COMPOSITION AND BIOLOGICAL ACTIVITIES OF *STROBILANTHES CRISPUS* LEAVES FOR ESSENTIAL OILS EXTRACTED BY HYDRODISTILLATION (HD) AND MICROWAVE-ASSISTED HYDRODISTILLATION (MAHD) METHODS

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Thesisi submitted in partial fulfilment of the requirements
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Dedicated to my supervisor, and my parents.

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ABSTRACT

Microwave-assisted hydrodistillation (MAHD), an advanced hydrodistillation (HD) method, in which a microwave oven is used to extract essential oil from medicinal plants. MAHD and HD methods have been compared and evaluated for their performance in the isolation of essentials oil from *Strobilanthes Crispus* leaves. The effect of microwave power, extraction time and water to raw material ratio were investigated to evaluate the best extraction conditions for obtaining maximum oil yield. As a result, the best condition that has been investigated for maximum essential oil production, 0.2016% (w/w) were 450W microwave power for 90 minutes at water to raw material ratio of 10:1. While, HD used 6 hours and water to raw material ratio of 10:1 to achieve the highest yield, 0.1886% (w/w). The composition of the extracted essential oils at different extraction time and microwave power was investigated by GC-MS to evaluate the quality of essential oil. Results shows that the constituent of essential oil from MAHD were different with essential oil from HD. The major constituent of *S. Crispus* oil from MAHD was Phthalic acid, bis(7-methyloctyl) ester and 1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester. However, the major compound of *S. Crispus* oil from HD were 1,2-Benzenedicarboxylic acid, decyl hexyl ester and 1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester. This indicates that the use of microwave irradiation influence the composition of essential oils. The maximum oil yield from *S. Crispus* were obtained at shorter extraction time period with significant influence on their chemical constituents. Therefore, for *S. Crispus* oil, the method of extraction depends on the uses of the oil. By reviewing the chemical constituents with other studies, Phthalic acid, bis(7-methyloctyl) ester has antimicrobial and antifouling activities whereas 1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester has anticancer activity. This may prove that MAHD can be used to extract large volume and high quality essential oil by using shorter time and saving energy.

ABSTRAK

Air penyulingan dengan bantuan gelombang mikro (MAHD) adalah teknologi yang maju daripada air penyulingan (HD). Kaedah ini menggunakan ketuhar gelombang mikro untuk mengeluarkan minyak pati dari tumbuhan. Kaedah MAHD dan HD telah dibandingkan dan dinilai untuk prestasi mereka dalam pengasingan minyak pati dari daun *Strobilanthes Crispus*. Kesan daripada kuasa ketuhar gelombang mikro, masa pengekstrakan dan air kepada nisbah bahan mentah telah disiasat untuk menilai keadaan operasi yang terbaik untuk maksimum hasil pengekstrakan minyak pati. Hasilnya, keadaan yang terbaik untuk pengeluaran minyak pati maksimum, 0.2016% (w/w) adalah 450W kuasa gelombang mikro selama 90 minit pada air dengan nisbah bahan mentah 10:1. Sebaliknya, HD menggunakan 6 jam dan air kepada nisbah bahan mentah daripada 10:1 untuk mencapai hasil yang tertinggi, 0.1886% (w/w). Komposis minyak pati yang dikeluarkan pada berbeza masa pengekstrakan dan berbeza kuasa gelombang mikro telah disiasat oleh GC-MS untuk menilai kualiti minyak pati itu. Keputusan menunjukkan bahawa komposisi minyak pati dari MAHD dan HD adalah berbeza. Konstituen utama minyak pati *S. Crispus* dari MAHD ialah *1,2-Benzenedicarboxylic acid, decyl hexyl ester* dan *1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester*. Walau bagaimanapun, konstituen utama minyak pati *S. Crispus* dari HD adalah *1,2-Benzenedicarboxylic acid, decyl hexyl ester* dan *1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester*. Ini menunjukkan bahawa pengguna penyinaran gelombang mikro dalam penyulingan boleh mempengaruhi komposisi minyak pati. Hasil maksimum minyak pati *S. Crispus* dapat diperolehi dengan kaedah MAHD dalam masa yang pendek dengan pengaruh yang besar ke atas komposisi minyak itu. Oleh itu, bagi minyak pati *S. Crispus*, kaedah pengekstrakan bergantung pada penggunaan minyak pati itu. Dengen mengkaji komposisi minyak pati *S. Crispus* dengan kajian lain, *Phthalic acid, bis(7-methyloctyl) ester* menpunyai aktiviti biologi antimicrobial dan antifouling sedangkan *1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester* menpunyai aktiviti biologi antikanser. Ini boleh membuktikan bahawa MAHD boleh digunakan untuk mengeluarkan jumlah minyak pati yang banyak dan berkualiti dengan menggunakan masa pengekstrakan pendek dan menjimatkan masa.

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LIST OF ABBREVIATIONS

DPPH	1, 1-diphenyl-2-picrylhydrazy
FTC	Ferric Thiocyanate
GC-MS	Gas-Chromatography-Mass-Spectrometry
HD	Conventional hydrodistillation
MAHD	Microwave-Assisted Hydrodistillation
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide
TBA	Thiobarbituric Acid

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole plant or plant part of known taxonomic origin (Franz, & Novak, 2015). Essential oils are sourced not only from flowers, but from barks, seeds, peels, roots, buds and various parts of plants. They can be extracted by several extraction methods, for example distillation, solvent extraction, solvent free microwave extraction, expression and so on (Tongnuanchan, & Benjakul, 2014; Schmidt, 2015). In general, essential oils mainly contain of terpenes (monoterpenes and sesquerpitenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids) (Bakkali et al. 2008; Mohamed et al., 2010).

Strobilanthes Crispus (Acanthaceae) plant is native to countries from Madagascar to Indonesia (Sunarto, 1977). *Strobilanthes Crispus* is a bush-like plant. S.Crispus is known as pecah beling (Malay) and Black Faced General (Mandarin). Traditionally, *S. Crispus* is used to treat diabetes and cancer, prevent lysis, as laxative and diuretic agent (Sunarto, 1977; Perry and Metzger, 1980). *S. Crispus* is also famous among the public because of its good biological activities. There are studies proven that *S. Crispus* has properties, such as anticancer activity, anti-diabetic, wound healing properties, antimicrobial activities, antioxidant and anti-ulcerogenic (Nurraihana, & Norfarizan-Hanoon, 2013). According Asmah et. al. (2006), by using hydrodistillation method, the essential oil of *S. Crispus* fresh leaves did not give any cytotoxic value against all the cell lines tested. However, the study showed *S. Crispus* essential oil has higher antioxidant activity compared to α-tocopherol (standard) but lower than *L. inermes*.

The methods to obtain essential oil from medicinal plant, including *S. Crispus,* are distillation (hydrodistillation, steam distillation and hydrodifussion), solvent extraction (solvent, supercritical carbon dioxide and subcritical water) and solvent-free microwave (Tongnuanchan, & Benjakul, 2014). The quality of essential oil mainly depends on their constituents. Extraction method is one of main factors that determine the quality of essential oil (Tongnuanchan, & Benjakul, 2014). Inappropriate extraction procedure can destroy and alter chemical signature of essential oil (Tongnuanchan, & Benjakul, 2014). According to Okoh & Afolayan (2011), steam distillation can lead to susceptible chemical changes on monoterpenes compound. Besides that, the essential oil extracted through solvent extraction contains solvent residues that pollute the fragrances. The removal of its solvent causes losses of more volatile compounds.

In this research, the extraction methods that have been choosen are hydrodistialltion (HD) and microwave-assisted hydrodistillation (MAHD). According to Tongnuanchan & Benjakul (2014), HD is often used to isolate nonwater-soluble natural products with high boiling point. This method protects the oils extracted to a certain degree because the surrounding water acts as a barrier to prevent it from overheating. In the other hand, MAHD is an advanced method of HD, which use microwave oven in the extraction process. MAHD have been used by several researchers to isolate essential oils from rosemary (Karakaya, et al., 2014), *Cinnamomum iners Reinw* (Phutdhawong et al., 2007), thyme (Golmakani, & Rezaei, 2008), mango flowers (Wang et al., 2010), lemongrass (Ranitha et al., 2014) and ginger (Abdurahman et al., 2013). Essential oils extracted by HD and MAHD have the same chemical composition but the differences between HD and MAHD are the effectiveness of extraction (Wang et al., 2010). Microwave-assisted hydrodistillation shorten extraction time, improve extraction yield, save energy consumption and it is an environmental friendly method. (Golmakani and Rezaei, 2008).

1.2 Motivation

The use of essential oils together with their therapeutic properties is an ancient tradition. In ancient, essential oils have been used for cosmetic purposes, as well as for their spiritually and emotionally uplifting properties. In recent years, there are growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants (Schwartsmann, et al., 2002). Therefore, application of essential oils which are extracted from natural plant gains growing attention in pharmaceutical and food industry. The world's total production of essential oils is estimated at about 100,000 – 110,000 tons (Gill et.al., 2014) . Besides, essential oils are famous with its biological activity, antioxidant, antibacterial and antifungal properties too.

There are many methods used to extract essential oils, such as distillation, solvent extraction, solvent free extraction, expression and so on (Tongnuanchan & Benjakul, 2014; Baser & Buchbauer, 2010). Among of the methods, hydrodistillation has become the standard method of essential oil extraction from plant material. However, oil extraction by using hydrodistillation need long extraction time and it is energy wasting method (Golmakani, & Rezaei, 2008). Recent, green and sustainable extraction methods of natural products is currently a hot research topic in the multidisciplinary area of applied chemistry, biology and technology. Therefore, an advanced hydrodistillation technique is developed which is known as microwave-assisted hydrodistillation. This technique saves energy and times, as well as increases the yield of essential oil (Golmakani, & Rezaei, 2008).

1.3 Problem Statement

Nowadays, essential oil of *S. Crispus* is still not available in the commercialized market now. *S. Crispus* leaves are commercialized as a healthy drinking tea in the health-food market. *S. Crispus* tea is the most common products that we can find in the market now. Besides that, *S. Crispus* are also available in the form of raw crude powder, as capsule and as an addictive mixed with coffee. *S. Crispus* have been proven showing several biological activities, such as anticancer, antidiabetic, antimicrobial, wound healing properties and so on. But, there is still a wide gap in looking for S. Crispus biological activities.Earlier studies on *S. Crispus* leaves were mostly focused on chemical

constituents from supercritical fluid extraction, hydrodistillation and solvent extraction method. To the best of the author's knowledge, however, there is no data on the MAHD of volatiles in *S. Crispus* leaves has been reported. For gain the clear knowledge of a method's efficiency, it is important to carry out the constituents as well as the biological activities comparison of the extracts so that its real clinical benefits can be determined. Therefore, the problem statement of this paper is to analyse the chemical composition and biological activities of *S. Crispus* for essential oil extracted by using hydrodistillation method (HD) and microwave assisted hydro-distillation method (MAHD) to enable of *S. Crispus* essential oil commercialize in the world market.

In general, essential oils are extracted by using steam or hydrodistillation methods. However, conventional hydrodistillation has several disadvantages, which are long extraction time, potential loss of volatile constituents and high energy use (Wang et al., 2010). Therefore, MAHD, which is an advanced extraction technique, is developed to shorten extraction time, improve extraction yield, save energy consumption and it is an environmental friendly method. (Golmakani and Rezaei, 2008).

1.4 Objectives

The objectives of this study are:

i. To evaluate the performance of hydrodistllation (HD) and microwave-assisted hyrodistillation (MAHD) method in extraction of essential oil from *S. Crispus* leaves.
ii. To evaluate the best condition (extraction time, water to raw material ratio and power supply) for essential oil extraction from *S. Crispus* leaves.
iii. To analyse the chemical compositions of essential oil obtained from *S. Crispus* leaves by using hydrodistillation method (HD) and microwave assisted hydro-distillation method (MAHD)

1.5 Scopes of Study

This research is an experimental study to compare hydro-distillation method (HD) with microwave-assisted hydro-distillation (MAHD) using *S. Crispus* as raw material. In order to study the objectives, several scopes have been identified. The scopes are:

i. To study the effect of microwave power on extraction of *S. Crispus* essential oil. Three different power supply (200W, 250W and 300W) are used to determine the effect of microwave power on the extraction yield.
ii. To study the effect of water to raw material ratio (v/w) on extraction of *S. Crispus* essential oil, three different water to raw material ratio are used (6:1, 8:1, 10:1 v/w). For example, for 50g of S. Crispus leaves are hydrodistilled with 400ml, 500ml and 600ml of distilled water.
iii. To study the effect of extraction time on extraction of *S. Crispus* essential oil for microwave-assisted hydrodistillation method, three different extraction time (HD: 2 hours, 4 hours, 6 hours, MAHD: 30min, 60min, 90min) are used.
iv. To analyse the chemical compositions of essential oil obtained from *S. Crispus* leaves by using hydrodistillation method (HD) and microwave assisted hydro-distillation method (MAHD), Gas-Chromatography-Mass-Spectrometry (GC-MS) is used.

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oil

Essential oils are complex mixtures of volatile compounds produced by living organisms and isolated by physical means only (pressing and distillation) from a whole plant or plant part of known taxonomic origin (Franz, & Novak, 2015). They are a mixture of volatile lipophilic (Sadgrove et al., 2015). A variety of other names are given to the essential oil. They are also known as essence, fragrant oil, volatile oil, etheric oil, aetheroleum or aromatic oil (Berger, 2007). They are lighter than water and have a density between 0.75 and 0.98g/cm^3 (Balz, 1996). Essential oils are soluble in alcohol, ether, and oils but practically insoluble in water and then only dispersible with the aid of emulsifiers (Balz, 1996). Although essential oils are only partially soluble in water, the aqueous solubility of individual essential oil component varies with respect to polarity (Sadgrove, et al., 2015). Generally, components with more polar functional groups are expected to be more soluble in water relative to other components (Sadgrove, et al., 2015).

Essential oils are sourced not only from flowers, but from barks, seeds, peels, roots, buds and various parts of plants. They can be extracted by several extraction methods, for example distillation, solvent extraction, solvent free microwave extraction, expression and so on (Tongnuanchan, & Benjakul, 2014; Schmidt, 2015). However, the most common methods are steam or hydrodistillation first developed in the Middle Ages by Arabs (Bakkali et al., 2008). Besides that, now essential oils can be extracted using modern microwave-assisted hydrodistillation (Karakaya et al., 2014) or microwave-assisted distillation techniques that requires no additional of water, other than cytosolic and vascular fluids already present in the source tissue (Mohamadi, et al., 2013).

Commonly, plant essential oils consists of the complex mixture of natural compounds, both polar and non-polar (Masango, 2005). In general, essential oils mainly contain of terpenes (monoterpenes and sesquerpitenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids) (Bakkali et al. 2008; Mohamed et al., 2010). According to Franz & Novak (2015), the respective main compounds are mainly derived from three biosynthetic pathways only, the mevalonate pathway leading to sesquiterpenes, the methyl-eruthrytol-pathway leading to mono and diterpenes, and the shikimic acid pathwat *en route* to phenylpropenes. Hemi- (1 isoprene), mono- (2-isoprenes), sesqui- (3 isoprenes) and di- (4 isoprenes) terpenes are the most common essential oil components, followed by the non-terpenoid group, phenylporpanoids (Sadgrove, et al., 2015).

Essential oils and their active components possess antiviral, antimycotic, antitoxigenic, and insecticidal properties. Therefore, essential oils play an important role in plants and act as antibacterial, antivirals, antifungals, insecticides, and protect the plants from herbivores. Those properties cause essential oils have been widely used in perfumes and make up products (creams, soaps, etc), sanitary products, dentistry, agriculture (antipests and herbicides), veterinary medicine (pest repellent, antiparasitic oil, in animal feed), as preservatives, and flavour additives for foods, as fragrances for household cleaning products and industrial solvents, as natural remedies (as mixtures with vegetal oil in massages or in baths, in aromatherapy, etc.) (Başer, & Franz, 2015; Buchbauer, & Hemetsberger, 2015; Burt, 2004).

2.2 *Strobilanthes Crispus*

Strobilanthes Crispus (Acanthaceae) plant is native to countries from Madagascar to Indonesia (Sunarto, 1977). In Malaysia, this plant is known as “pecah beling”, “bayam karang” “pecah kaca” or “jin batu” in Malaysia (Noraida, 2005). Besides that, in Indonesia, this plant is known as “daun picah beling” in Jakarta or “enyoh kelo”, “kecibeling”, “kejibeling”, or “ngokilo” in Java (Sunarto, 1977). This plant is also called as ‘Black Faced General’ which is derived from the direct Chinese translation of the name of the plant.

The scientific classification for *S. Crispus* is as follow in Table 2.1. Greek strobilus ‘a cone’ and Anthos ‘a flower’, leaves and bracts enclose the flowers (Blume, 1826)

Table 2.1: Scientific classification for *S. Crispus*

Kingdom	**Plantae**
Phylum	Angiosperms
Class	Dicotyledonae
Sub Class	Solanales
Family	Acanthaceae
Genus	*Strobilanthes*
Species	*Crispus*

Source: (Blume, 1826)

S. Crispus is an annual plant, which grows easily in forest, riverbanks and abandoned fields. It is commonly used as fence hedges. The bush-like plant can attain a height between 1 to 2m. The circular bark can be divided into segments and similar to its branches, they are hairy and green. The leaves of *S. Crispus* are oblong-lanceolate, rather obtuse and shallowly crenate crispate and have rough surface, covered with short hairs (Backer and Bakhuizen, 1963; Surnato, 1977). The upper surface of the leaves has a darker green colour and is less rough as compared to the underside (Surnato, 1977). The flowers are yellow in colour, short, dense and panicled spikes (Backer and Bakhuizen, 1963). Figure 2.1 shows the leaves of *S. Crispus*.

Figure 2.1: The leaves of *S. Crispus* (Nurraihana, & Norfarizan-Hanoon, 2013)

2.3 Volatile Compounds in *Strobilanthes Cripus*

According to Asmah et al. (2006), there were at least 28 volatile compounds extracted from *S. Crispus* leaves by hydrodistillation method. The main component for *S. Crispus* was phytol (46.01%) and other components of the oil were alpha cadinol (3.47%), taumurolol (2.49%), ledol (1.81%) and eugenol (1.08%) as shown in the Figure 2.2 (Asmah et al, 2006). Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is an a constituent of the chlorophyll molecule, a biomolecule that is involved in the production of energy from light (Van den Brink & Wanders, 2006). It is used as a precursor for manufacture of synthetic forms of vitamin E (Netscher, 2007). Figure 2.2 shows chemical constituents of essential oil from *S. Crispus* leaf.

Liza et al. (2010) found that by using supercritical carbon dioxide extraction, 3.98% bioactive flavonoid compound and eight flavonoid compounds were found in *S. Crispus* leaf under optimum condition. The optimum conditions were pressure at 200 bar, temperature at 50°C and dynamic time at 60 min (Liza, et al., 2010). The 8 flavonoid compounds were Catechin, Epicatechin, Rutin, Myricetin, Luteolin, Apigenin, Naringenin and Kaempferol. Kampferol contributed the highest percentage, 19.45% to the contents of flavonoid. Liza et al. (2012) optimized this supercritical carbon dioxide extraction method again. In the study, 10.04% flavonoid content were obtained from *S. Crispus* leaf. Same 8 flavonoids and other compounds were obtained. Kaempferol (21.54mg/g), was found as the highest flavonoid compound under the new optimum condition which were pressure at 200 bar, 50°C and 5g/min co-solvent flow rate (Liza et

al, 2012). Kaempferol is one of flavonoid as phytoestrogens (Kim, & Choi, 2013). Kaempferol has been suggested to have antioxidant, anti-inflammatory and anticancer effect (Kim, & Choi, 2013).

Peak	Retention time	Substance	(%)
1	10.830	2,3-dihydrobenzofuran	1.68
2	16.216	Megastigmatrienone	1.21
3	16.865	unknown	1.73
4	17.108	Alpha-cadinol	3.47
5	17.267	Tau-murolol	2.49
6	17.342	unknown	1.21
7	17.392	Ledol	1.81
8	17.525	1-Naphtalenol	1.97
9	17.992	Eugenol	1.08
10	19.133	2-Undecanone	5.84
11	19.308	Phenol	3.07
12	20.042	2-hexyl,1- decanol	3.69
13	20.283	Isophytol	0.96
14	20.800	Nonadecanoic acid	2.10
15	20.918	9,17-Octadecadienal	2.34
16	22.000	Hexyl octyl ether	1.77
17	22.082	Phytol	46.01
18	22.443	Tetradecanal	0.87
19	22.873	2,6,10-trimethyl pentadecane	0.93
20	23.839	Eicosane	1.10
21	24.071	13-tetradece-11-yn-1-ol	1.02
22	24.768	Heptadecane	1.26
23	25.691	Tridecyl iodide	1.70
24	26.243	Di-n-octyl phtalate	2.62
25	26.687	Tetratetracontane	1.45
26	27.804	Octacosane	1.88
27	30.614	Pentadecane	3.00
28	34.590	Heptacosane	1.73

Figure 2.2: Chemical constituents of essential oil from *S. Crispus* leaf (Asmah et al, 2006)

2.4 Biological Activities of *Strobilanthes Cripus*

Traditionally, *S. Crispus* is used for antidiabetic and anticancer, to prevent lysis, as laxative and diuretic agent (Sunarto, 1977; Perry and Metzger, 1980). This plant contains of cystoliths of calcium carbonate (Perry and Metzger, 1980). The high contain of calcium carbonate cause this plant becoming mildly alkaline and has a function in ease of urination (Perry and Metzger, 1980; Noraida, 2005). Besides that, according to Sunilson et al. (2010), the fresh of this plant was used by orang asli in Kampung Bawong, Perak, to enhance the immune system. In the Gemencheh Settlement, a survey revealed

that this plant can be used to treat kidney stones by placing the heated leaves on the hips (Ong and Norzalina, 1999).

In addition, Fadzelly et al. (2006) found that *S. Crispus* tea displayed anti-hyperglycaemic activities in experimental animal models by reduced blood glucose level and improved lipid profile. According to Norfarizan-Hanoon et al.(2009), the administration of *S. Crispus* juice also possesses antihyperglycemic, hypolipidemic and antioxidant effect in normal and streptozotocin-induced diabetic. In this study, different doses of *S. Crispus* juices were fed to normal and streptozotocin-induced diabetic male and female Sprague Dawley rats together with basal diet for 30 days. Besides, *S. Crispus* also has been proven to have wound healing properties. The ethanol extract of *S. Crispus* leaf significantly enhanced the acceleration of wound healing enclosure in rats (Al-Henhena, et al., 2011). Antioxidant properties also have been detected in *S. Crispus*. Asmah et al. (2006) found that *S. Crispus* essential oil has higher antioxidant activity compared to α-tocopherol (standard) by using FTC and TBA methods.

There are several studies reporting the possible inhibitory of cancer by the extract of *S. Crispus.* For the essential oil from *S. Crispus* fresh leaves, they did not give any cytotoxic value against the cell lines tested (Asmah et al, 2006). But, Muslim et al. (2010) found that both methanolic and aqueous extract of the leaves of *S. Crispus* have anti-angiogenic activity. In this study, the possible cytotoxicity effects were tested on MCF-7, colon carcinoma (HCT 116), liver cancer cell lines (HepG2), non-small cell lung adenocarcinoma (NCI-H23), human breast ductal carcinoma (T-47D) and normal colonic fibroblast cell line (CCD-18Co). MTT was used to evaluate the cell proliferation assay and ex vivo rat aortic was used to evaluate the inhibitory effect of the *S. Crispus* extracts.

Furthermore, *S. Crispus* extracts also showed antimicrobial activities. According to (Mustafa, et al., 2009), *S. Crispus* crude extract was active on *B. cereus* by showing the largest mean of diameter of inhibition zones at the concentration of 20mg/ml. The crude extract of *S. Crispus* also showed the Minimal Inhibition Concentration (MIC) value at 2mg/ml, whereas the Minimal Bactericidal Concentration (MBC) at much higher concentration with the MBC values at 6mg/ml.

A few research have shown that *S. Crispus* is enriched with strong in vitro and in vivo antioxidant properties. Mohd Fadzelly et al. (2006) have studied the antioxidant activity of several types of *S. Crispus* tea. The fermented and unfermented of *S. Crispus* tea from young to old leaves were screened for the possible antioxidant activity in vitro using Ferric Reducing/Antioxidant Power and DPPH free radical scavenging assay and were compared with green and black tea. The results showed both methods have the same trend for antioxidant activities. The green tea possesses the highest antioxidant activity, followed by black tea, *S. Crispus* unfermented tea (old leaves), *S. Crispus* unfermented tea (young leaves), *S. Crispus* fermented tea (old leaves) and *S. Crispus* fermented tea (young leaves). Asmah et al. (2006) used FTC and TBA methods to investigate the antioxidant activity of *S. Crispus* and *Lowsonia inermes* essential oil. *S. Crispus* essential oil showed higher antioxidant activity compared to α-tocopherol but lower than *L. inermes.*

In conclusion, *S. Crispus* showed several biological activities, such as anticancer, antidiabetic, antimicrobial, wound healing properties and so on. But, there is still a wide gap in looking for *S. Crispus* biological activities. Hence, further studies in biological activities are deserved.

2.5 Extraction Methods

Essential oils can be isolated by several extraction methods, for example distillation, solvent extraction, solvent free microwave extraction, expression and so on (Tongnuanchan & Benjakul, 2014; Baser & Buchbauer, 2010). The two methods used in this study are hydrodistillation method and microwave-assisted hydrodistillation method.

2.5.1 Hydrodistillation Method (HD)

HD has become the general method of essential oil extraction from plant. There are three types of hydrodistillation, which are water distillation, steam distillation and direct steam distillation (Sinha, et al., 2007). The distillation used in this study is water distillation. This method always used to isolate non-water-soluble natural products with

high boiling point (Tongnuanchan, & Benjakul, 2014). The process involves the complete immersion of plant materials in water, followed by boiling (Tongnuanchan, & Benjakul, 2014). The oils extracted by this method is protected to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. (Tongnuanchan, & Benjakul, 2014) By using HD method, the required material can be distilled at a temperature below 100°C (Tongnuanchan, & Benjakul, 2014).

There is a special continuous form of water distillation named a Clevenger apparatus (Walton, & Brown, 1999). Clevenger apparatus is used for the quantitative determination of essential oils according to the European Pharmacopeia (Walton, & Brown, 1999). Clevenger apparatus is shown as Figure 2.3. For Clevenger apparatus, oil which is lighter than water is collected in the conical bulb while water returns to the distillation flask (Walton, & Brown, 1999). The distilled essential oil, after separation from water, it must be dried by using anhydrous sodium sulphate (Sinha, et al., 2007). This hydrodistillation process can be done at a reduced pressure (under vacuum) to decrease the temperature to less than 100°C (Gill, et al., 2014). This can be advantageous in protecting heat sensitive chemical compounds from rearrangement or complete decomposition which will influence the essential oil quality (Gill, et al., 2014).

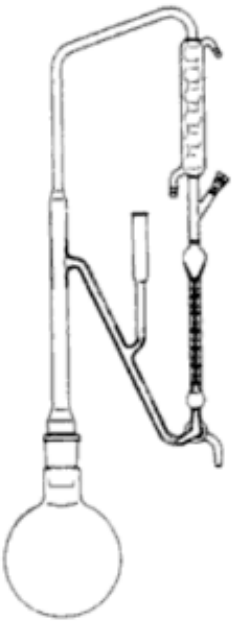

Figure 2.3: Clevenger apparatus (Walton, & Brown, 1999)

2.5.2 Microwave-assisted Hydrodistillation (MAHD)

Microwave are non-ionizing electromagnetic waves of frequency between 300MHz to 300GHz (Letellier and Budzinski, 1999; Routray & Orsat, 2012). Its frequency is positioned between the X-ray and infrared rays in the electromagnetic spectrum (Letellier, & Budzinski, 1999). 2450 MHz frequency is commonly utilized in dosmetic microwave ovens and for extraction application with a wide range of commercial units designed for analytical chemistry purposes (Letellier & Budzinski, 1999; Routray & Orsat, 2012). Microwave are electromagnetic waves which was made up of two oscillating perpendicular fields, they are electrical field and magnetic field (Letellier and Budzinski, 1999). The electrical field is responsible for heating (Letellier and Budzinski, 1999). One of applications of microwaves is the direct action of waves on material which is able to transform absorbed electromagnetic energy into heat energy (Letellier, & Budzinski, 1999).

Unlike conventional heating, microwave heating happens in a targeted and selective manner with practically no heat being lost to the surrounding environment as the heating occurs in a closed system rotation (Mandal et al., 2007). This superior heating mechanism can significantly reduce the extraction time as compare to hydrodistillation (Golmakani, & Rezaei, 2008). Heating using microwave is based upon its direct effect with polar substances/solvents and is governed by ionic conduction and dipole rotation (Letellier, & Budzinski, 1999). The dissipation factor (tan σ) is the measure of the ability of the solvent to absorb microwave energy and pass it on as heat to the surrounding molecules, which is used to determine the efficiency with which different solvents heat up under microwave (Mandal, et al., 2007). The dissipation factor is given by the equation:

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'} \qquad (2.1)$$

where ε'' is the dielectric loss which indicates the efficiency of converting microwave energy into heat, ε' is the dielectric constant which is the measure of the ability to absorb microwave energy (Mandal, et al., 2007). Figure 2.4 shows dissipation factor and dielectric constants for some solvents commonly used in microwave-assisted extraction

Solvent	Dielectric constant[a] (ε')	Dielectric loss (tanδ) × 10^{-4}
Acetone	20.7	
Acetonitrile	37.5	
Ethanol	24.3	2500
Hexane	1.89	
Methanol	32.6	6400
2- propanol	19.9	6700
Water	78.3	1570

a: determined at 20°C

Figure 2.4: Dissipation factor and dielectric constants for some solvents commonly used in microwave-assisted extraction (Mandal, et al., 2007).

By using microwave for heating, the moisture inside the plant cell evaporates and generates tremendous pressure on the cell wall due to swelling of the plant cell (Wang & Weller, 2006). The pressure pushes the cell wall from inside, stretching and rupturing it (Mandal, et al., 2007). These actions help to leach out the active constituents from rupture cells to surrounding water thus improving the yield of phyto-constituents (Mandal, et al., 2007). Besides, temperature increases penetration of the solvent into the matrix of cells and constituents are released into the hot solvent (Mandal, et al., 2007). By microwave assisted hydrostillation, the extraction time and solvent usage will be reduced as well as the extraction yield will be increased (Wang & Weller, 2006).

2.6 Factors Affecting HD and MAHD

2.6.1 Solvent (Water) to Raw Material (Leaves) Ratio

The solvent volume must be sufficient to ensure that the solvent volume is always entirely immersed in the solvent throughout the entire irradiation time (Mandal, et al., 2007). In general, a higher ratio of solvent volume to solid matrix (leaves) may be effective in conventional extraction methods (Mandal, et al., 2007). But, in microwave assisted extraction, a higher ratio may yield lower recoveries, which may be due to inadequate stirring of the solvent by microwaves (Mandal, et al., 2007). According to Ranitha, et al. (2014), the yield from the plant material increased when the amount of water as solvent was reduced. Presence of excess amount of water also cause excess

thermal stress due to rapid heating of the solution on account of effective absorption of microwaves by water (Dhobi et al., 2009). Besides, according to Gao et al. (2006), extraction with high liquid/solid ratio, the concentration of flavonoids in extraction solution was low due to more energy and time were needed to condense the extraction solution in later separation and purification process. Thus, the liquid/solid ratio of 50:1 (ml/gm) was found suitable to reach the high yield of flavonoids from the dried *S. medusa* cell cultures (Gao, et al., 2006). However, there was a ratio 10:1 (ml/mg) to 20:1 (ml/mg) found to be optimum in many application (Li et.al. 2004; Pan et. al., 2001, 2003; Talebi et. al., 2004).

2.6.2 Microwave Power Level (Only for MAHD)

The amount of energy supplied to the sample which is converted to heat energy in the dielectric material is controlled by the intensity of the incident microwave power to increase its temperature (Ma, et al., 2009). It affects interactions and equilibrium rate and controls partition of analytes between sample and solvent (Ma et al., 2009). According to Hu, et al. (2008), the yield of the extracted compound increases with the microwave power. The study revealed that when microwave power was increased from 200 to 1000W, the extraction yield of flavonoid from Radix Astragali was increased. But, high microwave power might increase the temperature overly high, and decrease the extraction yield due to product damage or compound breakdown (Ma et al., 2009). Besides, there was report showing that varying of power from 400W to 1200W had no significant effects on the yield of flavonoids extraction (Gao, et al., 2006). However, it was seen that extraction time was shortened by 45 min when using 1200W. At higher power, rapid rupture of cell wall occur at higher temperature causing the desired analytes or impurities leaching out into the solvent (Mandal, et al., 2007). On the other hand, at lower power level, the cell wall rupture takes place gradually and this enables selective microwave assisted extraction (Mandal, et al., 2007). The power must be chosen correctly to avoid excessive temperature, which could lead to solute degradation and overpressure inside the vessel (Mandal, et al., 2007).

2.6.3 Extraction Time

As in other extraction technique, time is another parameter which its influence needs to be taken into account. Commonly, the quantity of analytes extracted is increased by increasing the extraction time, although there is risk that degradation may occur. A proper study on optimization of extraction time is vital because extraction time may vary with different plant part used (Mandal, et al., 2007). Irradiation time is also affected by the dielectric properties of the solvent (Mandal, et al., 2007). Solvents like water, ethanol, and methanol may heat up tremendously on longer exposure thus risking the future of thermolabile constituents (Mandal, et al., 2007). According to Asmah et. al. (2006), the *S. Crispus* leaves was hydrodistilled by using conventional hydrodistillation for 6 hours to get 0.43% (v/w) of essential oil.

2.6.4 Matrix Characteristics

The plant particle size and the status in which it is presented for microwave assisted extraction can have a significant effect on the recoveries of the compounds (Mandal, et al., 2007). The extraction can be enhanced by providing larger surface area. Larger surface area provides better contact between the plant matrix, and the solvent, also finer particles will allow improved or much deeper penetration of the microwave (Mandal, et al., 2007). According to Kwon et al., (2003), in the extraction of ginseng saponins, extraction yield increased with the decrease in particles size. However, it was also found that particles less than 60 meshes are not suitable for the filtration of the extracts. Besides that, water soaked samples were used for the extraction of paclitaxel which resulted in better recovery. In many cases, the extract recoveries are improved by the natural moisture content of the plant matrix, as in the case of essential oil (Lucchesi et al., 2004; Wang et al., 2006). In some cases soaking of the dried plant material in the extracting solvent improve the extraction yield (Mandal, et al., 2007).

2.7 Technique for Determination and Analysis of Essential Oils

2.7.1 Gas Chromatography-Mass Spectrometry (GC-MS)

Capillary Gas Chromatography is an important analytical method in organic chemical analysis for the determination of individual low molecular substances in complex mixture (Watson, & Sparkman, 2008). Mass spectrometry (MS) as detection method gives the useful and meaningful data, arises from the direct determination of the substance molecule or of fragments (Watson, & Sparkman, 2008). So, the results obtained from MS are always used as a reference for other indirect processes and finally for confirmation of the facts (Watson, & Sparkman, 2008). The combination GC with MS make us easy to get data from the analysis. However, the area of application of GC-MS is limited to substances which are volatile enough to be analysed by gas chromatography (Watson, & Sparkman, 2008).

Chromatography is a solute fractional technique which relies on the dynamic distribution of molecules to be separated between two phases: a stationary (or binding) phase and a mobile (or carrier) phase (Ghosh, 2006). In its simplest form, the stationary phase is particulate phase while the mobile phase is passed through the column with fixed velocity (Ghosh, 2006). For GC, the mobile phase is a gas and the samples need to have sufficient volatility to be carried through the column (Lundanes et al., 2014). The mobile phase must be an inert gas, reacting with neither the stationary phase nor the sample components (Lundanes et al., 2014). The gas that is used must be of high purity (Lundanes et al., 2014).

The velocities at which these molecules move through the column depend on their respective interactions with the stationary phase (Ghosh, 2006). Due to different interactions between the sample components and the stationary phase, the sample components migrate through the system at different speeds and elute from the column with different retention times (Lundanes et al., 2014). The retention times is defined as the time between the sample introduction and the elution from the column (Lundanes et al., 2014). A chromatogram is obtained when the sample components are separated and detected by a detector connected to the outlet of the column (Lundanes et al., 2014). In a chromatogram, the elution time is found at the x-axis while the size of constitutes is found

on y-axis (Lundanes et al., 2014). With isocratic elution (constant composition of the mobile phase), the peak width will increase with increasing elution time (Lundanes et al., 2014).

The basic principle of mass spectrometry (MS) is to generate ions from either inorganic or organic compounds by any suitable method, to separate these ions by their mass-to-charge ratio (m/z) and to detect them qualitatively and quantitatively by their respective m/z and abundance (Gross, 2011). The analyte may be ionized thermally, by electric field or by impacting energetic electrons, ions or photons (Gross, 2011). The ions can be single ionized atoms, clusters, molecules or their fragments or associates (Gross, 2011). A mass spectrum of molecule is produced. The mass spectrum provides the result as a plot of ion abundance versus mass-to-charge ratio (Hoffmann, & Stroobant, 2007). Mass spectra can be presented as a bar graph or as a table. The most intense peak is called the base peak and is arbitrarily assigned the relative abundance of 100% (Hoffmann, & Stroobant, 2007). The abundances of all the other peaks are given their proportionate values, as percentages of all the base peak (Hoffmann, & Stroobant, 2007).

When a mass spectrometer is employed as the chromatographic detector (GC-MS), its output represent the chromatogram that would have been obtained with other chromatographic detectors (Gross, 2011). The chromatogram as produced by the mass spectrometer is composed of a large set of consecutively acquired mass spectra, each of which containing spectral data of the eluting species, for example, each component can be identified from its mass spectrum (Gross, 2011). This is because mass spectral chromatograms represent ionic abundances as a function of retention time, these are called ion chromatograms (Gross, 2011). Figure 2.5 shows the typical GC-MS chromatogram

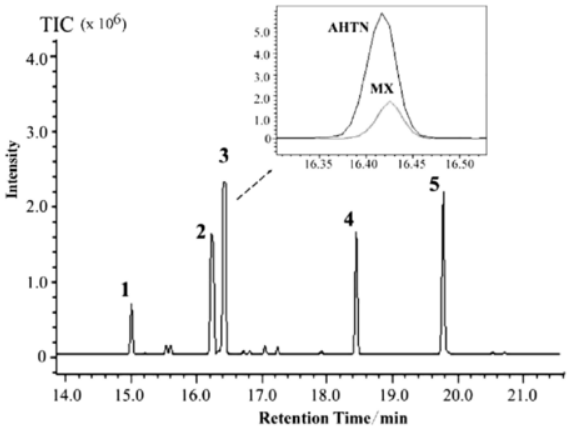

Figure 2.5: Typical GC-MS chromatogram

(Gross, 2011)

CHAPTER 3

METHODOLOGY

3.1 Raw Material

Blended *S.Crispus* dry leaves was purchased from herbs supplier (Malaysia Herbal Shop or Delima Jelita Herbs). Blended leaves were used because extraction yield increased by decreasing the particle size and increasing the contact surface area. The purchased plant materials was kept in a dry sealed plastic bag at ambient temperature and protected from the light. In order to increase the collection efficiency, the plant materials were soaked in distilled water for 30min before the extraction (Ranitha et al., 2014).

Figure 3.1: Dry leaves of *S. Crispus* in a sealed plastic bag

3.2 Hydrodistillation

One hundred grams of dried *S. Crispus* leaves were placed in a 2L rotatory flask containing 1000ml of distilled water and hydrodistilled for 6 hours using a Clevenger apparatus. The apparatus was set up as shown in Figure 3.2. The distillate was observed for every 30 minutes for 6 hours. If there were particulates or oil droplets, there was the oil extract. After 6 hours, the distillate was decanted and prepared for water separation and GC-MS analysis. The system was operated at a fixed power 500W and under atmospheric pressure (Ranitha et al., 2014).

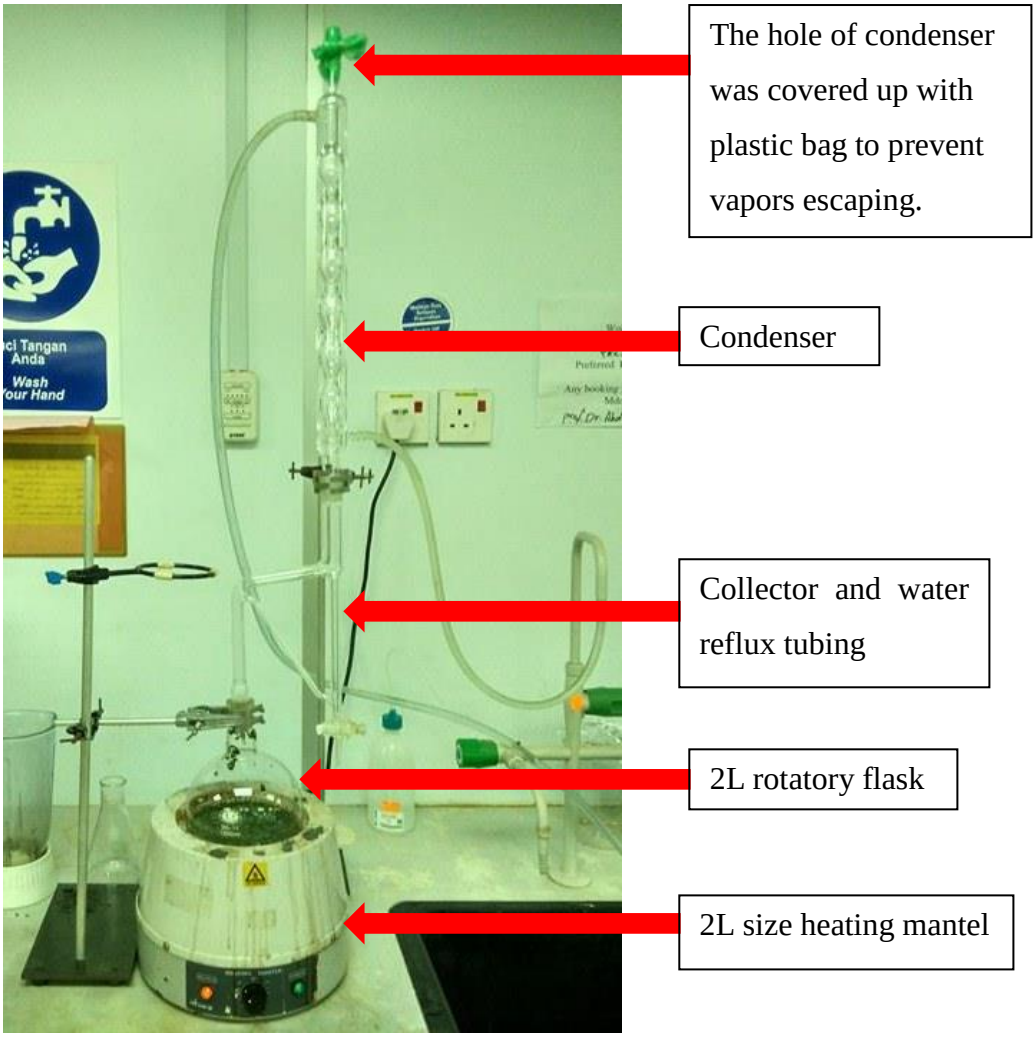

Figure 3.2: Clevenger apparatus set up

To evaluate the best water to raw material ratio and extraction time, the experiment was repeated by fixing the duration time (2 hours, 4 hours and 6 hours) but using 400ml (8:1 v/w) and 300ml (6:1 v/w) of distilled water. For instances, as shown in Figure 3.3, every water to raw material ratio, three extraction time were used to get three different samples. Therefore, there were totally 9 samples of extracted essential oil.

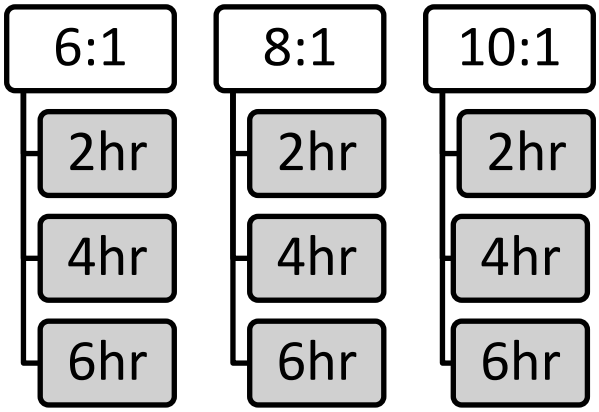

Figure 3.3: Three water to raw material ratios with three extraction time for HD.

3.3 Microwave-Assisted Hydrodistillation

A domestic microwave oven (Samsung GE71M, 1150 Watt power consumption, 750 Watt output power with 240V-50Hz power source; 2450MHz) was modified and connected to the Clevenger apparatus for MAHD operation. The cavity dimension of the microwave is 330 x 211 x 309 mm. Fifty grams of dried *S. Crispus* leaves was placed in a 1L rotatory flask containing 500ml of distilled water. The flask was set up within the microwave oven cavity and a condenser was used on the top (outside the oven) to collect the extracted essential oils. The apparatus was set up as shown in Figure 3.4. The essential oil was decanted from the condenser after 90 minutes extraction time. The decanted essential oil was prepared for further separation and analysis (Ranitha et al., 2014; Jeyaratnam et al., 2016).

To evaluate the best condition for MAHD, the experiment were repeated by using different water to raw material ratio (6:1, 8:1, 10:1 v/w), different power supply (180W, 300W, 450W) and different extraction time (30min, 60min, 90min) were used. As shown

in Figure 3.5, Figure 3.6 and Figure 3.7, every water to raw material ratio, the experiment was repeated by replacing three different power supply and three different extraction time. There were totally 27 samples of decanted essential oil for further analysis.

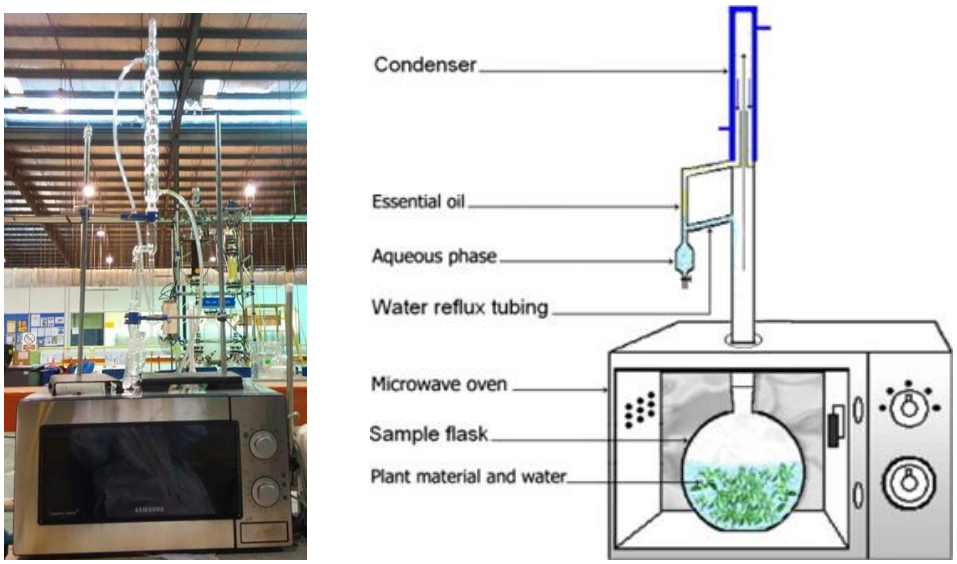

Figure 3.4: Microwave-assisted hydrodistillation apparatus (Golmakani, & Rezaei, 2008)

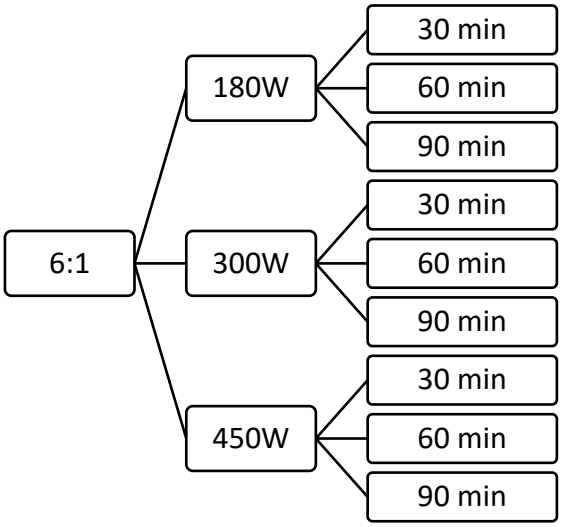

Figure 3.5: Water to raw material ratio (6:1) with different power supply and different extraction for MAHD.

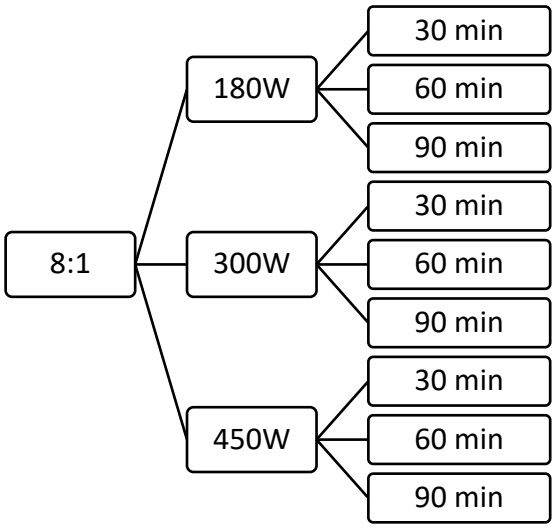

Figure 3.6: Water to raw material ratio (8:1) with different power supply and different extraction for MAHD.

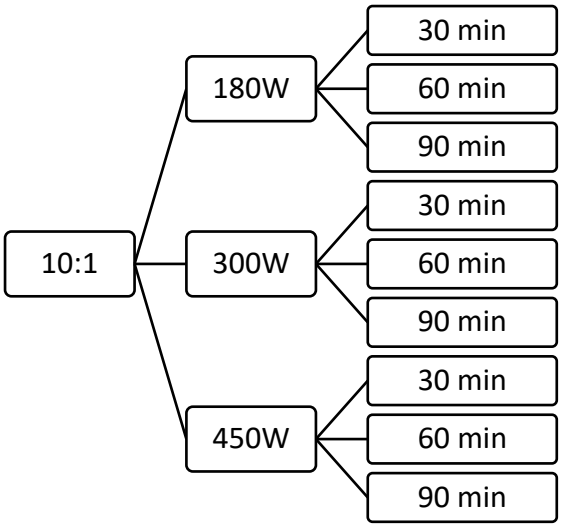

Figure 3.7: Water to raw material ratio (10:1) with different power supply and different extraction for MAHD.

3.4 Yield Analysis

The decanted essential oil was separated by using GC-MS grade hexane. After separation, the separated oil was dried by using anhydrous sodium sulphate (Asmah, et al., 2006). Hexane was evaporated by using rotary evaporator. Evaporation flask was weighed and the weight was recorded. After evaporation, the evaporation flask with

essential oil was weighed again. Then, the essential oils was dissolved in hexane and stored in vial at 4°C prior to analysis. The amount of yield obtained from the extraction was analysed to evaluate the performance of MAHD in *S. Crispus* oil extraction. According to Ranitha et al. (2014), yield of the oil obtained for every run was calculated by using below equation:

$$Weight\ of\ essential\ oil = Weight\ with\ flask - weight\ of\ rotary\ flask \quad \textbf{(3.1)}$$

$$Yield\ of\ essential\ oil = \frac{amount\ of\ essential\ oil\ (g)\ obtained}{amount\ of\ raw\ materials\ (g)\ used}\ x\ 100\% \quad \textbf{(3.2)}$$

3.5 Chemical Composition Analysis

A GC-MS instrument (5973N, Agilent Technologies, Wilmington, DE, USA) equipped with a mass selective detector operating in the electron impact mode (70eV) was used to study the composition of the essential oil at various parameter condition to analyse its quality. The GC part (6890N, Agilent Technologies, Palo Alto, CA, USA) is equipped with an HP-5MS (Agilent Btechnologies) capillary column (30m long, 0.25mm id and 0.25mm film thickness). Temperature-programming of the oven included an initial hold at 50°C for 5 min and a rise to 220°C at 3°C min^{-1} followed by additional rise to 300°C at 5°C min^{-1} hold for another 15 minutes. A post-run of 5 minutes at 300°C was sufficient for the next sample injection. Mass analyser was used in full scan mode scanning from m/z 40-550 and mass spectra were taken at 70 eV. The samples were diluted with n-hexane (1/10, v/v) and a volume of 1.0 µl was injected to the GC with the injector in the split mode (split ratio: 1/10). Carrier gas, He, was adjusted to a linear velocity of 1 ml min^{-1} employed in a splitless mode with injector temperature 250°C and ion source 280°C. For the compound identification, manual spectral matching was ascertained by using the mass spectral library of National Institute Standard and Technology (NIST) library data from GC-MS, database (Wiley/NBS library) or with published mass spectra (Asmah et. al., 2006). The percentage of each constituent was calculated using the normalization technique, based on the area of each peak from a total area peak value estimated to be 100%.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Result for Yield Analysis

Yield of essential oil for *Strobilanthes Crispus* was calculated by using equations as shown below.

$$Weight\ of\ essential\ oil = Weight\ with\ flask - weight\ of\ rotary\ flask \quad \textbf{(3.1)}$$

$$Yield\ of\ essential\ oil = \frac{amount\ of\ essential\ oil\ (g)\ obtained}{amount\ of\ raw\ materials\ (g)\ used}\ x\ 100\% \quad (\mathbf{3.2})$$

4.1.1 Yield Analysis for HD

Yield analysis for HD are shown from Table 4.1 to Table 4.3.

Table 4.1: Yield analysis for 2 hours HD in different water to leaves ratio.

Time	2 hours (120 minutes)			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7113	159.7768	0.0655	0.0655
8 : 1	159.7103	159.781	0.0707	0.0707
10 : 1	159.7078	159.7845	0.0767	0.0767

Table 4.2: Yield analysis for 4 hours HD in different water to leaves ratio.

Time	4 hours (240 minutes)			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7133	159.8677	0.1544	0.1544
8 : 1	159.7115	159.8767	0.1652	0.1652
10 : 1	159.7124	159.8849	0.1725	0.1725

Table 4.3: Yield analysis for 6 hours HD in different water to leaves ratio.

Time	6 hours (360 minutes)			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7089	159.8791	0.1702	0.1702
8 : 1	159.7134	159.8913	0.1779	0.1779
10 : 1	159.7126	159.9012	0.1886	0.1886

4.1.2 Yield Analysis for MAHD

Yield analysis for HD are shown from Table 4.4 to Table 4.12.

Table 4.4: Yield analysis for 30 minutes and 180W MAHD in different water to leaves ratio.

Power (W)	180			
Time (min)	30			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7093	159.7502	0.0409	0.0818
8 : 1	159.7123	159.7608	0.0485	0.0970
10 : 1	159.7112	159.7621	0.0509	0.1018

Table 4.5: Yield analysis for 60 minutes and 180W MAHD in different water to leaves ratio.

Power (W)	180			
Time (min)	60			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7202	159.7990	0.0788	0.1576
8 : 1	159.7143	159.7982	0.0839	0.1678
10 : 1	189.7122	189.8002	0.0880	0.1760

Table 4.6: Yield analysis for 90 minutes and 180W MAHD in different water to leaves ratio.

Power (W)	180			
Time (min)	90			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7201	159.8025	0.0824	0.1648
8 : 1	159.7153	159.8046	0.0893	0.1786
10 : 1	159.7093	159.8012	0.0919	0.1838

Table 4.7: Yield analysis for 30 minutes and 300W MAHD in different water to leaves ratio.

Power (W)	300			
Time (min)	30			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7156	159.7614	0.0458	0.0916
8 : 1	159.7145	159.7675	0.0530	0.1060
10 : 1	189.7206	189.7756	0.0550	0.1100

Table 4.8: Yield analysis for 60 minutes and 300W MAHD in different water to leaves ratio.

Power (W)	300			
Time (min)	60			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7202	159.7990	0.0788	0.1576
8 : 1	159.7143	159.7982	0.0839	0.1678
10 : 1	189.7122	189.8002	0.0880	0.1760

Table 4.9: Yield analysis for 90 minutes and 300W MAHD in different water to leaves ratio.

Power (W)	300			
Time (min)	90			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7201	159.8025	0.0824	0.1648
8 : 1	159.7153	159.8046	0.0893	0.1786
10 : 1	159.7093	159.8012	0.0919	0.1838

Table 4.10: Yield analysis for 30 minutes and 450W MAHD in different water to leaves ratio.

Power (W)	450			
Time (min)	30			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7113	159.7614	0.0501	0.1002
8 : 1	159.7103	159.7655	0.0552	0.1104
10 : 1	159.7123	159.7709	0.0586	0.1172

Table 4.11: Yield analysis for 60 minutes and 450W MAHD in different water to leaves ratio.

Power (W)	450			
Time (min)	60			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7153	159.7978	0.0825	0.1650
8 : 1	159.7088	159.7949	0.0861	0.1722
10 : 1	159.7079	159.8021	0.0942	0.1884

Table 4.12: Yield analysis for 90 minutes and 450W MAHD in different water to leaves ratio.

Power (W)	450			
Time (min)	90			
Ratio (water:leaves)	**Weight of rotary flask (g)**	**Weight with essential oil (g)**	**Weight of essential oil (g)**	**Yield (%)**
6 : 1	159.7113	159.7974	0.0861	0.1722
8 : 1	159.7099	159.8011	0.0912	0.1824
10 : 1	159.7203	159.8211	0.1008	0.2016

4.1.3 Induction Time of HD and MAHD

Induction time used of HD was approximately 22 minutes while the induction time for MAHD were 12 minutes, 8 minutes and 5 minutes for microwave power 180W, 300W and 450W respectively.

4.2 GC-MS Result

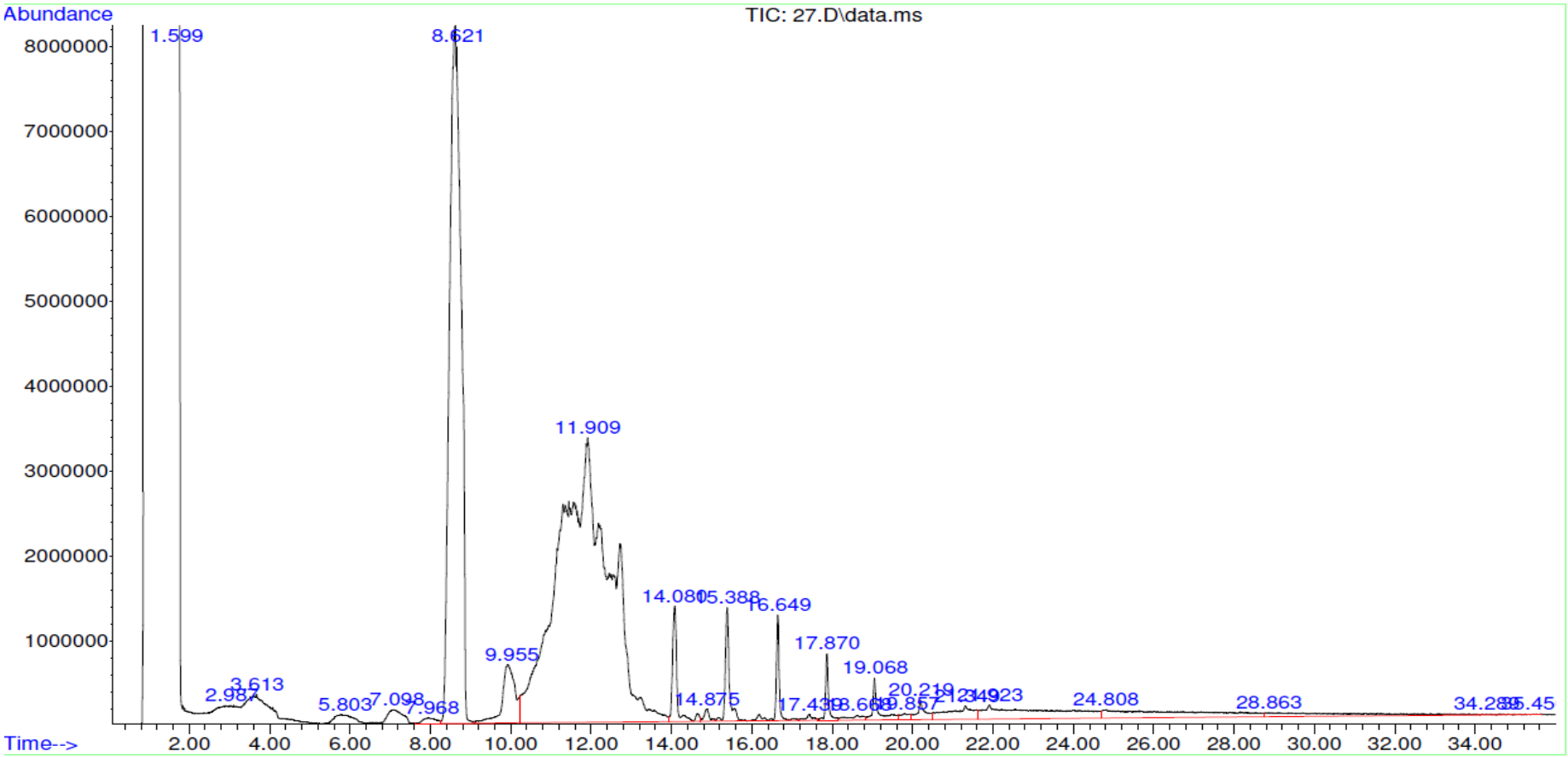

Figure 4.1: Chromatograph of *S. Crispus* Oil for 90 minutes MAHD by using 450W microwave power with 10:1 ratio.

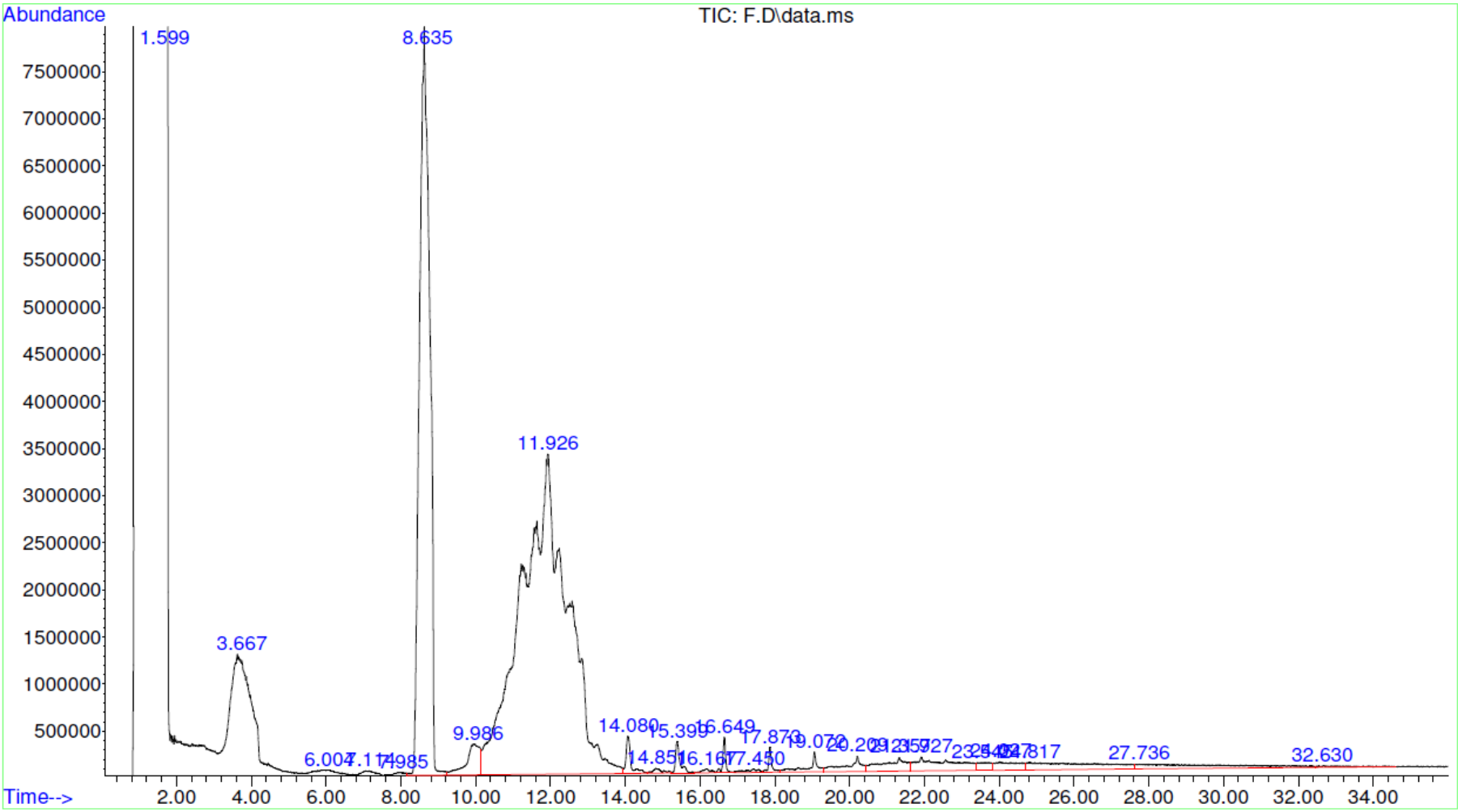

Figure 4.2: Chromatograph of *S. Crispus* Oil for 360 minutes HD with 10:1 ratio.

4.2.1 Chemical Constituents of *S. Crispus* Oil

The detailed GC-MS analysis reports are attached in Appendix A. Sample of *S. Crispus* oil for 90 minutes MAHD by using 450W microwave power with 10:1 ratio was denoted as sample 27 whereas sample of *S. Crispus* oil for 360 minutes HD with 10:1 ratio was denoted as sample F. Blank was denoted as standard 1 in the analysis report. The compounds which overlapped with pure hexane (blank) were cancelled off as *S. Crispus* oil compounds. Table 4.13 shows compounds in pure hexane. Table 4.14 shows compounds in *S. Crispus* oil for 90 minutes MAHD by using 450W microwave power with 10:1 ratio. Table 4.15 shows compounds in *S. Crispus* oil for 360 minutes HD with 10:1 ratio.

Table 4.13: Compounds in pure hexane (Blank).

No.	Retention Time (min)	Area (%)	Compounds
1.	1.604	97.87	Hexane
2.	3.986	0	1,1,3,3-Tetraallyl-1,3-disilacyclobutane
3.	6.945	0	Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl-
4.	21.889	1.1	Cyclotrisiloxane, hexamethyl
5.	23.93	0.76	Tetrasiloxane, decamethyl-
6.	29.73	0.07	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-
7.	30.638	0.07	Cyclotrisiloxane, hexamethyl
8.	31.61	0.04	5-Methyl-2-phenylindolizine
9.	32.411	0.01	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene
10.	32.657	0.02	Cyclotrisiloxane, hexamethyl
11.	33.148	0.02	Cyclotrisiloxane, hexamethyl
12.	33.639	0.02	5-Methyl-2-phenylindolizine
13.	34.644	0.01	Cyclotrisiloxane, hexamethyl
14.	35.52	0.01	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-

Table 4.14: Compounds in *S. Crispus* oil for 90 minutes MAHD by using 450W microwave power with 10:1 ratio.

No.	Retention Time (min)	Area (%)	Compounds	Relative Peak Area (%)
1.	2.982	0.4	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	2.04
2.	3.613	0.59	Phytol	3.01
3.	5.802	0.14	Pentadecane	0.72
4.	7.095	0.16	Tetracosane	0.82
5.	7.971	0.05	1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester	0.26
6.	8.622	5.61	1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester	28.67
7.	9.958	0.53	Heptadecane	2.71
8.	11.912	9.91	Phthalic acid, bis(7-methyloctyl) ester	50.64
9.	14.081	0.35	Nonacosane	1.79
10.	14.871	0.04	Ethanone, 1-(4-bromophenyl)-, (2E)-[1-(4-phenoxyphenyl)ethylidene]hydrazone, (1E)-	0.20
11.	15.384	0.32	Triacontane	1.64
12.	16.645	0.23	Hexadecane, 1-iodo-	1.18
13.	17.435	0.03	5,5'-Di(ethoxycarbonyl)-3,3'-dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethane	0.15
14.	17.873	0.14	Eicosane	0.72
15.	18.663	0.05	Benzo[h]quinoline,2,4-dimethyl-	0.26
16.	19.069	0.15	Eicosane	0.77
17.	19.86	0.04	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane	0.20
18.	21.345	0.22	4-Methyl-2-trimethylsilyloxy-acetophenone	1.12
19.	21.921	0.6	4-Methyl-2-trimethylsilyloxy-acetophenone	3.07
20.	35.456	0.01	Benzo[h]quinoline,2,4-dimethyl-	0.05

Table 4.15: Compounds in *S. Crispus* oil for 360 minutes HD with 10:1 ratio.

No.	Retention Time (min)	Area (%)	Compounds	Relative Peak Area (%)
1.	3.666	1.95	Phytol	10.71
2.	6.005	0.11	Octane, 5-ethyl-2-methyl-Heneiocsane	0.60
3.	7.116	0.05	Eicosane	0.27
4.	7.981	0.02	9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2-7-bis-[2-(diethylamini)-ethoxyl]fluorene	0.11
5.	8.633	5.09	1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester	27.95
6.	9.989	0.28	Phthalic acid, bis(7-methyloctyl) ester	1.54
7.	11.923	9.7	1,2-Benzenedicarboxylic acid, decyl hexyl ester	53.27
8.	14.081	0.12	Nonacosane	0.66
9.	14.85	0.03	1' H-Androst-16-eno[16,17-b]indol-3-ol, 1'-methyl-, (3.beta.,5.alpha.)-	0.16
10.	15.395	0.1	Eicosane	0.55
11.	16.164	0.02	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	0.11
12.	16.644	0.07	Eicosane	0.38
13.	17.445	0.03	Silane,trimethyl[5-methyl-2-(1-methylethyl)phenoxyl]-	0.16
14.	17.873	0.05	Octacosane	0.27
15.	19.069	0.1	13-Methylhentriacontane	0.55
16.	23.545	0.07	4-Methyl-2-trimethylsilyloxy-acetophenone	0.38
17.	24.026	0.13	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	0.71
18.	27.732	0.29	1H-Indole, 1-methyl-2-phenyl-1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	1.59

4.3 Observation of Essential Oil

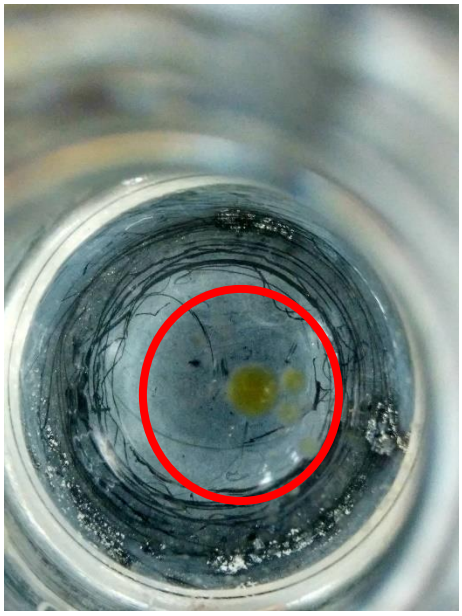

Figure 4.3: Yellow *S. Crispus* oil from HD

Figure 4.4: Pale yellow *S. Crispus* oil from MAHD.

The difference in colour may be caused by the different constituents in *S. Crispus* oil. However, difference in colour does not affect the biological activities of essential oil as long as the quantity and quality of essential oil were determined.

4.4 Effect of Water to Raw Material Ratio on Yield

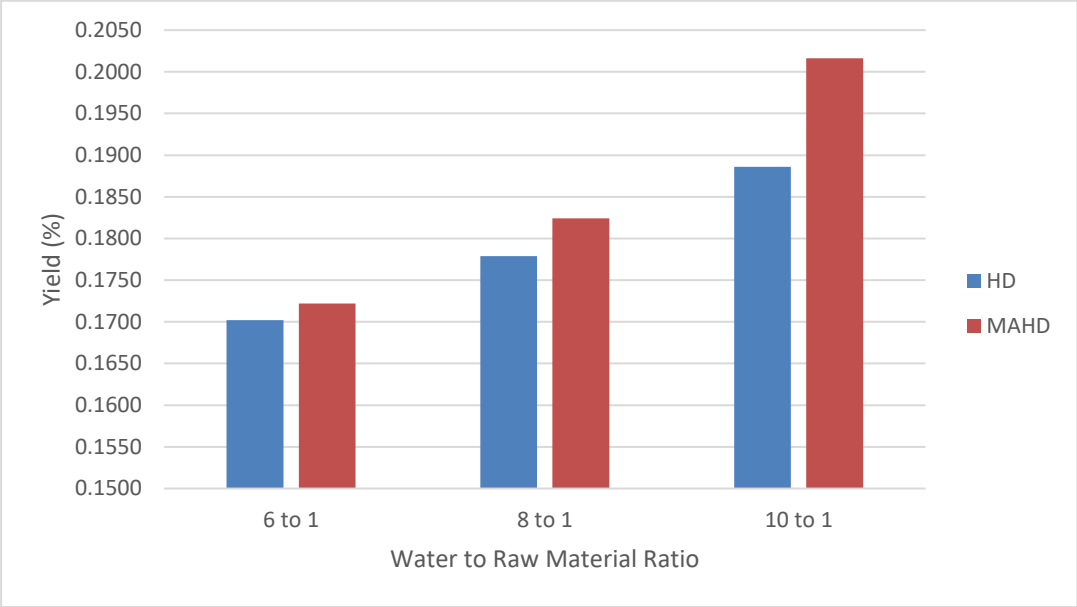

Figure 4.5: Yield versus water to raw material ratio for 360 minutes HD and 90 minutes MAHD of 450W.

Figure 4.5 shows the effect of different water to raw material ratio on yield of *S. Crispus* oil at constant extraction time of 90 minutes and constant microwave power 450W for MAHD, and the corresponding yield from HD using 360 minutes extraction time at different water to raw material ratio. The mass of *S. Crispus* leaves used in HD and MAHD were 100g and 50g respectively. The highest yield obtained at ratio of 10:1 followed by 8:1 and 6:1. The yield extracted from Strobilanthes *Crispus* at 6:1, 8:1 and 10:1 were 0.1722%, 0.1824% and 0.2016% respectively for MAHD. On the other hand, the yield for HD were 0.1702%, 0.1779% and 0.1886% respectively. Therefore, it can be concluded that the yield from *S. Crispus* increased with the amount of water as solvent.

According to Mandal et al. (2007), a higher ratio of solvent volume to solid matrix (raw material) may be effective in conventional extraction methods. However, in microwave assisted extraction, a higher ratio yield lower recoveries. This is because inadequate stirring of the solvent may be caused by the microwave (Mandal, et al., 2007). Extraction with high liquid/solid ratio, more energy and time were used to condense the extraction solution and purification process (Mandal, et al., 2007). However, there were

study showing 10:1 (ml/mg) to 20:1 (ml/mg) found to be optimum in many applications (Li et.al. 2004; Pan et. al., 2001, 2003; Talebi et. al., 2004).

Basically, the optimum yield for MAHD and HD was obtained at water to raw material ratio of 10:1. However, MAHD used 90 minutes to obtain 0.2016% yield while HD consumed 360 minutes to reach 0.1886% yield. This suggests that MAHD requires less energy and time to produce high yield of essential oil compared to HD. Thus, the water to raw material ratio of 10:1 was used as the criteria for further analysis.

4.5 Effect of Microwave Power on Yield

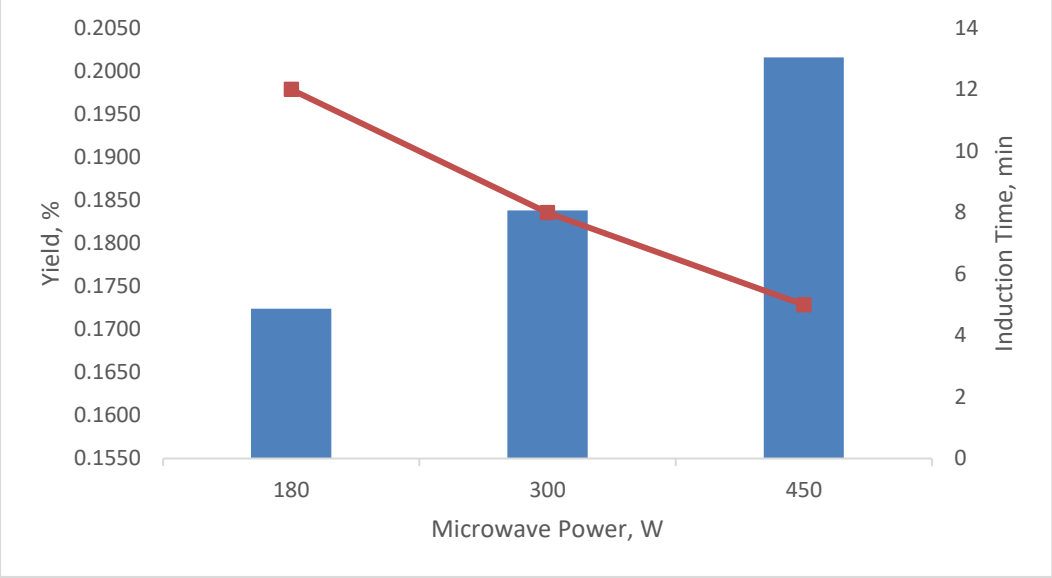

Figure 4.6: Yield and induction time versus microwave power for 90 minutes MAHD with 10 to 1 water to raw material ratio.

Figure 4.6 illustrates the effect of microwave power on extraction yield and induction time of MAHD at a fixed water to raw material ratio of 10 to 1 as well as at a fixed extraction time 90 minutes. From Figure 4.6, the yield of extracted *S. Crispus* increased with an increasing microwave power level. The yield of oil at 180W, 300W and 450W were 0.1724%, 0.1838% and 0.2016% respectively. Similar trend was reported in a previous research work by Hu et. al. (2008). The study showed that increasing microwave power from 200 to 1000W, the extraction yield of flavonoid from Radix Astragali was increased.

However, high microwave power might increase the temperature overly high, and decrease the extraction yield. Overly high temperature can damage product or cause compound breakdown (Ma et al., 2009). At higher power, rapid rupture of cell wall occur causing the desired compound or undesired impurities leaching out into the solvent (Mandal, et al., 2007). On the other hand, cell wall rupture occur slowly at lower power level. This enables selective microwave selective extraction (Mandal, et al., 2007).

Besides, induction time was reached faster for higher microwave power level. Induction time is the time consumed for a mixture to reach its boiling point. At constant water to raw material ratio of 10:1 and at a duration of 90 minutes, the induction time for 180W, 300W and 450W were 12 minutes, 8 minutes and 5 minutes respectively. In the case of HD, the induction time was 22 minutes at same condition. This means that MAHD use shorter time to reach the boiling point, as well as began to produce the essential oil earlier.

This is because microwave heating occurs in a closed system rotation, no heat being lost to the surrounding environment (Mandal et al., 2007). In addition, water, which is a polar solvent, it possesses high dielectric constant (Mandal et al., 2007). With high dielectric constant, water can efficiently convert microwave energy into heat. Therefore, induction time was shorter by using MAHD. Longer induction time happened in lower microwave power because lower density waves produced at lower power level. On the other hand, for HD, the heat is supplied particularly to the bottom of the distillation flask and the heat localized at one fixed point. Besides, HD also happened in an opened system (Jeyaratnam et. al., 2016), heat might be lost into the environment. Thus, HD takes longer time to reach induction time. Based on the result obtained, 450W was determined as the optimum microwave power for further analysis.

4.6 Effect of Extraction Time

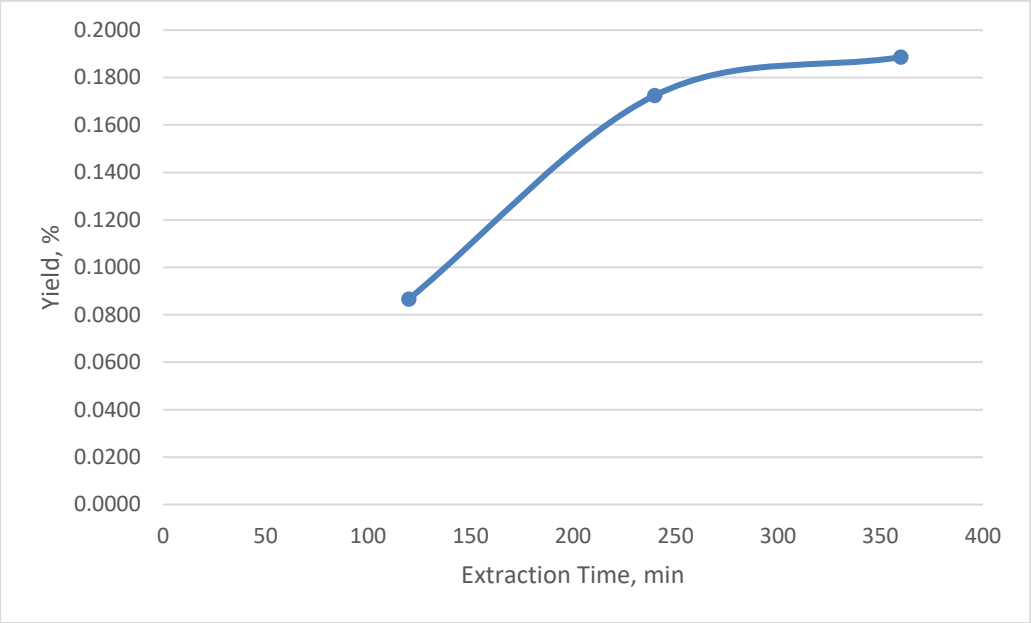

Figure 4.7: Yield analysis versus extraction time for HD at water to raw material ratio of 10:1.

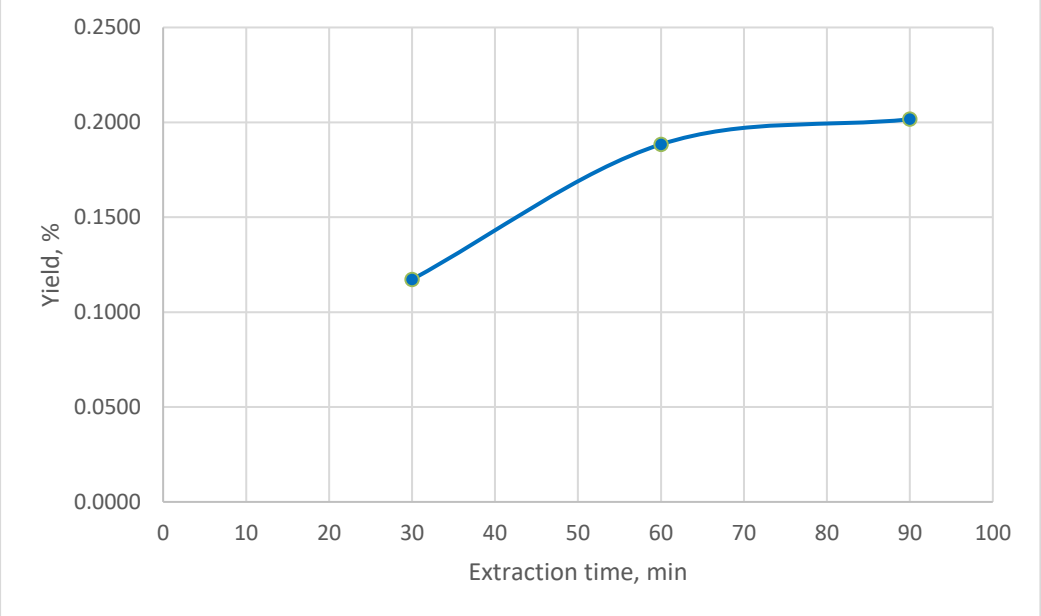

Figure 4.8: Yield analysis versus extraction time for MAHD at water to raw material ratio of 10:1.

Figure 4.7 illustrates the effect of extraction time on yield of *S. Crispus* essential oil at water to raw material ratio of 10:1 in HD. Figure 4.8 shows variation in extracted *S. Crispus* essential oil with extraction time at a constant microwave power of 450W, and at water to raw material ratio of 10:1 in MAHD. For HD, the yield of *S. Crispus* oil increased with increasing extraction time. At extraction time of 120 minutes, 240 minutes and 360 minutes, the extracted oil yields were 0.0867%, 0.1725% and 0.1886% respectively. The yield increased slowly when the extraction time extended beyond 240 minutes.

On the other hand, in the case of MAHD, an increasing trend can be observed for the *S. Crispus* oil yield as the extraction time was increased from 30 minutes to 90 minutes. The yield for extraction time of 30 minutes, 60 minutes and 90 minutes were 0.1172%, 0.1884% and 0.2016% respectively. From the result obtained, it is obvious that MAHD used shorter extraction time to reach the same yield as HD. The highest yield obtained from MAHD used 90 minutes whereas HD used 360 minutes.

Many of the reported studies showed the same trend, an increase of extraction time increases the extraction yield (Jeyaratnam et al., 2016; Wang et. al., 2010; Zheljazkov et. al., 2014). The rate of heating of water is quite high. This is because the high dielectric properties. Therefore, prolonged application of microwave may cause the degradation of the target compounds with overheating of the system (Routray, & Orsat, 2012).

From all the yield analysis, it can be noticed that the yield of *S. Crispus* were very low. According to Asmah et. al. (2006), the yield of *S. Crispus* oil was 0.43% (v/w) for 6 hours HD. The yield were among 0.0655% and 0.1886% for HD while the yield for MAHD were among 0.0818% and 0.2016%. This study showed that water as solvent used in HD and MAHD is hard to yield essential oil from *S. Crispus* leaves. This may be caused by essential oil losses occurred during water evaporation, and incomplete evaporation of the solvent during separation may inflate the essential oil yield (Charles, & Simon, 1990).

4.7 Quantity and Quality of *S. Crispus* Oil

At the best conditions as stated in the previous sections, *S. Crispus* oil from MAHD, were compared with *S. Crispus* oil from conventional method of HD in terms of quantity and quality of the oil. The yields of *S. Crispus* oil obtained at optimum conditions for the MAHD and HD were 0.1886% and 0.2016% respectively. MAHD obtained higher yield in shorter time compared to HD method. For qualitative analysis, the identified components in *S. Crispus* oil are shown in Table 4.16.

Table 4.16: Comparison of *S.Crispus* oil obtained by MAHD and HD.

Compounds	Relative Peak Area (%)	
	MAHD	HD
7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	2.04	-
Phytol	3.01	10.71
Pentadecane	0.72	-
Tetracosane	0.82	-
1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester	28.93	27.95
Heptadecane	2.71	-
Phthalic acid, bis(7-methyloctyl) ester	50.64	1.54
Nonacosane	1.79	0.66
Ethanone, 1-(4-bromophenyl)-, (2E)-[1-(4-phenoxyphenyl)ethylidene]hydrazone, (1E)-	0.20	-
Triacontane	1.64	-
Hexadecane, 1-iodo-	1.18	-
5,5'-Di(ethoxycarbonyl)-3,3'-dimethyl-4,4'-dipropyl-2,2'-dipyrrylmethane	0.15	-
Eicosane	1.49	1.20
Benzo[h]quinoline,2,4-dimethyl-	0.31	-
3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane	0.20	-
4-Methyl-2-trimethylsilyloxy-acetophenone	4.19	0.38
Octane, 5-ethyl-2-methyl-Heneicosane	-	0.6
9-(2',2'-Dimethylpropanoilhydrazono)-3,6-dichloro-2-7-bis-[2-(diethylamini)-ethoxyl]fluorene	-	0.11
1,2-Benzenedicarboxylic acid, decyl hexyl ester	-	53.27
1' H-Androst-16-eno[16,17-b]indol-3-ol, 1'-methyl-, (3.beta.,5.alpha.)-	-	0.16
1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	-	0.11

Silane,trimethyl[5-methyl-2-(1-methylethyl)phenoxyl]-	-	0.16
Octacosane	-	0.27
13-Methylhentriacontane	-	0.55
2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	-	0.71
1H-Indole, 1-methyl-2-phenyl-1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	-	1.59

From Table 4.16, it shows that 16 compounds were found for MAHD and HD. However, among those 16 compounds, there are 6 common compounds present in MAHD and HD *S.Crispus* oil, which were phytol, 1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester, phthalic acid, bis(7-methyloctyl) ester, nonacosane, eicosane and 4-Methyl-2-trimethylsilyloxy-acetophenone. The constituent of *S.Crispus* oil extracted by HD and MAHD were different. The highest compound found in *S.Crispus* oil were 1,2-Benzenedicarboxylic acid, decyl hexyl ester for HD and Phthalic acid, bis(7-methyloctyl) ester for MAHD, which are 53.27% and 50.64% respectively. Similar situation was reported in a previous research works by Wang et. al. (2012) and Mohammadhossein et. al. (2013). In both of the research, the major components of oil for HD and MAHD were different. In work of Mohammadhossein et. al. (2013), the major constituent of HD oil is borneol whereas the major compound of MAHD oil is yomogi alcohol.

In addition, *S.Crispus* oil from MAHD contain more n-alkanes compared to *S.Crispus* oil from HD. *S.Crispus* oil from MAHD contain 9.17% of n-alkanes whereas *S.Crispus* oil from HD contain 1.53% of n-alkanes. The n-alkanes in *S.Crispus* oil from MAHD are pentadecane, tetracosane, heptadecane, nonacosane, triacontane and eicosane while *S.Crispus* oil from HD consists of nonacosane, eicosane and Octane, 5-ethyl-2-methyl-Heneicosane.

The variability in chemical constituent of *S.Crispus* oil from MAHD and HD may be due to difference power level used in HD and MAHD. At higher power, rapid rupture of cell wall occurs at higher temperature causing compounds leaching out into the solvent (Mandal, et al., 2007). On the other hand, high microwave power might increase the product temperature overly high causing product damage or compound breakdown (Mandal, et al., 2007). Besides that, the extraction time of HD was longer than MAHD, which are 6 hours and 90 minutes. According Mandal et al. (2007), solvents like water,

ethanol, and methanol may heat up tremendously on longer exposure thus risking the future of thermolabile constituents. In addition, the loss of some compounds in MAHD may be caused by the reduction in extraction time. This reduces the degradation of compounds by hydrolysis, transesterification or oxidation (Lucchesi et al., 2004).

According to Asmah et. al. (2006), phytol is the key constituent to evaluate the quality of *S.Crispus* oil. However, in this study, the major compound were 1,2-Benzenedicarboxylic acid, decyl hexyl ester for HD and Phthalic acid, bis(7-methyloctyl) ester for MAHD, whereas phytol is the minor compound. This variability may be caused by different climatic, geographical and soil growing conditions (Ghasemzadeh et al., 2015; Jamshidi et al., 2009). Therefore, for different use of *S.Crispus* oil, different geographic for grown is necessary. Plant growing condition may contribute to this variability. 2-Benzenedicarboxylic acid, decyl hexyl ester for HD and Phthalic acid, bis(7-methyloctyl) ester are plasticizer compound (Lowell Center for Sustainable Production, 2011). These two compounds may appear in plant chemical constituent by absorbing the compounds from the contaminated air or polluted soil and water (Manayi, & Saeidnia, 2015; Wu, et al., 2013). According to Manayi & Saeidnia (2015), accumulation of phthalates may occur in medicinal plants which grow near the water and rivers.

4.8 Biological Activities of *S. Crispus* Oil

The highest compound found in *S. Crispus* oil was phthalic acid, bis(7-methyloctyl) ester for MAHD. According to Saeidnia & Abdollahi (2013), phthalic acid ester is known as phthalates, which is a group of plasticizers widely used with, for example polyvinyl chloride (PVC). Phthalates are synthetic chemicals with ubiquitous human exposures because of their wide use in plastics. Since phthalates are not in chemical binding to polymers, they can easily leach out to the environment. However, in the study of Ibrahim Husein et al (2014), high accumulation of phthalates in *A.palaestinum* leaves was discovered. In addition, some medicinal plants of the genus Phyllanthus produce phthalates, which are bis(2-ethyloctyl) phthalate and bis(2-ethylicosyl) phthalate. These two phthalates exhibited antimicrobial activities. The phthalates found in *S.Crispus* oil was bis(7-methyloctyl) ester phthalates. As reported by

Vennila & Udayakumar (2015), phthalic acid, bis(7-methyloctyl) ester exhibited antimicrobial and antifouling activity. Thus, by this statement, *S. Crispus* oil by MAHD has antimicrobial and antifouling biological activities.

On the other hand, the major compound found in *S. Crispus* oil from HD was 1,2-Benzenedicarboxylic acid, decyl hexyl ester. According to PubChem Database, 1,2-Benzenedicarboxylic acid, decyl hexyl ester is known as decyl hexyl phthalate. It is also a kind of plasticizer compound. However, this compound never appear in any plant composition report. Therefore, the function and biological activity are unknown. In addition, accumulation of phthalate can occur in medicinal plants that grows in water flow in rivers and canals (Manayi, & Saeidnia, 2015). In such situation, wastewater might be the origin of pollution and phthalate exposure to these plants.

Furthermore, 1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester showed high percentage in both *S. Crispus* oil of MAHD and HD, which were 28.93% and 27.95% respectively. This bioactive compound which isolated from *Streptomyces* sp. exhibited cytotoxic activity against cancer cell and low toxicity against normal cell lines (Krishnan, et al., 2014). In another word, *S. Crispus* oil exhibit cytotoxic activity against cancer cell as *S. Crispus* oil contains 1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl)ester as second major constituent.

In conclusion, *S. Crispus* oil from MAHD exhibits antimicrobial, antifouling and anticancer biological activities, whereas *S. Crispus* oil from HD exhibits anticancer biological activity. However, further research is needed to isolate, identify, characterize and elucidate the structure of these bioactive compounds responsible for medicinal values of *S. Crispus* oil.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research focused on effect of water to raw material ratio, extraction time and microwave power in comparison with conventional method, hydrodistillation. The yield of extracted *S. Crispus* essential oil and its constituents in the *S. Crispus* oil were evaluated and compared for the both methods. The optimum conditions for MAHD obtained were 450W, 90 min of extraction and 10:1 of water to raw material ratio. The optimum yield of *S. Crispus* oil obtained at the optimum condition was 0.2016%. On the other hand, the optimum condition for HD were 360 minutes of extraction and 10:1 of water to raw material ratio. The yield of *S. Crispus* oil was 0.1886%.

In addition, the biological activities of the constituents were studied by reviewing previous studies. From this current research, *S. Crispus* oil from MAHD exhibits antimicrobial, antifouling and anticancer biological activities, whereas *S. Crispus* oil from HD exhibits anticancer biological activity only.

This indicates that advance technology, MAHD has better performance compared with conventional method, HD. MAHD which had been reported for its superiority of rapid essential oil production, had also been proven herein as result shown that MAHD required a shorter extraction time to obtain higher yield of essential oil compared to conventional method.

But, the chemical constituents present in the *S. Crispus* oil by both method were different in the quantity and quality of active compounds. Phthalic acid, bis(7-methyloctyl) ester was the major constituent of *S. Crispus* oil from MAHD, whereas 1,2-

Benzenedicarboxylic acid, decyl hexyl ester was the major constituents of *S. Crispus* oil from HD. It is worthy to note that *S. Crispus* oil from MAHD and HD consists of different chemical constituents. Therefore, for different uses of *S. Crispus* oil, different extraction method should be used based on the oil purpose.

Thus, microwave assisted hydrodistillation possesses highly desirable features compared to the conventional method. Some of these features includes time saving during extraction of essential oil, indirectly leading to reduced cost and energy. Besides that, there is possibility for obtaining better quality and quantity of *S. Crispus* oil by using MAHD method. In conclusion, MAHD is suggested as an alternative convenient, effective and efficient method for extracting essential oil both at laboratory and industrial scale production.

5.2 Recommendation

In order to improve the research results, it is recommended that the apparatus used for MAHD and HD should be cleaned with distilled water to remove any impurity residues. Besides, raw material used should be increased in order to obtain a suitable yield for chemical constituents analysis. Low yield of oil essential oil will cause analysis more inaccurate because little impurity also can be counted as the compound of essential oil and increase the weight of oil. Fresh dried material is suggested. Raw material should be stored in refrigerator to maintain the freshness. For every separation of essential oil, the apparatus should be washed thoroughly and dried by using oven to remove moisture in the apparatus. When using microwave oven, the modified hole should be covered with aluminum foil in order to minimize radiation and escaping heat. Finally, solvent used to separate essential oil should be in GC-MS grade to minimize impurities appearing in the essential oil. The yield obtained from this research is too low. Therefore, it is suggested to change other extraction methods, such as vacuum distillation, supercritical carbon dioxide extraction and organic solvent extraction.

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APPENDIX

Figure A.1: Samples of *S. Crispus* oil

Figure A.2: Samples of *S. Crispus* oil.

Figure A.3: Samples of *S. Crispus* oil.

Figure A.4: Samples of *S. Crispus* oil.