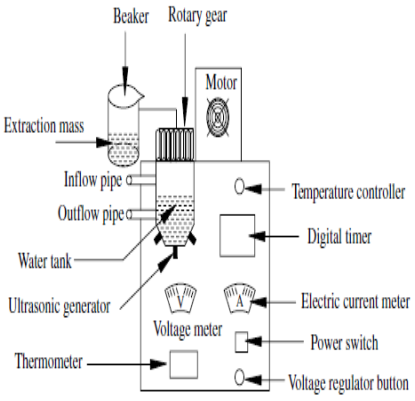

Figure 2.5: Schematic diagram UAE

Source: Ma et al., 2008, pp: 228

Additionally, there are large number of researches are now available on the extraction process as to get bio-active compound in herbs, crops ,vegetables and fruits

using ultrasonic assisted extraction method (Kadkhodaee & Hemmati-Kakhki.,2003; Albu et al.,2004; Ma et al.,2008). Moreover, most of the researchers conclude that the ultrasonic assisted extraction method could reduce the extraction time in comparison with the use of conventional method.Above statement have been supported by Ishtiag et al.,2009,Wang & Weller.,2006.

2.2.2 Conventional Soxhlet extraction

Generally, Soxhlet extractor is a very useful tool for separation purposes where the analyte is concentrated from the matrix as a whole or separated from particular interfering substances. Solvent extraction of solid samples, which is commonly known as solid-liquid extraction also referred to leaching or Lixiviation in a more correct use of the physicochemical terminology, is one of the oldest process that still remains using it. Wang & Weller (2006) said that, Soxhlet is actually a standard technique which has been used for a long time and the main reference for evaluating the performance of other solid-liquid extraction method. Usually, the conventional Soxhlet extraction process is commonly applied in most of the separation process which involving oil removing from the solid matrix because of it is more straightforward and inexpensive method but also slow and tiresome (Garcia-Ayuso & Lurue de Castro, 1999).

2.2.3 Comparison Advantages and disadvantages for Ultrasonic assisted extraction and Conventional Soxhlet extraction method

Obviously, both methods have their own advantages and disadvantages according to the process requirement as shown in table 2.1.

Table 2.1: Method comparison

COMPARISON DISADVANTAGES & ADVANTAGES FOR BOTH METHODS			
NO	CONVENTIONAL SOXHLET EXTRACTION METHOD		ULTRASONIC ASSISTED EXTRACTION METHOD
1	Extraction time is long	Time	Less time as for best result
2	Large amount of solvent used	Volume solvent used	Less amount of solvent used
3	Agitation cannot be provided in the Soxhlet device to accelerate the process	Process efficient	Additional agitation or shaking is used to avoid formation solid free regions
4	Large amount of solvent requires an evaporation procedure	Evaporation process	Less temperature used avoid too much evaporation process
5	Possibility of thermal decomposition as the extraction occurs at the boiling point of the solvent for a long time	Possibility to decompose	Low tendency to decomposed due to less temperature used
6	High electricity used make high production cost	Production Cost	Lower electricity used thus make lower production cost
7	Maintaining a relative high extraction temperature with heat from the distillation flask	Temperature	Occur at lower temperature as for best results
8	No filtration requirement after leaching	Filtration procedure	Requirement of filtration process after leaching
9	Method is simple and cheap	Complexity	Additional of multiple step but still simple
10	The displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with solid matrix.	Operation efficient	-

Sources: Wang, L & Weller, C.L (2006) pg: 300-312

2.3 FACTOR AFFECTING EXTRACTION PROCESS

Optimization of different parameters toward the extraction process is a prime factor that should be considered as to enhance the high extraction efficiency. There are several factors that could affect high production yield. Those are diameter particle size distributions, extraction time, extraction temperature, volume or types of solvent, sonic power apply frequency or amount of samples. However, the effects on each of the factors are different depending on the method used.

2.3.1 Diameter particle size distributions

From point view of the diameter particle size distributions, the rate of extraction efficiency is increases according to the increases diameter size of particle distributions. Generally, small particle size could increase the solid-liquid interface at the same time will increases the extraction efficiency (Martinez, J.L.C, 2009). However, as for volatile analyte the diameter particle size should not be too small (ground) as it can be liberated during grinding process.

2.3.2 Extraction temperature

Effect of the extraction temperature is quite same for the effect of extraction time. It is because both of the factors are dependent to each others. Usually, temperature has a positive effect on extraction efficiencies when it is not too high where the active components in the samples could degrade at high temperature. This is due to the increase number of cavitations bubbles in the surface contact area (Martinez, 2009). Additionally, at high temperature solvent have higher capacity to solubilise the analyte, while the surface tension and the solvent viscosity decrease with temperature which will improve sample wetting and matrix penetration (Prasad, 2011). On the other hand, according to Ma et al (2006) as for ultrasonic assisted extraction, high temperature is not favourable because of the solvent tend to evaporate at high temperature. Thus, the selection of extraction temperature and time should be carefully consider the evaporation of solvent and the solubility of the compound in order avoid loss of solvent in high temperature plus could reduce the cost.

2.3.3 Extraction time

The use of different time in the extraction of bio-active components of plant materials has been reported by many researchers (Kadkhodaee & Hemmati-Kahki., 2003; Albu et al., 2004; Ma et al., 2008; Prasad et al., 2011).Based from the study, the yield of the active compound after extraction process occurred is high when increasing the extraction time. However, longer extraction time will spoil the precious bio-active compound in it. It is because of the content easily decomposed if kept at high temperature for a long period. It was also observed that degradation of the active compound in the samples and conversion of analyte will occurred due to longer time (Prasad et al, 2011). One study reported by Grigonis et al (2005), by using Soxhlet extraction method, the compound containing are possible to degradations due to local overheating effects and longer extraction time besides limited solvent choice. On the other hand, Abdullah et al. (2010) noted that increases of the sonication time will increase the duration of cavitations processes while the extraction running.

2.3.4 Volume solvent

Solvent volume also plays an important role in increasing the yield of product. The chosen of solvent used in the extraction process should be compatible with the samples otherwise the interaction between the solvent and the samples could not occur. In addition, the volume of solvent should also be concordance with the amount of samples as to extract efficiently (Martinez, 2009). It can be supported by Prasad. (2011) that the matrix will be swelling during the extraction process thus as to ensure that, the solvent volume must be sufficient to ensure that the entire sample is immersed. Plus, increases the volume of solvent in the extraction process could enhance the yield of product. According to Abdullah et al. (2010) larger solvent volume promotes an increasing concentration gradient between solvent and solid samples thus provide larger mass transfer between solid and solvent. However, larger amount solvent after it achieving the extraction optimum is not improving the yield of the product.

CHAPTER 3

PROJECT METHODOLOGY

3.1 PROCESS FLOW DIAGRAM

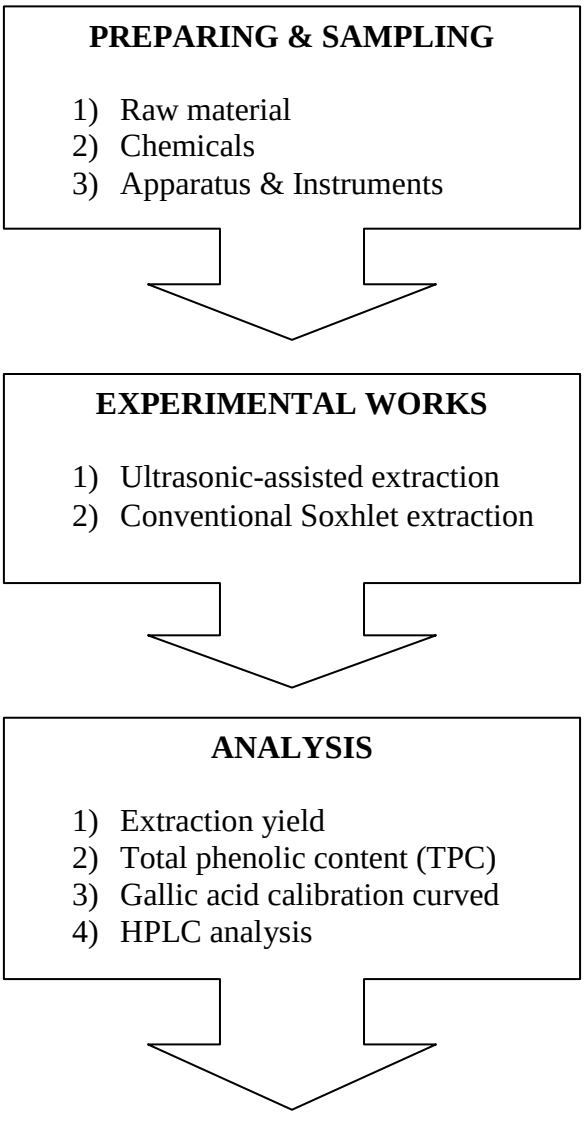

Figure 3.1: Project Flow Chart

3.2 PREPARING & SAMPLING

Briefly, the fresh leaves of Tulsi plant (Ocimum Sanctum) as Figure 3.2 were collected from any near botanical farm in urban area at Temerloh, Pahang, Malaysia. These fresh leaves were washed initially with the tap water (Figure 3.3) as to remove the undesired materials on the leaves. The clean Tulsi leaves were shade air dried by exposing them at ambient temperature for two weeks (Figure 3.4) without using sun light as to prevent loss of the quality. Then, the leaves were ground into fine powder using a grinder. Lastly, the dried ground leaves were sieving to produce different size particle (315,630, 800 and 1000 µm) before stored in dry labelled sealed plastic as Figure 3.5 for next step. Apart from above, listed of chemicals and apparatus used as shown as at table 3.1 & 3.2.

Figure 3.2: Fresh Tulsi leaves

Figure 3.3: leaves after cut & clean

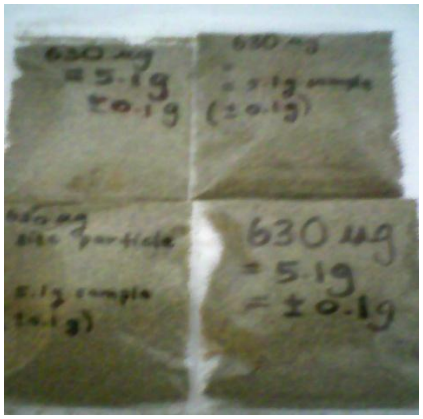

Figure 3.5: sample with different sizing

Figure 3.4: Tulsi leaves after dried

Table 3.1: Chemicals

NO	CHEMICAL USED	PURITY (%)	PURPOSE
1	Ethanol	99.4	As a solvent to extract the leaves
2	Gallic acid monohydrate	99.8	As a standard of phenolic content
3	Folin-ciocalteu reagent	100	As a reagent
4	Sodium carbonate (Na2CO3)	100	As a standard of phenolic content
5	Methanol (HPLC grade)	99.9	As a mobile phase in HPLC analysis
6	Acetic acid (HPLC grade)	100	As a mobile phase in HPLC analysis

Table 3.2: Apparatus and Instruments

NO	EQUIPMENT USED	BRAND	PURPOSE
1	Soxhlet extractor • Condenser • Soxhlet chamber • Extractor thimble • Extraction flask	Favorit	-As to do the extraction process
2	Ultrasonic Assisted Extractor	-	- As to do the extraction process
3	Rotary Evaporator	Buchi Rotavapor R-200	-As to get the crude extract when evaporate the solvent
4	UV-Vis Spectrophotometer	Hitachi, U-1800 Spectrophotometer	- To get absorbance value for the standard calibration data
5	HPLC (High performance Liquid chromatography)	HP 1100 system Agilent-Technologies (U.S.A)	-To get the value of peak area and concentration of the samples and standard calibration data

3.3 EXPERIMENTAL WORK

3.3.1 Ultrasonic Assisted Extraction

Firstly, 5 g of dried Tulsi powder were soaked with 50 ml of ethanol at room temperature in a conical flask which were sealed by Para-film and aluminium foil as to avoid loss of solvent. Then the conical flasks were immersed into the Ultrasonic assisted bath extractor as Figure 3.6 for irradiation under different extraction conditions. As for the first parameter, the ultrasonic extraction was performed at different size of particle (315,630,800 and 1000 µm) and secondly for different temperature (30, 40 and 50˚C). After that, the extraction was performed for varies of time which are in (20 min, 40 min, 60 min and 80 min). Finally, each extracted were filtered off (Figure 3.7 & 3.8) through filter paper (Whatman: 125mm) and the filtrate was collected before transferred into clean Schott bottle as Figure 3.9 below for further step.

Figure 3.6: UAE extraction process

Figure 3.7: Filtering Process

Figure 3.9: Crude extract in Schott bottle

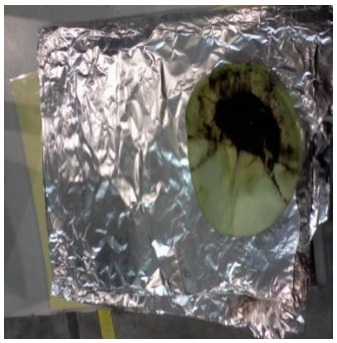

Figure 3.8: Sample after filtered

3.3.2 Soxhlet Extraction

The conventional Soxhlet extraction apparatus consisting of a condenser, Soxhlet chamber, thimble extractor and extraction flask (Figure 3.10) were used. Briefly, 5 g of dry grounded powder sample was loaded in the thimble before being extracted by 200 ml of ethanol solvent .The solvent was put in the 500ml round-bottom two neck flask where one neck were put the cotton wool surround the thermometer. Thermometer was attached as to measure the temperature simultaneously. Then, the thimble was put in the extraction chamber. The other one neck flask was attached to the extraction chamber with the condenser .The flask then was put in the heating mantle. As for the first parameter, the ultrasonic extraction was performed at different size of particle (315,630 and 800 µm) and the time periods for these extractions are for 1hr, 2hr, 3hr, and 4hr at 70˚C depending on varies size of particle. Constant heat was provided by the heating mantle for recycling the solvent. After complete extraction process, the extract in round bottom two neck flasks was transferred into clean Schott bottle.

Figure 3.10: Soxhlet extraction process

Figure 3.11: Product recycled back to the flask

3.4 ANALYSIS

3.4.1 Extraction yield

The extraction yield was analyzed by the method describe by Ahmad et al. (2010).The extract was evaporated to dryness using a rotary evaporator under a vacuum at 78˚C to give a dark crude green colour. The mass of the dark crude green extract were weighed as to get the yield. Finally, the crude extracts were stored in the vial and kept it in the dark placed. Each evaporated thick and vicious extract was diluted with 20 mL ethanol for further tests to determine the total phenolic contents in the HPLC analysis. Above of all, the results are expressed as the yield of extract:

$$\textbf{Yield extract (\%)} = \frac{Weight\ gallic\ acid\ (g)}{Weight\ of\ the\ sample\ (g)}\ x\ 100 \qquad (3.1)$$

3.4.2 Total Phenolic Content (TPC)

Total Phenolic contents (TPC) of the Tulsi plant extracts were determined using the Folin-Ciocalteau assay. At first, 100 µL of crude extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2.0mL of 7.5% sodium carbonate. Then, the mixture was measured at 765nm UV-VIS Spectrophotometer after half hour at the room temperature. Gallic acid was used as the standard and the concentrations of phenolic compound in the extracts of Tulsi plant were expressed as Gallic acid equivalents (GAE), were measured according to the Folin-ciocalteu method described by Prasad et al. (2011).

3.4.3 Preparation Gallic Acid Calibration Curve

Briefly, a calibration curve plot was conducted using Gallic acid ranging from 0.0 to 1.0 mg/mL which were made as previously described (Sim.S.K, Sri Nurestri.M.A, & Norhanom .W.A, 2010) with some modifications. Firstly, three stock solutions were prepared which are Gallic acids (1 mg/mL), sodium carbonate (Na_2CO_3) and Folin-ciocalteu reagent. Then, the Gallic acid was pipette out according to the ranging concentration into the test tubes. The final volume was made to 1 mL with

methanol. Different concentrations of the resultant Gallic acid solution were mixed with 2.5 mL Folin-ciocalteu reagent. After the colour turned to yellow-green colour around five minutes, 2.0 mL of saturated sodium carbonate was added to the mixture. As the mixture turned to dark-blue colour (five minutes), all the test tubes were incubated at 50˚C for 10 minutes. Lastly, all the different concentration mixtures were put into the cuvettes with the blank that containing ethanol for UV-Vis Spectrometer meanwhile all the rest mixtures were put in the vial for HPLC standard analysis. As for UV-Vis Spectrophotometer, the absorbance was determined at 765 nm thus the calibration curved plot as figure 3.12 was obtained by plotting the absorbance against concentration of Gallic acid (mg/mL) meanwhile for HPLC analysis the calibration curved plot as figure 3.13 was obtained by plotting the peak area against concentration of Gallic acid (ng/uL).

UV-Vis Spectrophotometer calibration curved

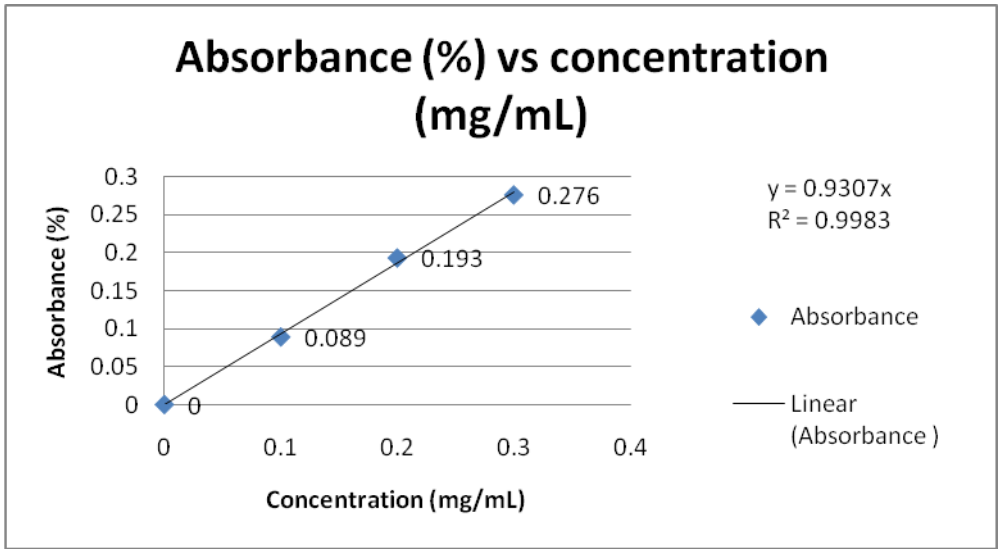

Figure 3.12: Graph Absorbance vs. Concentration

The standards calibration curved was shown as in figure above as for different types of analysis which are UV Vis Spectrophotometer and HPLC analysis. As for figure 3.12, the regression gives the linear relationship:

$$Y = 0.9307\ X,\ (R^2 = 0.9983) \qquad (3.2)$$

Where Y (%) is the absorbance at 765 nm and X (mg/mL) is the concentration of the Gallic acid of (phenolic content) for the UV Vis Spectrophotometer analysis.

HPLC calibration curved

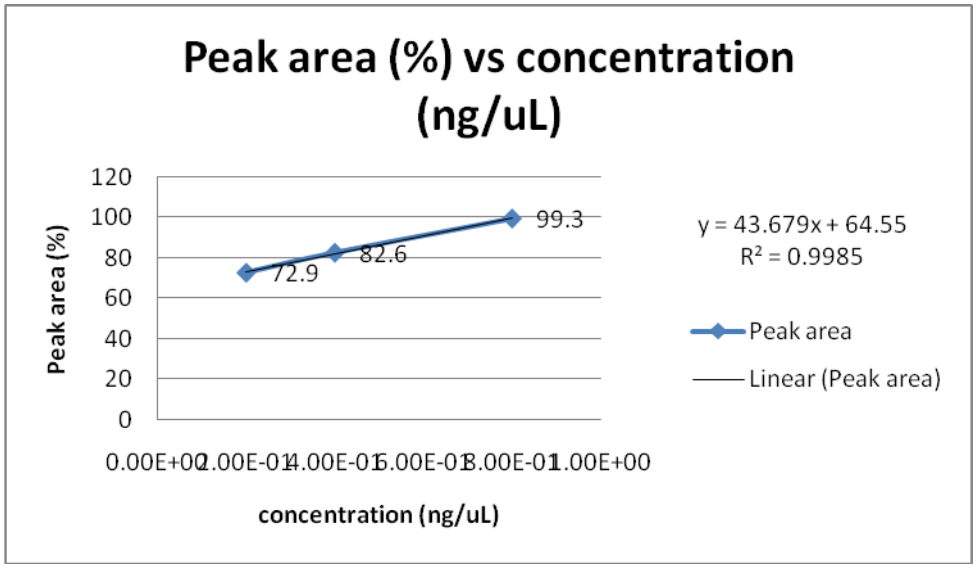

Figure 3.13: Graph Peak area vs. Concentration

As for figure above, the regression gives the linear relationship:

$$Y = 43.679\ X + 64.55,\ (R^2 = 0.9985) \qquad (3.3)$$

Where Y (%) is the peak area reading for the standard samples, the y-intercept is 64.55 and X (mg/mL) is the concentration of the Gallic acid of (phenolic content) for the HPLC analysis.

3.4.4 HPLC Analysis

The High Performance Liquid Chromatography apparatus was performed with an HP1100 system (Agilent Technologies, U.S.A) with isocratic delivery and UV-Vis detector monitored at 290nm.The separation was carried out in isocratic condition at 25˚C using Zorbax Eclipse XDB-C18 column (250 x 4.6mm x i.d , 5µm particle size) with a guard column C18.Running conditions included injection volume of the extract was 5 µL and flow rate at 1mL/min .The mobile phase contained methanol-0.4% acetic acid at the ratio of (80:20 v/v).Above of all, the samples were filtered through organic solvent compatible membrane filters (pore size 0.20 µm, Millipore) prior to injection in sample vials. Gallic acid was used as internal and externals standards besides phenolic compounds present in the samples were identified by comparing the retention time (Rt) of the standards and by the co- injection. All the methods were made with some modifications as previously described (Singh.U.P et al, 2009).

CHAPTER 4

RESULT & DISCUSSION

4.1 INTRODUCTION

In this research, the total phenolic compound extract from Ultrasonic-assisted extraction or conventional Soxhlet method was analyzed. Besides, the purpose of this research is to compare the effectiveness of extraction method between these two methods by manipulating four different parameters. The amounts of extraction yield were studied by verifying parameters such as particle size distribution, temperature, extraction time and volume of ethanol solvent.

4.2 PROCESS PARAMETER SETUP

The parameters that have been manipulated in ultrasonic assisted extraction were particle size distribution, different extraction temperature, different extraction time, and also different volume of ethanol solvent .However, only two parameters that have being verified in conventional Soxhlet method which were particle size distributions and different extraction time. Next, the results were based on one fixed parameter at one time. The result of mass of Gallic acid (g), concentration Gallic acid (g/L) ,Yield of Gallic acid (%) for each material were placed on the table refer at appendix .The table A.1 shown data collection from the analysis as for Ultrasonic assisted extraction and the table A.2 as for conventional Soxhlet extraction .

4.3 OPTIMIZATION

4.3.1 Effect of diameter particle size distribution on the Yield Gallic acid extraction for UAE and Soxhlet extraction from Tulsi plant

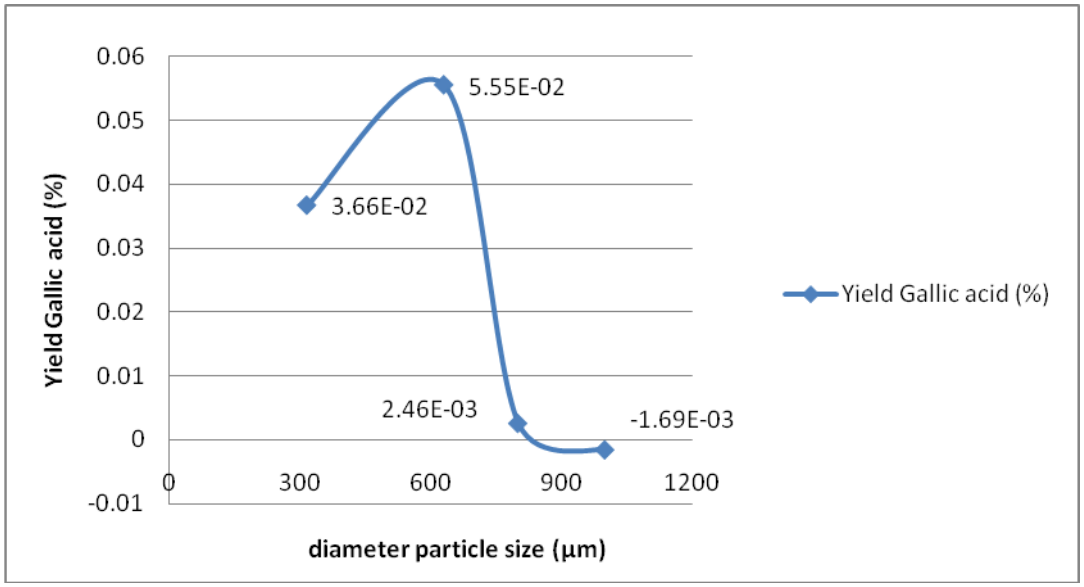

Figure 4.1: Graph on the effect of different particle size on Yield Gallic acid for UAE: extraction time (20 min) & extraction temperature (40˚C).

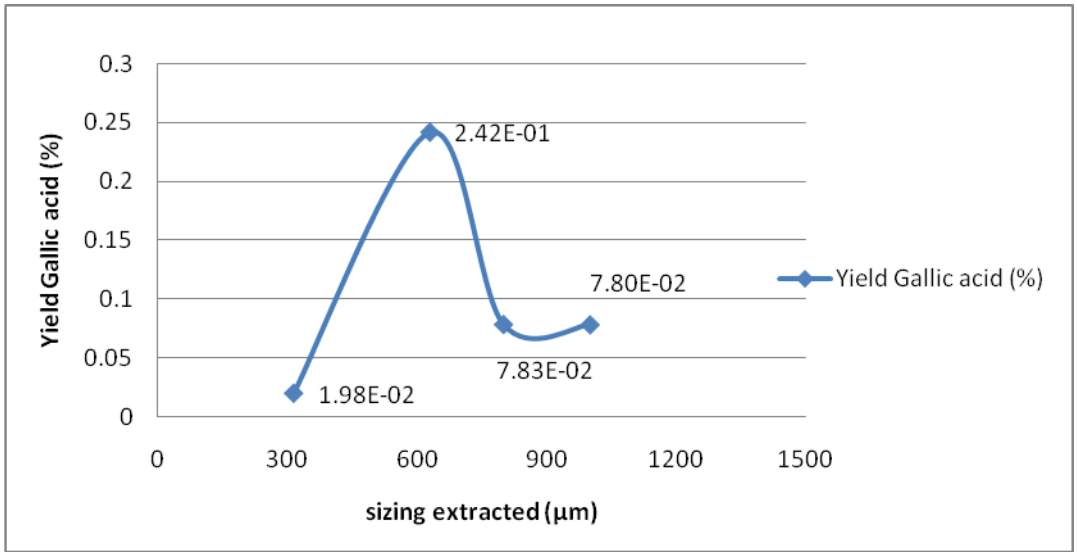

Figure 4.2: Graph on the effect of different particle size on Yield Gallic acid for Soxhlet: extraction time (240 min) & extraction temperature (70˚C)

Figure 4.1 and 4.2 shows the effect of different diameter size particle on the yield of Gallic acid extracted from Ultrasonic assisted extraction and conventional Soxhlet extraction method. It indicates that the yield of Gallic acid is increases with the increase of diameter particle size after achieving optimum yield for both figures. The optimum yield for UAE is 5.55E-2 % meanwhile for Soxhlet extraction method is 2.42E-1 % which both are at 630 µm of diameter particle size of Tulsi plant.

This phenomena occurred because of the particle is easily extracted when it is in small diameter. It is supported by Martinez (2009), where small particle size could increases the solid-liquid interface at the same time will increases the extraction efficiency. As a matter of fact, as the size particle is more than 630 µm of diameter, the yield of ultrasonic extraction were starting to decrease. It is maybe because of easily to decomposed when particle are in small diameter due to longer time.

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4.3.2 Effect of extraction temperature on the yield Gallic acid extraction for UAE and Soxhlet extraction from Tulsi plant

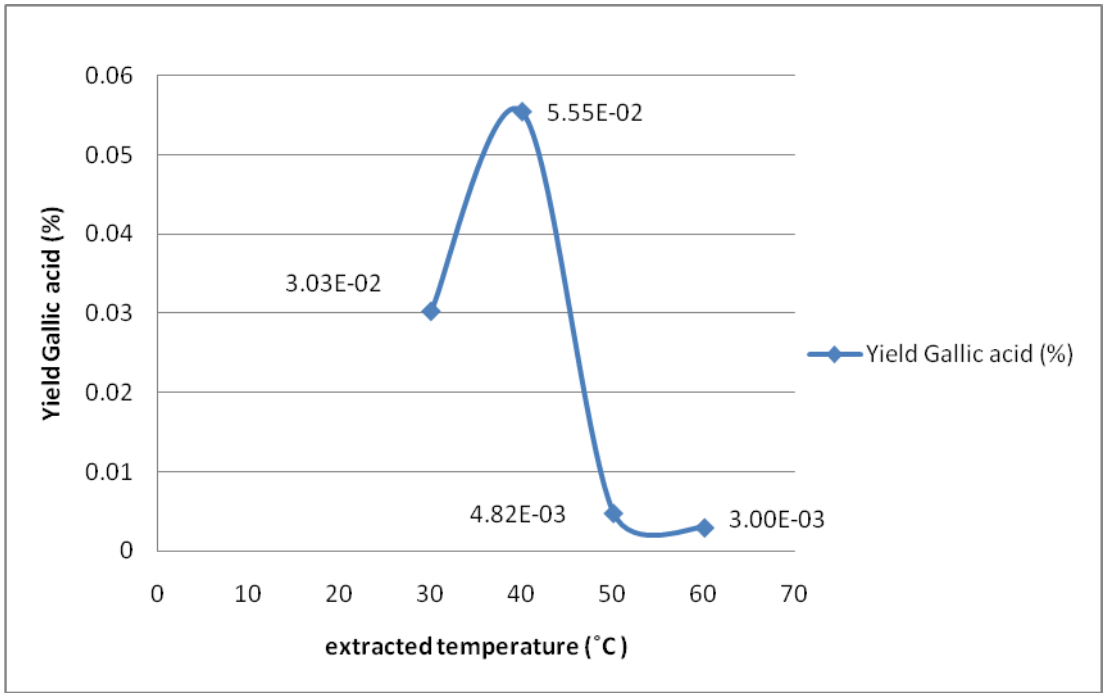

Figure 4.3: Graph on the effect of extraction temperature on Yield of Gallic acid for Soxhlet: extraction time (20 min) & particle sizing (630 μm)

Experiments were conducted to study the effect of temperature on the yield of Gallic acid extracted. Generally, the extraction yield is increases with the rise of the extraction temperature. This is due to the increase number of cavitations bubbles in the surface contact area (Martinez, 2009). Additionally, at high temperature solvent have higher capacity to solubilise the analyte, while the surface tension and the solvent viscosity decrease with temperature which will improve sample wetting and matrix penetration (Prasad, 2011). On the other hand, according to Ma et al (2006) as for ultrasonic assisted extraction, high temperature is not favourable because of the solvent tend to evaporate at high temperature.

Figure 4.3 shows the effect of different temperature on the percentage of yield of Gallic acid extracted. It indicates that the yield Gallic acid is increases steadily with the rise of extraction temperature and start to decrease as a point achieves the optimum yield at temperature 40˚C. Longer extraction temperature and time was not investigated because it may have negative effects resulting from degradation of the analyte .Besides, it probably due to increased amount of matrix components would be co-isolated at high temperature (Prasad, 2011). Therefore, 40˚C is chosen as an optimal temperature in the following procedures.

In case of conventional Soxhlet extraction, the different extraction temperature is not favourable parameters to be done. It is because the samples only can be extracted after it achieves 70˚C and remains constant which of the reading is closely to the boiling points of the ethanol solvent. Due to time extracted even for different particle size diameter done, the temperature reading is still the same at 70˚C. These phenomena occurred because of, only after the solvent reached closely to their boiling point, the solvent will begin to evaporate and move to the extraction chamber through the samples contained in the thimbles. Again the process will repeated as the solvent from the round bottom flask and to the extraction chamber until there are no more compounds in the samples to be extracted.

4.3.3 Effect of extraction time on the yield Gallic acid extraction for UAE and Soxhlet extraction from Tulsi plant

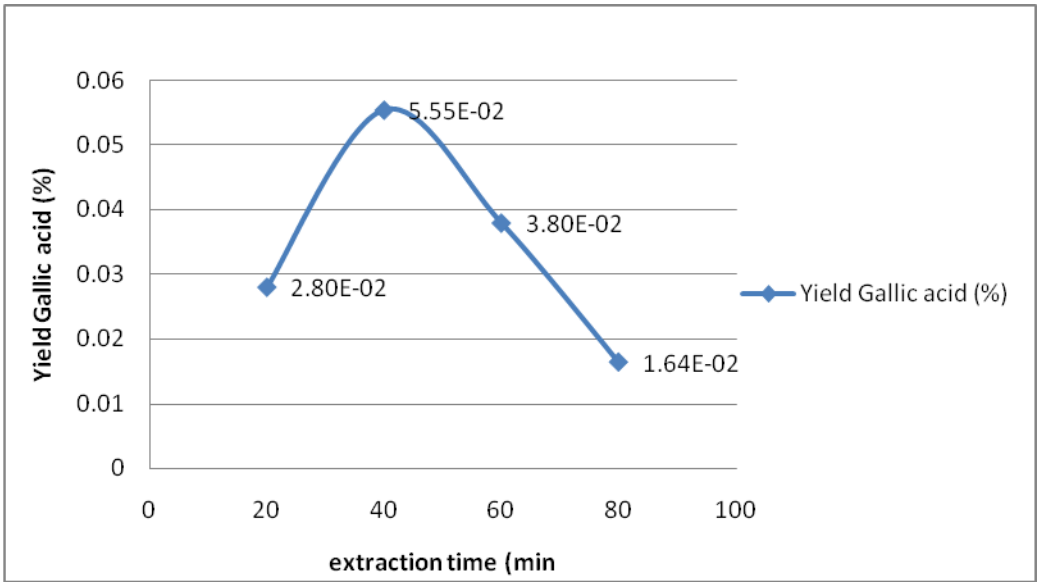

Figure 4.4: Graph on the effect of extraction time on Yield of Gallic acid for UAE: Extraction temperature (40 ˚C) & particle sizing (630 μm)

The results illustrated in figure 4.4 show the yield Gallic acid extraction are dependent on the extraction time as for ultrasonic assisted extraction method. It indicated that the yield of Gallic acid is increased with extended ultrasonic extraction time in the beginning of extraction from 20 to 40 min and start to decrease as after achieving the optimum extraction time which is above 40 min. This is because of longer interaction of the particle with the solvent could occur when increases the extraction time. The statements above prove by Abdullah et al. (2010) that increases of the sonication time will increase the duration of cavitations process while the extraction running.

Besides that, the results indicated that the efficient extraction period for achieving maximum yield Gallic acid extraction which is 5.55E-2 % was about at 40 min for UAE. However, when longer time were experimented on the samples at 60 min and above , the graph pattern start to change which is decreasing the yield of UAE extraction. It is because of the Gallic acid content easily decomposed if kept at high temperature for a long period. It was also observed that degradation of the active

compound in the samples and conversion of analyte will occurred due to longer time (Prasad et al, 2011). Therefore, 40 min were chosen as the optimal time for the UAE with the highest yield.

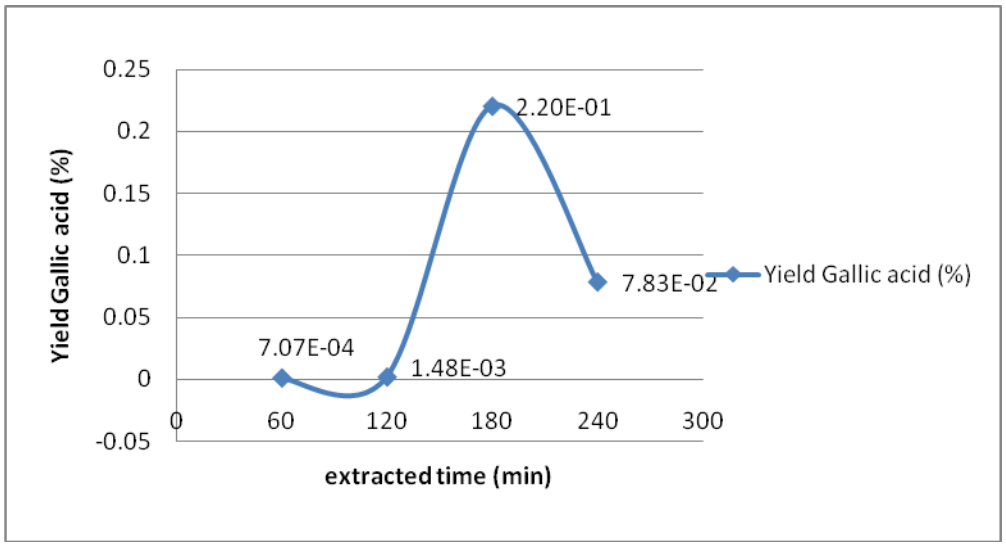

Figure 4.5: Graph on the effect of extraction time on Yield of Gallic acid for Soxhlet: Extraction temperature (70 ˚C) & particle sizing (800 μm)

In conventional Soxhlet extraction method, the yield Gallic acid extraction increase with the increase of duration of Soxhlet extraction as figure 4.5. This pattern occurred because of when longer time applied; solvent has been recycling back to the extraction chamber from the round bottom flask repeatedly causing it to extract more efficient of the desired compound from the raw material. However, longer extraction time was not investigated because due to time phenolic content easily decomposed at high temperature. One study reported by Grigonis et al (2005), by using Soxhlet extraction method, the compound containing are possible to degradations due to local overheating effects and longer extraction time besides limited solvent choice.

4.3.4 Effect of volume of solvent on the extraction crude yield for UAE and Soxhlet extraction from Tulsi plant

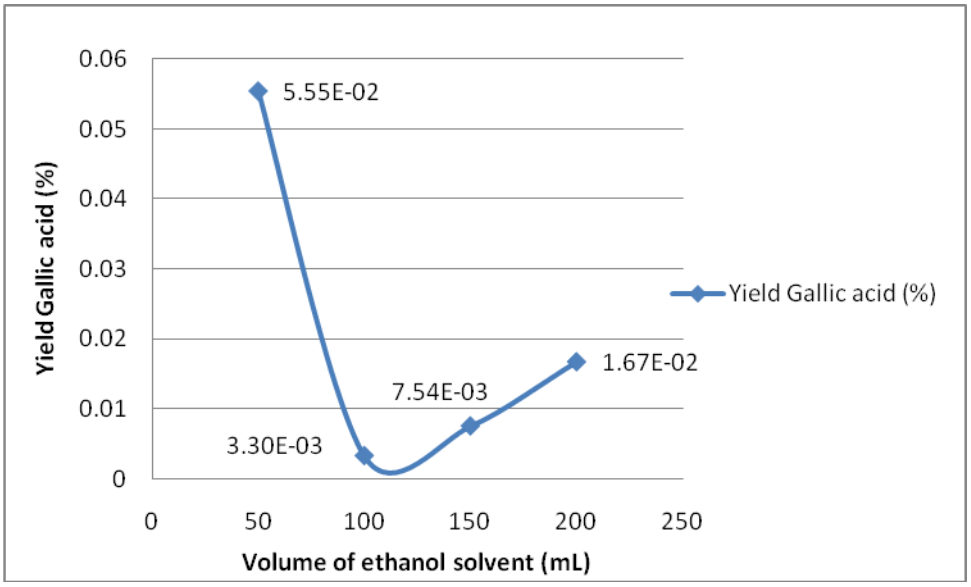

Figure 4.6: Graph on the effect of volume solvent on Yield of Gallic acid for UAE: extraction time (40 min), temperature (40˚C) & particle sizing (630 μm)

Figure 4.6 shows that yield Gallic acid extraction from Tulsi plant are dependent on ratio of solvent .Generally, the volume of solvent should be concordance with the amount of sample does not matter the type of samples as to extract efficiently (Martinez, 2009). Besides that, the matrix will be swelling during the extraction process thus as to ensure that, the solvent volume must be sufficient to ensure that the entire sample is immersed (Prasad et al, 2011). From the figure above the yield of the Gallic acid shows highly at 50 mL volume ethanol solvent which is 5.55E-2 % yield and starting to decrease after that. So it can be concluded that the yield at 50 mL is optimum ratio of solvent for the Tulsi plant samples to be extracted by using UAE.

According to Abdullah et al. (2010) larger solvent volume promotes an increasing concentration gradient between solvent and solid samples thus provide larger mass transfer between solid and solvent. However, too larger amount of volume solvent will decreases the yield as for ultrasonic assisted extraction as indicated in the figure above. The yield of the Gallic cid will decreases when uses above 50 ml volume of

ethanol solvent. According to Martinez. (2009), the used of the high amount solvent after it achieving the optimum is not improving the recoveries.

As for conventional Soxhlet extraction, the different volume of ethanol solvent is not favourable parameters to be done. It is because the samples only can be extracted at high amount of volume solvent. This is one of the negative points taken as high amount of solvent needed to run the experiment. There are many numbers of researches proved that Traditional Soxhlet method are time consuming and require relatively large quantities of solvents (Garcia-Ayuso & Lurge de Castro, 1999;Prasad et al.,2011).Thus, as it is already shown the disadvantages of the conventional Soxhlet method , the experiment were not carried on with this parameters.

4.6 METHOD COMPARISON

The result comparison is last process in this project analysis. Based from all data collections, the choice of the efficient extraction method would mainly depend on their merits and demerits along the process occurred such as the amount of extraction yield, complexity, production cost, safety and environmental prevention. Besides, the effective extraction should be inexpensive, safe and recover as much possible of the antioxidants present in the plants (Grigonis et al., 2005). Generally, Soxhlet extraction is quite well established oldest method that has been used for many decades (Wang, L & Weller, C.L, 2006). Solvent extraction of solid samples, which is commonly known as solid-liquid extraction also referred to as leaching or Lixiviation in a more correct use of the physicochemical terminology, is one of the common process that still remains using it.

There are a few advantages of this method such as better reproducibility and efficiency, sample fresh solvent contact during the whole process , no filtration procedure and simple manipulation should be retained have made it a trusted techniques used in widely industrial applications (Wang ,L & Weller, C.L , 2006). However, these traditional methods are solvent and time consuming (Prasad et al, 2011). So far, according to Grigonis, D et al (2005), this method is not always acceptable for industrial applications due to long extraction time, large consumptions of hazardous and some other disadvantages. In simple words, more usage of solvents means, a lot of organic

chemical will be wasted after the extraction process completed. Thus, it will increase up the level of pollution to our environment and safety especially for the use in big scale as for the industrial process needed.

Plus, time consuming is make our production cost highly maybe more than our product. Unfortunately, it is only considering about the time and volume consuming, how about the temperature? The temperature also plays a big role in the extraction process. Since, this method needed to run at high temperature, it is nothing only increased up our cost and power. Considering of that reasons, there is increasingly demand for the new techniques with less time consuming, low organic solvent consumption, and higher pollution prevention such as Ultrasonic assisted extraction (Wang ,L & Weller, C.L , 2006).

Ultrasonic assisted extraction is one of the technique recently has been predominantly in many research. It has its own merits make it more favourable techniques in used. In theory, the mechanical effects of ultrasound induce a greater penetration of solvent into cellular materials and improve mass transfer (Wang, L & Weller, C.L, 2006).The technique is commonly used in industry not for extraction process only but also to clean the optical parts, jewellery, surgical instrument, lenses and biological cells which bacteria can be disintegrated. One study led by Ishtiag.F (2009) said that ultrasound also has been used in life sciences such as medical imaging , diagnostics ,and fermentation process.

In this study, UAE method is used as to compare the effectiveness with the Soxhlet extraction method in kind of extraction yield of the Gallic acid, complexity of the procedure taking, the merits and demerits for both methods besides the pollution prevention taking. It show in terms of yield of the production, both methods can obtained at the same level of extraction yield thus make the second choice is taken. However in terms of complexity of the procedure taking and pollution prevention, the positive results led to the ultrasonic assisted extraction method. In simple word, the best results could be obtained of UAE method only in less time, temperature and volume of solvent compare with the conventional Soxhlet method. This means it could save the production cost greatly.

CHAPTER 5

CONCLUSION AND FUTURE RECOMENDATION

5.1 INTRODUCTION

At the end, the project should be done successfully according to the period on the Gantt chart with achieving the project objective. However, during the project stage a few problem were occurred and the problem need to solve in way to done the project. It is because limitation of time and equipment to done the project. Besides, there a lot of disturbances occur while handling the experiment. Above of all, the project still can be continued based on future recommendation in way to achieve the better result for the project. The project that has better result can be significant to the next researcher and as the references.

5.2 PROBLEM OCCURED

While running the experiment, there a lot of disturbances occurred thus may affected the result beside could make the process become slow. Firstly, when handling the conventional Soxhlet method the water supply should be smoothly flow along the process at the exchanger flask otherwise the glass were broke due to overheating put on it. This is because of the conventional Soxhlet method could be run only as it achieving boiling point of the ethanol solvent. Along the process, the pipe which flowing the water supply were closed thus broke the glass use.As a result, the product were spoiled due to spilled out some of the product. Thus, the process should be set up once again. Thus, the extraction process becomes slow.

5.3 FUTURE RECOMENDATIONS

Several future recommendations would like to express to the faculty for future final year project are:

(i) Provide a space for extraction process with smoothly flow of water.
(ii) The apparatus as HPLC and UV-Vis should be regularly check as to avoid the error while taking the results.
(iii) Raw material, chemicals and apparatus should be provide as it could smooth the stage of experiment because of the process quite delayed due to materials problems.

5.4 CONCLUSIONS

As conclusion, at the end of analysis all the project objective was achieve. The problem had been occur also can be solve by alternative method in way to done the project. From the analysis, the ultrasonic assisted extraction is one of the potential method should be used in many chemical industry as it could lowering the time used, require less solvent besides high pollution prevention. As a result, we could lowering the production cost besides could get high yield of product. From the project also, we can know the effect of different parameters to the product yield. Based on result also, it can conclude that, the diameter of materials, time, temperature and volume of solvent could affect the production of high yield.

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