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# A COMPARATIVE STUDY OF *CINNAMOMUM CASSIA* (CINNAMON) BARK OIL EXTRACTION THROUGH HYDRODISTILLATION (HD) AND MICROWAVE ASSISTED HYDRODISTILLATION (MAHD)

NITTHIYAH A/P JEYARATNAM

Thesis submitted in fulfilment of the requirements
for the award of the degree of
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Dedicated

to the Glory of

The Almighty God

and to

My lovely parents.

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The Author.

# ABSTRACT

The use of natural origin essential oils has recently gained much attention in many fields such as food flavouring, pesticides and in pharmaceutical industries. However, limitations to the use of existing conventional extraction methods are the inherent time consumption, high cost, high power consumption, potential loss of volatile compounds from essential oils as well as environmental concerns. Therefore new innovative extraction techniques are required to overcome these limitations. In this research, essential oil was extracted from *Cinnamomum cassia* bark using conventional hydrodistialltion (HD) and non-conventional microwave assisted hydrodistillation (MAHD) methods. The influence of pre-treatment on the *C. cassia* bark prior to extraction was investigated. Also, changes in morphology of the cinnamon bark powder before and after extraction by the two methods was observed through SEM. Milder disruption of cinnamon bark oil gland was observed for MAHD compared to HD. This is associated with the effective heat distribution obtainable from MAHD. The efficiency of MAHD extraction technique was compared with HD in terms of chemical composition and biological activity of the oil obtained as well as cost implication of the extraction process. To justify the performance of MAHD technique, the three main influencing factors such as solvent-to-plant material ratio, extraction time and irradiation microwave power were analysed. Analysis of these factors was initially carried out using one factor at a time (OFAT) method. Furthermore, screening and optimization of the factors was conducted with the help of Design expert software via factorial analysis and central composite design (CCD) respectively. The optimum conditions obtained through CCD for MAHD is 75 min extraction time, 8:1 of water to raw material ratio and 225 W irradiation power. The maximum yield obtained is 2.75 %. On the other hand, the extraction parameters for HD are 150 min extraction time, 8:1 of water to raw material ratio and a constant operating power of 350 W. The maximum yield obtained for HD is 2.02 %. The essential oil obtained at the optimum operating conditions for both methods was subjected to further qualitative analysis. Compositional analysis was conducted through gas chromatography-mass spectrometry (GC-MS). A total of 36 compounds were found for both extraction methods which were supported by FTIR analysis. However, the active compound present in *C. cassia* bark oil (trans-cinnamaldehyde) manifested a mean value of 84.43 ± 0.70 and 79.55 ± 0.45 for MAHD and HD respectively. Furthermore, cassia crude extract obtained through MAHD exhibits larger proportion of oxygenated compounds (9 % higher) than HD method. Energy consumption analysis shows that MAHD is more energy saving and environmental friendly. It reduced the overall carbon dioxide emission from HD by 59 %. In addition, cytotoxicity studies revealed that essential oil obtained through MAHD showed lower $LC_{50}$ value (51.21 ppm) than HD (68.88 ppm). This indicates that the MAHD technique is suitable for obtaining volatile oils from cinnamon cassia bark and the oil obtained can offer great pharmaceutical benefits.

# ABSTRAK

Penggunaan minyak pati telah mendapat perhatian yang luas dalam pelbagai industri seperti perisa makanan, racun perosak dan farmaseutikal. Walau bagaimanapun, kaedah pengekstrakan secara konvensional mempunyai kelemahan seperti masa pengekstrakan yang panjang, kos yang tinggi, penggunaan tenaga yang tinggi, kemungkinan pemeruapan sebatian yang penting serta berpotensi memberi kesan samping kepada alam sekitar. Ciri-ciri sedemikian membuatkan kaedah tersebut tidak kompeten dengan keperluan industri kini. Oleh itu teknik pengekstrakan inovatif baru diperlukan untuk mengatasi batasan-batasan tersebut. Dalam kajian ini dua kaedah ekstrasi, konvensional dan berteknologi, telah dikaji untuk memperoleh minyak pati daripada kulit kayu *Cinnamomum cassia* iaitu melalui kaedah penyulingan hidro (HD) dan kaedah penyulingan hidro dengan bantuan gelombang mikro (MAHD). Pengaruh pra-rawatan terhadap kulit kayu *C. cassia* sebelum pengekstrakan telah disiasat. Selain itu, perubahan morfologi terhadap serbuk kulit kayu manis, sebelum dan selepas pengekstrakan melalui kedua-dua kaedah, telah analisis melalui Pengimbas Mikroskop Elektron (SEM). Gangguan yang lebih ringan terhadap kelenjar minyak kulit kayu manis dikenalpasti dengan menggunakan MAHD berbanding HD. Ini disebabkan oleh ciri-ciri MAHD yang membolehkan pengedaran tenaga haba yang berkesan. Seterusnya, keberkesanan teknik pengekstrakan MAHD telah dibandingkan dengan HD dari segi komposisi bahan kimia, aktiviti biologi serta, kos pengekstrakan minyak pati. Bagi menjustifikasikan prestasi teknik MAHD, tiga faktor utama yang mempengaruhi proses pengekstrakan seperti nisbah bahan air-kepada-bahan mentah, masa pengekstrakan dan kuasa penyinaran ketuhar gelombang mikro telah dianalisa dan interaksi kajian telah dinilai dengan menggunakan ujikaji satu-faktor-di-satu-masa (OFAT). Keputusan daripada eksperimen OFAT telah dianalisis dalam kajian pengoptimuman sistem MAHD menggunakan Kaedah Gerak Balas Permukaan ( RSM ) berdasarkan Reka Bentuk Komposit Berpusat ( CCD). Keadaan optimum yang diperolehi melalui CCD untuk MAHD ialah 75 min masa pengekstrakan, nisbah air dengan bahan mentah ialah 8:1 dan kuasa penyinaran adalah 225 W. Hasil minyak pati maksimum yang diperolehi adalah sebanyak 2.75 %. Manakala bagi parameter pengekstrakan untuk HD, 150 min masa pengekstrakan, nisbah bagi air dengan bahan mentah adalah 8:1 dan kuasa operasi tetap adalah 350 W, dikenalpasti sebagai konfigurasi optimum dan hasil minyak patinya adalah 2.02 %. Minyak pati yang diperolehi daripada keadaan operasi optimum kedua-dua kaedah telah digunakan untuk analisis kualitatif. Analisis komposisi telah dijalankan menggunakan gas kromotografi-jisim gas spektrometri (GC-MS). Sejumlah 36 bahan telah ditemui dalam minyak pati dari kedua-dua kaedah pengekstrakan yang mana disokong oleh analisis FTIR. Walaubagaimanapun, bahan aktif yang ditemui dalam minyak kulit kayu *C. cassia* (trans-cinnamaldehyde) dimanifestasikan nilai minima iaitu 84.43 ± 0.70 dan 79.55 ± 0.45 untuk MAHD dan HD masing-masing. Disamping itu, hasil ekstrak mentah *C. cassia* diperolehi melalui MAHD menunjukkan komposisi sebatian beroksida yang lebih tinggi (9 % tertinggi) berbanding kaedah HD. Analisis penggunaan tenaga membuktikan MAHD dapat menjimatkan tenaga dan lebih mesra alam sekitar. Ianya boleh menggurangkan pembebasan karbon dioksida dari HD sebanyak 59 %. Tambahan pula, kajian sitotoksik mendedahkan bahawa minyak pati yang diperoleh melalui MAHD mempunyai nilai $LC_{50}$ yang lebih rendah (51.21 ppm) berbanding HD (68.88 ppm). Ini menunjukkan bahawa teknik MAHD lebih bersesuaian bagi memperolehi minyak pati daripada kulit kayu manis *C. cassia* dan minyak pati tersebut berpotensi untuk memberi lebih banyak faedah farmaseutikal.

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# LIST OF SYMBOLS

| | |
|---|---|
| $\tan \delta$ | Dielectric loss tangent |
| $\varepsilon'$ | Dielectric constant |
| $\varepsilon''$ | Dielectric loss |
| $E$ | Electric field strength |
| $P$ | Microwave power distribution per volume unit |
| $f$ | Frequency |
| $q_{mw}$ | Volume of heat generation |
| $\rho$ | Density |
| $C_p$ | Heat capacity |
| $\Phi$ | Volume fraction |
| $T$ | Temperature ($^{0}$C) |
| $D_p$ | Penetration depth |
| $\lambda$ | Wavelength |
| µm | Micrometer |
| $+\alpha$ | Positive axial |
| $-\alpha$ | Negative axial |
| $n_i$ | Number of interactions |

# LIST OF ABBREVATIONS

| | |
|---|---|
| AACC | American Association of Cereal Chemists |
| ANOVA | Analysis of Variance |
| BBD | Box-Behken Design |
| BLT | Brine Shrimp Lethality Test |
| CAMD | Compressed Air Microwave Distillation |
| CCD | Central Composite Design |
| DCM | Dichloromethane |
| DMSO | Dimethyl Sulfoxide |
| EM | Electromagnetic |
| FMASE | Focused Microwave Assisted Soxhlet or Solvent Extraction |
| FTIR | Fourier Transform Infrared Spectroscopy |
| GC-MS | Gas Chromatography-Mass Spectrometry |
| GRAS | Generally Response as Safe |
| HD | Hydrodistillation |
| MAE | Microwave-Assisted Extraction |
| MAHD | Microwave Assisted Hydrodistillation |
| MASD | Microwave Accelerated Steam Distillation |
| MDG | Microwave Dry-Diffusion and Gravity Process |
| MHG | Microwave Hydrodiffusion and Gravity |
| MSD | Microwave Steam Distillation |
| MSDf | Microwave Steam Diffusion |
| NIST | National Institute of Standards and Technology |
| OFAT | One Factor at a Time |

| | |
|---|---|
| PMAE | Portable Microwave Assisted Extraction |
| PTFE | Polytetraflouro Ethylene |
| RSM | Response Surface Methodology |
| RT | Retention Time |
| SEM | Scanning Electron Microscopy |
| SFE | Supercritical Fluid Extraction |
| SFME | Solvent Free Microwave Extraction |
| TDS | Triple Distribution System |
| UAE | Ultrasound Assisted Extraction |
| VMHD | Vacuum Microwave Hydrodistillation |

# LIST OF APPENDICES

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Study

Essential oil is generally referred to as concentrated volatile oils which are lipophilic and hydrophobic in nature. They exhibit very distinct scent and extend through various parts of a plant or herbs. Initially, essential oil has been used mainly in applications such as flavouring in foodstuffs, cosmetics, cleaning products, pesticides and fragrances. Recently, applications of essential oil have extended into aromatherapy. This is based on the discovery that components of essential oil possess some curative properties and can be used as alternative medicines (Bousbia et al., 2009).

Essential oil may be obtained from large variety of plant sources as well as from various parts of the plant. Among the possible plant sources of essential oil, *Cinnamomum cassia* (cinnamon) is of particular interest. Cinnamon belongs to the *Lauraceae* family and it is one of the very important spices and traditional herbal medicine in the world (Geng et al., 2011). Wide distribution of cinnamon plant can be found in India, Srilanka, China, Seychelles, Vietnam, Malaysia and Madagascar (Jantan et al., 2008 and Li et al., 2013b).

Essential oils have been found to be a significant component of *C. cassia* and it can be extracted from different parts of the plant such as twigs, seeds, calyxs, leaves, and barks (Li et al., 2013b and Ravindran et al., 2004). Oil obtained from *C. cassia* contains large amount of terpenes and other aromatic compounds which are notable for different activities such as antioxidant, antiallergy, anticancer and antibacterial (Prasad et al., 2009 and Unlu et al., 2010). Specifically, the bark of *C. cassia* is a very important source of commercial raw materials based on its high content of essential oils and trans-cinnamaldehyde (Chinese Pharmacopoeia Commission, 2010; Geng et al., 2011 and Li et al., 2013a). It has therefore been observed to possess high pharmaceutical properties (Li et al., 2013a).

In order to investigate the pharmaceutical properties of cinnamon bark oil, one technique which may be used for evaluating the cytotoxicity of the crude bark extract is brine shrimp lethality test (Michael et al., 1956). This assay was initially designed to study the mortality of laboratory invertebrate model, *Artemia salina*. It was later perceived to be an easy, fast, reliable and cost effective method to determine the acute toxicity of essential oils and other related products. Several experimental work have been conducted to explore this technique for evaluating cytotoxicity of different plant and spices (Bajracharya and Tuladhar, 2012; Krishnaraju et al., 2005 and Montanher et al., 2002).

On the other hand, there is an increasing range in the areas of cinnamon application such as in seasonings, drinks, cosmetics, foods, chemical industries, commodity essences and most importantly in pharmaceutical preparations. It had therefore become one important natural resource with great economic values (Li et al., 2013a). Several researches have been carried out on volatile oils obtained from cinnamon barks (Ding et al., 2011 and Li et al., 2013b). One of such researches focused on variation in yield and chemical components of oils from cassia bark at different growth stages (Geng et al., 2011) while another researcher investigated variations based on origins and species (Li et al., 2013b). It had been observed however that there could be wide variation in the components of the oils if they are obtained through different extraction methods (Li et al., 2013a). Due to this there is need for a deliberate and well-orchestrated effort to obtain volatile oils with minimal disruption to its components.

The conventional techniques for obtaining volatile oils from plant materials include steam distillation, expression, hydrodistillation (HD), empyreumatic (otherwise called destructive) distillation, organic solvent distillation, steam and water distillation and maceration (Djouahri et al., 2013). Among these methods, HD is the most frequently used technique (Djouahri et al., 2013 and Gavahian et al., 2012). However, there are several shortcomings associated with the conventional methods. This includes high energy consumption, long extraction time and potential loss or damage of volatile compounds (de Rijke et al., 2006; Gavahian et al., 2012 and Lo Presti et al., 2005). Over time, there had been various improvements on the extraction methods. These are aimed especially for the purpose of reducing the extraction time, reducing the operation cost, improving oil quality and increasing extraction yield. Some of these new approaches include pressurized solvent extraction, ultrasound-assisted extraction, supercritical fluid extraction, microwave-assisted extraction (MAE), and microwave assisted hydrodistillation (MAHD) (Djouahri et al., 2013).

Recently, much attention is being focused on MAHD, exploiting the dielectric heating of microwave for reduced extraction time, for easy work-up and manipulation as well as to obtain higher purity of extracts (Liu et al., 2012). Different class of compounds including aromas, phenols, pesticides and especially essential oils have been extracted through this technique and there is report on the speed up of extraction through this technique (Petrakis et al., 2014). In fact, the yield of volatile essential oil obtained after 30 min extraction with MAHD is comparable to what was obtained after 4 h extraction via HD (Golmakani and Rezaei, 2008). Generally, the benefits associated with MAHD includes but not limited to selective heating, increased production, effective heating, elimination of process steps, reduced thermal gradients, reduced equipment size and faster start-up time. Most importantly is the fact that oils obtained through MAHD are environmental friendly (Filly et al., 2014).

Some studies have been carried out on extraction of essential oils from *C. cassia* barks using MAHD. Liu and co-workers (2012) carried out a comparative study on the extraction of cinnamomi cortex using ionic liquid-based microwave assisted simultaneous extraction and distillation (ILMSED), hydrodistillation (HD) and microwave hydrodistillation (MHD) (Liu et al., 2012). In their research, cassia oil yield from the three extraction methods were compared and operating parameters were optimized. It was reported that simultaneous microwave and distillation extraction led to reduced energy consumption as well as improved extraction yield (Liu et al., 2012). Similar observation was also reported from a recent research on the effect of microwave treatment on yield of essential oil from *C. cassia* bark. In this same research, the impact of microwave on polyphenols and flavour compounds was also investigated. Higher extraction yield, polyphenols and flavour compounds were obtained from microwave assisted extraction compared to conventional hydrodistillation extraction method

(Sowbhagya et al., 2016). MAHD technique can therefore be suitably investigated for further detailed working principles.

As reported, MAHD is being explored for extracting cassia oil. However, despite the many researches on MAHD, there have not been reports on the cytotoxicity of oils obtained via MAHD. Moreover, there has not been any report on the effect of pre-treatment of raw material as well as functional group analysis of *C. cassia* bark oil compounds extracted through MAHD. Furthermore, there have not been reports on comparison between functional groups of *C. cassia* bark oil obtained through MAHD and its comparison with conventional HD method. Therefore, these gaps have been identified as suitable areas for potential research.

## 1.2 Problem Statement

Recently, there have been increasing demands for essential oils. This is based on its great versatile potential benefits and applications such as flavouring in foodstuffs, cosmetics, cleaning products, pesticides, fragrances, aromatherapy, commodity essences and most importantly in pharmaceutical preparations. The most commonly used conventional method for obtaining essential oils from aroma plants and medicinal herbs is hydrodistillation (HD). However, there are several shortcomings associated with this method such as long extraction time, potential loss or damage of volatile compounds and high energy consumption. This therefore necessitates the need for better innovative and advance extraction technique which can mitigate these challenges.

Also, hydrodistillation method has been observed to pollute the environment due to the large amount of carbon dioxide which is being released during a single extraction process. Environmental concerns therefore require that extraction technique which is safe and more environmental friendly should be exploited in order to protect the integrity of the environment.

One evolving and advanced extraction technique which is being used nowadays is microwave assisted hydrodistillation (MAHD). Although MAHD extraction technique has been conducted on a number of plant materials, there has not been any report on the cytotoxicity of oils obtained via this method.

On the other hand, few experimental works have been conducted on *C. cassia* bark through different extraction techniques. However, there is no sufficient report on the effect of raw material pre-treatment on the efficiency of the extraction method. Also, functional group analysis of *C. cassia* bark oil compounds extracted through MAHD has not been reported. Most importantly, there have not been reports on comparison between functional groups of *C. cassia* bark oil obtained through MAHD and its comparison with conventional HD method. These gaps have been identified as suitable areas for potential research. There is therefore the need for research work to cover these gaps and to proffer useful information on the potentiality of MAHD extraction technique.

## 1.3 Objectives

The main objective of this research is to compare the qualitative properties of *C. cassia* bark essential oil obtained through HD and MAHD techniques. The aims of the research are as follows:

1. To extract essential oil from *Cinnamomum cassia* bark through Microwave Assisted Hydrodistillation (MAHD) and conventional Hydrodistilation (HD) and optimize the operating parameters by applying Response Surface Methodology (RSM).
2. To investigate the potential of MAHD and compare the chemical composition of cassia bark oil extracted through HD and MAHD.
3. To provide substantial information about the toxicity properties of the extracted oil through MAHD, as compared with HD.

## 1.4 Scope of Study

The following scope shall be used to achieve the first objective:

i. Conduct preliminary studies to identify the parameters which would influence the extraction process.
ii. Extract essential oil from *C. cassia* barks through MAHD and HD methods by varying the extraction time, extraction power and solvent volume.

iii. Evaluate the effects of influencing parameters on the cinnamon oil yield through One Factor at a Time (OFAT) method.
iv. Screen and optimize the extraction parameters based on cinnamon oil yield by applying 2-Level Factorial Design and Central Composite Design (CCD) of Response Surface Methodology (RSM) using Design Expert computer software.

To achieve the second objective, the following scope shall be followed:

i. Morphological studies of *C. cassia* bark powder before and after extraction through MAHD and HD by using Scanning Electron Microscopy (SEM).
ii. Evaluate the dielectric properties of solvent used, volume of heat generation and penetration depth of microwave.
iii. Evaluate the influence of extraction technique on the yield of *C. cassia* bark oil.
iv. Analyse the chemical composition of cinnamon oil by using Gas Chromatography-Mass Spectrometry (GC-MS).
v. Examine the structural similarities between essential oil extracted through HD and MAHD by using Fourier Transform Infrared Spectroscopy (FTIR).
vi. Deduce the cost and energy implication as well as environmental impact of each extraction technique.

To achieve the third objective, the following scope shall be followed:

i. Cytotoxicity of cassia bark oil extracted under optimized conditions from both conventional HD and MAHD methods.
ii. Bioassay lethality test of the extracted essential oil using *Artemia salina* brine shrimp.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Introduction

This chapter presents a review of the previous research works which have been conducted on essential oil, particularly those obtained from *C. cassia*. Numerous researches have been done on extraction of essential oil by using various techniques however, there are some rooms for improvement based on the previous studies, and especially on the extraction techniques for *C. cassia* bark oil. This chapter therefore focuses on the improvement of extraction technique of *C. cassia* bark oil. Other areas which are covered include characterization techniques, bioassay and statistical model of essential oil obtained from *C. cassia*.

## 2.2 Cinnamomum Cassia

*Cinnamomum cassia* belongs to the Lauraceae family which is one of the largest families of plant. The Lauraceae consists of about 50 genera and more than 2000 species distributed across tropical to subtropical regions including Southeast Asia and tropical America (Kochummen, 1989). Some genera can be found at pantropical region like *Cassytha*, *Beilschmiedia*, *Litsea*, *Cryptocarya*, *Ocotea* and *Persea*. On the other hand, several others are distributed throughout Australia and Asia like *Endiandra*, *Alseodaphne*, *Dehaasia*, *Hexapora*, *Eusiderosylon* and *Neolitsea*. The *Eusiderosylon* species is confined to Sumatra and Borneo but *Dehaasia* and *Hexapora* species are native to Peninsular Malaysia. Whereas, *Ravensara* and *Potemeia* are restricted to Madagascar (Gentry, 1988 and Wiart, 2006). Generally, the Lauraceae family are famous for their variant floral morphologies.

In Malaysia, the Lauraceae family comprises of 16 genera and 213 species (Corner, 1988). Most of the plants from this family are used in timber industries due to their suitability for plywood manufacturing and decorative applications. Also, in Malaysia plant materials from four genera are being actively used for research purposes. These are *Lindera*, *Litsea*, *Beilschmiedia* and *Cinnamomum* species. Among these four, the *Cinnamomum* species are of particular interest.

About 21 species of the *Cinnamomum* genus are distributed in Peninsular Malaysia. These species are shrubs and the size of the tree could range from small to medium sized (Jantan et al., 2004). Among these species, 14 have been studied through different extraction methods for their volatile oil chemical constituents, major chemical group and yield. These species are *C. aureoflavum, C. subavenium, C. rhyncophyllum*, *C. pubescens*, *C. mollissimum*, *C. impressicostatum*, *C. zeylanicum, C. parthenoxylon*, *C. cordatum, C. sintoc*, *C. scortechinii*, *C. altissimum, C. cassia* and *C. microphyllum*. Among these investigated species, *C. cassia* (cinnamon) is of particular interest.

*Cinnamomum cassia* also known as Chinese cinnamon, is one of the most importantly used spices and traditional herbal medicine in the world (Geng et al., 2011). It can be widely found in Madagascar, Srilanka, China, India, Malaysia, Vietnam and Seychelles (Jantan et al., 2008 and Li et al., 2013a). Essential oils have been reported to be a significant component of *C. cassia* with the possibility for obtaining the oil from various parts of the plant such as twigs, leaves, seeds, calyxs and also barks (Li et al., 2013b and Ravindran et al., 2004). Oils obtained from *C. cassia* bark have been specifically reported to possess large quantity of terpenes and other aromatic compounds which make it a good material for research purposes. This is because terpenes and aromatic compounds have been observed to manifest several desirable activities including antifungal (Giordani et al., 2006; Lee et al., 2007 and Ooi et al., 2006), antibacterial (Prasad et al., 2009 and Unlu et al., 2010), antioxidant (Lin at al., 2003), potential repellent and anticancer agent (Chang et al., 2006 and Shin et al., 2006) and antibacterial properties (Prasad et al., 2009 and Unlu et al., 2010). Therefore, due to the high essential oil content of *C. cassia*, it has been perceived to be a major source of commercial raw materials (Chinese Pharmacopoeia Commission, 2010; Geng et al., 2011 and Li et al., 2013a). Trans-cinnamaldehyde which is the main active chemical component of *C. cassia* oil has been observed to exhibit highly desirable pharmaceutical properties (Li et al., 2013a). The chemical formular of trans-cinnamaldehyde is $C_9H_8O$. It has a boling point of around 248-250 $^{o}$C, density of 1.05 g mL$^{-1}$ at 20 $^{o}$C and molecular weight of about 133.16 g mol$^{-1}$ (Ye et al., 2013). The chemical structure of trans-cinnamaldehyde is illustrated in Figure 2.1.

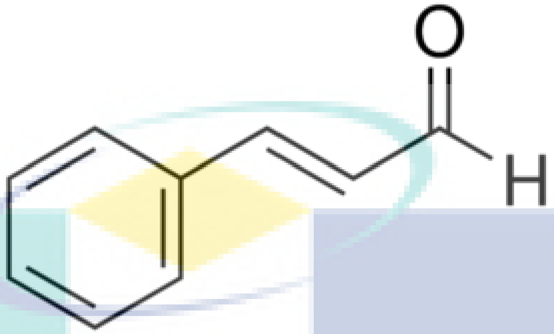


Figure 2.1: Chemical structure of trans-cinnamaldehyde.

In order to obtain the good quality and quantity oils from *C. cassia*, it is necessary to extract it through a suitable extraction technique. A variety of emerging extraction techniques are being investigated for the separation and purification of bioactive compounds from cassia bark and other parts of the plant. The commonly used methods for obtaining volatile oils from cassia bark include hydrodistillation (HD) (Geng et al., 2011 and Li et al., 2013b), solvent extraction (Yang et al., 2012), maceration at room temperature (Tabassum et al., 2013) and Soxhlet extraction (Kasim et al., 2014). These methods are generally referred to as conventional methods of extraction. Among these, HD is the most frequently used method (Djouahri et al., 2013 and Gavahian et al., 2012).

It has been generally observed however that there are several shortcomings associated with the conventional methods. These include long extraction time, potential damage or total loss of volatile compounds as well as high energy consumption (de Rijke et al., 2006; Gavahian et al., 2012 and Lo Presti et al., 2005). Over time, there were several modification and improvement to the extraction methods specifically with the aim of reducing the extraction time, reducing the cost of operation, improving oil quality and increasing extraction yield. Some of the newly developed approaches are supercritical fluid extraction (SFE), pressurized solvent extraction (PSE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE) and microwave assisted hydrodistillation (MAHD) (Djouahri et al., 2013). The use of these advanced techniques has the potential to overcome the challenges associated with the conventional methods. However, until they are confirmed through series of chemical, biological and other important test and characterization techniques, they cannot be suitably acceptable.

Recently, a lot of research is being carried out on MAHD method. These researches take advantage of the dielectric heating capacity of microwave to obtain reduced extraction time, ease of work-up and manipulation as well as higher purity of extracts (Liu et al., 2012). Various classes of chemical compounds such as pesticides, phenols, aromas and most importantly essential oils have been obtained through this technique and the extraction process was confirmed to be rapid (Petrakis et al., 2014). In fact, the yield of volatile essential oil obtained after 30 min extraction with MAHD is comparable to what was obtained after 4 h extraction via HD (Golmakani and Rezaei, 2008). Generally, the benefits which may be derived from MAHD includes but not limited to effective heating, reduced thermal gradients, selective heating, reduced equipment size, increased production, faster start-up time and elimination of process steps. Beyond these benefits, the oils obtained through MAHD have been observed to be environmental friendly (Filly et al., 2014). If these set of information is anything to go by, then MAHD should not only be effective towards the reported species of plants. Rather, it should be suitable for other plants materials such as *Cinnamomum cassia* which is the subject for this study.

## 2.3 Cinnamomum Cassia Essential Oil

*Cinnamomum cassia* oil have been obtained through methods such as steam distillation, hydrodistillation, solvent extraction and microwave assisted extraction. The yield and chemical composition of the cassia oil may be influenced by the extraction method and types of plant materials (Hussain et al., 2008). Yang and co-workers (2012) carried out research on the various parts of *C. cassia* and reported that the bark of cassia oil possessed higher antioxidant activity compared other parts of cassia like bud and leaves (Yang et al., 2012).

Generally, *C. cassia* bark consists of about 0.5 to 5 % essential oils, comprising about 87 % of trans-cinnamaldehyde and several other chemical compounds (Haley and

McDonald, 2016). However, the yield and chemical composition of *C. cassia* bark oil may also vary according to the growth and part of the cassia plant being extracted. According to the research carried out by Geng and co-workers (2011), it was reported that the cassia oil extracted from stem bark ranged from 0.41–2.61 % whereas those obtained from the branch bark ranged between 2.70–3.11 % (Geng et al., 2011).

As stated, extraction method often influence the yield of cassia oil. Each extraction method has its own peculiar features in extraction of essential oil from *C. cassia*. Since, essential oils are volatile, fragile and unstable, they can be easily evaporated, decomposed or degraded easily if they are not properly protected from external factors including harsh extraction techniques (Asbahani et al., 2014). Therefore, it is necessary to choose the best extraction method in order to recover all the essential compounds present in the cassia oil with maximum yield. Recently, advanced extraction techniques such as MAHD have received much attention especially due to long term health and environmental issues. Cassia oil obtained through MAHD technique have been observed to possess high potential as pharmaceutical raw materials (Liu et al., 2012).

Literature review revealed that cinnamon oil primarily comprises of monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated monoterpene, oxygenated hydrocarbons etc (Cheng et al., 2006). Specifically, the major chemical compounds exhibit in cinnamon oil are trans-cinnamaldehyde, cis-cinnamaldehyde, caryophyllene, camphor, cinnamyl-acetate, caryophillene oxide, linalool, geraniol, α-cubebene, bornyl acetate, α-copaene, eugenol and γ-elemene, among others (Cheng et al., 2006 and Jayaprakasha et al., 2000). Due to these various chemical compounds which are present in cinnamon oil, there is need for articulated research to investigate the potential of its components. This can be made through studies on the biological activities of the extracted oil and to relate the properties with its chemical components (Salleh et al., 2016). This is necessary in order to ascertain the potential pharmaceutical applications of the extracted oil. Thus toxicity studies of cassia oil is expedient for the development of new active and modern medicine. To maintain good quality of the essential oil, selection of suitable and efficient extraction methods is a matter of great importance (Dhobi et al., 2009).

## 2.4 Conventional Methods of Essential Oil Extraction

The frequently used techniques for obtaining volatile oils from plant materials are hydrodistillation (HD), organic solvent distillation, steam distillation, expression, empyreumatic (otherwise called destructive) distillation, steam and water distillation and maceration (Djouahri et al., 2013). These techniques are often referred to as conventional methods of extraction and among these; HD is the most frequently used technique (Djouahri et al., 2013 and Gavahian et al., 2012). It is therefore logical to test every emerging technique against HD and compare their properties. This is necessary to justify and ascertain the prospect of the new extraction method over the existing ones.

### 2.4.1 Hydrodistillation

Hydrodistillation (HD) is an ancient, traditional and simple method that has been used for extraction of volatile oils and active compounds from natural aroma plants materials (Meyer Warnod, 1984). Generally, organic solvents are not being used during extraction unless it might be used for pre-treatment purposes. The principle of this method is to completely immerse the plant material in water prior to the extraction. Later the plant material mixture with water is heated by using a direct heating source until the mixture boils. During the boiling period, the plant material absorb sufficient amount of heat to expand the phloem and burst the oil glands that contain essential oils. These volatile oils are release from the secretory oil glands of the plant material and are transferred to the water through mass transfer. As the boiling continues, the essential oil containing water becomes steam and it is carried over a condenser after which it is distilled out. The mixture of water and crude extract is then collected and further separated to obtain the concentrated oil. The major benefit of using water in distillation process is that it is immiscible with most of the terpene compounds that is majorly consisted in essential oils. Therefore, after condensation process, the essential oil becomes clearly separated from the water and could be easily separated by decantation.

As time went on, the HD method evolved into HD by Clevenger system. The uniqueness of this new system is that it permits recycling of the condensates through the cohobate system. This method is highly preferable for the extraction of plant materials like flower and petals as it avoids clustering during extraction. Also HD is frequently used to extract seeds, roots, barks and stems. An essential element to be considered in HD process is that the amount of water inside the tank must be sufficient to completely run the distillation process. Otherwise, the plant materials may be burnt off. Usually, the HD extracted essential oil is slightly darker in colour and exhibits the certain burning odours when compared to other extraction methods. Therefore, there should be an extra care when carrying out extraction on powdered plant materials. This is because they tend to settle more on the bottom of the reactor making them prone to thermal degradation. Furthermore, plant materials that form mucilage and raise the viscosity of the water may increase the possibility for burning or charring. Thus one of the advantages of the HD method is that it practically it shields the extracted essential oils to certain degree since the surrounding water acts as cooling agent to avoid them from being thermally degraded. This method is highly suitable compared to other conventional methods for plant materials that are hard and tough such as root, bark, stem and nuts. Hence, this method is still being widely used in industries till date because of the simplicity of the process, cheap running cost, ease of handling and portable equipment.

However, this HD method has several drawbacks such as long extraction periods (3 to 6 h for cinnamon bark) and difficulty of controlling the continually increasing temperature which may lead to overheating of the plant materials. Prolonged extraction may lead to chemical alteration of terpene molecules especially monoterpene substance via hydrolysis, oxidation and cyclization. This can make them less odoriferous which may result to reduction in quality of the volatile oils. Similarly, due to the longer induction time and long extraction period, large amount of energy is being consumed. Also this method releases huge amount of carbon dioxide to environment which make it somewhat environmental unfriendly.

### 2.4.2 Basic Principles of Hydrodistillation

The fundamental scientific principle of hydrodistillation process is the conversion of water (solvent) into a vapour (steam) through the application of energy. This energy is being applied in the form of heat which is often referred to as latent heat of vaporization. Generally, water boils at the atmospheric pressure where its vapour pressure is equals to the atmospheric pressure. Therefore, two immiscible liquids as in the case of water and essential oil, exhibit the total vapour pressure of the mixture by sum up their partial pressure. The boiling point for most of the volatile oil falls in the range of 150 $^0$C to 300 $^0$C at atmospheric pressure (Stahl et al., 2012). For instance, if a sample of volatile oil primarily having a component ‘A’ which exhibit boiling point of 190 $^0$C and the boiling point of the water is commonly 100 $^0$C, these two immiscible liquid are being mixed together. At the point when each of the vapour has reached saturation level, the temperature may suddenly decline to 99.5 $^0$C, where this temperature indicates the sum of the two vapour pressure equal to 760 mmHg. This have been reported in literature by Guenther (1972) that the mixing of two immiscible liquids generally may lead to a lower boiling point for the mixture substances compared to the boiling point of the individual components (Guenther, 1972). Thus the temperature may be lowered such that the total pressure of the mixture is being represented by the vapour mixture which is equal to the operating pressure (Himmelblau and Riggs, 2012). This phenomena may be simplified by Eq. 2.1.

$$P_T = p_A + p_W \tag{2.1}$$

where,

$P_T$ is the total vapour pressure of the mixture, kPa

$p_A$ is the partial vapour pressure of component ‘A’, kPa

$p_W$ is the partial vapour pressure of water, kPa

The individual partial pressure of the components can be calculated by using Dalton’s law of partial pressure (Coulson et al., 1991). When the essential oil is being distilled by steam during hydrodistillation process, the composition of the distillate is equal to the composition of vapour pressure A and water, which also would be equivalent to the condensed mixture. Hence, if the distillate mixture consists of water and volatile oil at saturated vapour the vapour pressure described in Eq. 2.2 can be manipulated to give the partial pressure of the individual component as follows:

$$\frac{wt_A}{wt_W} = \frac{p_A MW_A}{p_W MW_W} \tag{2.2}$$

where,

$wt_A$ is the weight of component ‘A’, kg

$wt_W$ is the weight of water, kg
$p_A$ is the partial vapour pressure of component 'A', kPa
$p_W$ is the partial vapour pressure of water, kPa
$MW_A$ is the molecular weight of component 'A', kg/mol
$MW_W$ is the molecular weight of water, kg/mol

Hence, this concept could be applied to any volatile oils having high boiling point range which could be evaporated with the aid of steam distillation in a ratio such that their total combined vapour pressure should be equal to the atmospheric pressure. Before selecting HD technique as an extraction method to obtain essential oils, factors such as sensitivity, volatility and water solubility of the volatile oils should necessarily be put into consideration. If the essential oil to be extracted is very sensitive to heat and steam, then HD is not a suitable method. This is because the fragile oil may be easily damaged by prolonged heating (Asbahani et al., 2014). On the other hand, for an efficient HD process, the degree of volatility of the essential oils should be higher than the boiling point of the solvent used for extraction. Otherwise the extracted oil may be loss via volatilization. In another vein, solubility of the oil in water needs to be well known. If the active compound of essential oils is highly soluble in water, hydrodistillation method is not suitable to extract the oils. It would be the best choice to use HD technique for extraction of volatile oils that are less soluble in water. Generally, volatile oils constituents with lower molecular weights are highly soluble in water. Therefore, most of the time it could not be distilled out through steam thereby making it prone to damage. Trans-cinnamaldehyde which is the active compound in cinnamon barks have been observed to be practically stable to the action of heat and reasonably insoluble in water, therefore, this plant material is suitable to be extracted through HD method.

A number of researches have been carried out to extract essential oil from *Cinnamomum cassia* bark through HD method (Adinew, 2014; Baseri et al., 2010; Geng et al., 2011; Geng et al., 2012; Li et al., 2013b; Tian et al., 2011 and Wang et al., 2011). However, reports from most of these researches show that there are some shortcomings associated with this conventional methods. One of such shortcoming is the potential alterations of the oils' chemical compounds especially the monoterpene compounds. Other shortcomings include long extraction period, high power consumption and total loss or thermal degradation of fragile volatile chemical compounds (Asbahani et al., 2014 and Gavahian et al., 2012). More innovative and advanced extraction techniques such as MAHD may therefore be exploited for highly desirable properties.

## 2.5 Non-Conventional Methods of Essential Oil Extraction

There are several drawbacks associated with the conventional methods such as long extraction time, potential loss or damage of volatile compounds and high energy consumption (de Rijke et al., 2006; Gavahian et al., 2012 and Lo Presti et al., 2005). Over time, there had been improvement on the extraction methods especially for the purpose of extraction time reduction, operation cost reduction, enhancement of oil quality and increased extraction yield. Some of the new approaches include ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), pressurized solvent extraction (PSE) and microwave assisted hydrodistillation (MAHD) (Djouahri et al., 2013).

### 2.5.1 Ultrasound-Assisted Extraction

Ultrasound refers to sounds waves with frequency above the threshold of human hearing (20 kHz). Ultrasound-assisted extraction (UAE) is one of the emerging technology in extraction field because it is simple, cheap, reduces processing time, enhance oil quality, saves energy helps to maintain the flavours and safety of products (Awad et al., 2012 and Ana and Mar, 2010). Recently, many researches have been carried out using ultrasound in order to extract essential oils from various plant materials. Mainly, UAE have been used in extraction of herbal oils (essential oils), polysaccharides, protein and bioactive ingredients (Awad et al., 2012). Some of the recent studies conducted using UAE includes the  extraction of capsaicinoids from chili peppers (Deng et al., 2016), polyphenols from spruce wood bark (Ghitescu et al., 2015), pectin from sisal waste (Maran and Priya, 2015) and oil from papaya seed (Samaram et al., 2015). From these researches, it was observed that the mechanical wave effects provides better penetration into cellular materials, via propagates through a series of rarefaction and compression motion on the molecules of the medium travelled.  These effects can improve the release of essential oils from the secretory oil glands into the bulk medium of solvents (Mason, 1998).

Generally, ultrasound requires sound waves with frequencies in the range of 20 kHz to 2000 kHz in order to enhance the permeability of sounds waves to penetrate into the oil cells of plant materials. The mechanism of UAE is cavitation which is produced with the aid of ultrasound. This generates high shear forces and microbubbles that enhance the surface erosion, fragmentation and mass transfer between solvent and plant material. The parameters involved in this method are frequency of ultrasound, extraction time and temperature. The advantages of this method includes the faster rate of extraction at which high yield of essential oils can be obtained. Besides that, instead of using organic solvents, generally response as safe (GRAS) solvents is mainly being used in extraction through UAE. In addition, UAE method helps to protect the products from being thermally degraded (usage of lower temperature) (Vilkhu et al., 2008). These highlighted advantages have been confirmed through different researches. Da Porto and co-workers (2013), compared UAE with conventional extraction method (Soxhlet extraction and maceration) for obtaining essential oils from grape seeds (da Porto et al., 2013). It was reported that UAE produced the highest polyphenols concentration and antioxidant activity at shorter extraction period (15 min) compared to the conventional methods (da Porto et al., 2013). Other researchers have also highlighted better performance of UAE than conventional method. One of these is in the research on the extraction of hypericins, hyperforin and flavonoids from *Hypericum perforatum L* (Smelcerovic et al., 2006). Another one is the extraction of valuable bioactive compounds from some aromatic plants and flowers. UAE was reported to be more rapid than steam distillation and supercritical water extraction (Roldan Gutierrez et al., 2008). However, despite these advantages, the major drawback of UAE is the usage of organic solvents in some cases (Romero et al., 2010). Also there have been occasional reports on damage of some bioactive constituents of medicinal plants via formation of free radicals due to high ultrasound frequency (20 kHz).

### 2.5.2 Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) also known as supercritical carbon dioxide extraction uses carbon dioxide under very high pressure to extract essential oils from plant materials. A supercritical fluid is defined as any substance that is beyond its critical pressure and temperature. The critical parameters of carbon dioxide are 304.2 K and 7.38 MPa. SFE is a new technique in extraction which started to evolve in the year 2000 and almost more than 300 plant species have been reported to have been extracted via this technique. Among these 28 % of the researches concentrated on seeds, 17 % on leaves and the remaining comprises of other parts of plant materials which have been extracted (de Melo et al., 2014). Some of the studies which have been conducted on the SFE method include extraction of essential oils from tomato (*Solanum lycopersicum* L.) (Machmudah et al., 2012; Saldana et al., 2010; Yi et al., 2009 and Vagi et al., 2007), grape (*Vitis vinifera* L.) (Agostini et al., 2012; da Silva et al., 2008; Ghafoor et al., 2012 and Prado et al., 2012), tyme (*Thymus vulgaris* L.) (Grosso et al., 2010; Petrovic et al., 2012 and Diaz Maroto et al., 2005), rice (*Oryza* variety), (Manosroi et al., 2010 and Wang et al., 2008), coffee (*Coffea* spp.) (Andrade et al., 2012 and Tello et al., 2011), rosemary (*Rosmarinus officinalis* L.) (Bensebia et al., 2009 and Irmak et al., 2010) and sunflower (*Heliantus annuus* L.) (Casas et al., 2009 and Salgin et al., 2006). Based on the mentioned studies, the major drawbacks found from this particular technique are high operating cost and many operating parameters required to be optimized. In another vein, the full recovery of the essential oil is low due to high pressure and temperature usage in this technique.

Mainly, the types of chemical compounds obtained through this extraction method is flavonoids, triterpenoids, tetraterpenoids, monoterpenoids, sesquiterpenoids, triglycerides, fatty acids, diterpenoids, fatty alcohols, tocopherols, phytosterols, tocotrienols and phenolics (de Melo et al., 2014). This method exhibits some environmental benefits such as replacing conventional organic solvents (chemicals) with environmental friendly substance. It also has the advantage of low energy consumption (Brunner, 2010; Jessop and Leitner, 1999 and Loppinet-Serani et al., 2010). As a result, SFE method is also known as “green solvent for future method” (Knez et al., 2014). In another vein, this technique could offer great safety and health advantages. This is because it makes use of supercritical water and carbon dioxide which is inert, non-carcinogenic, non-mutagenic, thermodynamically stable and non-flammable (Sahena et al., 2009). Although the extracts obtained via this method possesses high yield of essential oils and excellent quality of oils, the drawbacks of this particular method is its economical non-feasibility and complicated operating methods.

### 2.5.3 Microwave Extraction

Microwave is non-ionizing electromagnetic wave that follows the chronological of radio < microwave < infrared frequencies in the electromagnetic spectrum. The range of microwave frequency falls between 300 MHz to 300 GHz. 915 MHz frequency is commonly used in industrial applications because of its greater penetration depth while 2450 MHz frequency is used in domestic microwave ovens and specifically for extraction purposes. Initially in the past decades, microwave was used for extraction of organic compound for digestion of samples for metal analysis (Abu Samra et al., 1975). This technique was later developed and studied for the potential method as non-conventional source of energy. High demand for new extraction techniques with special features opened the room for more advanced extraction techniques such as the microwave extraction. Some of these features include short extraction period, less organic solvent consumption, easy extraction process, suitable to automation, economically feasible, technically compatible, energy saving and reduced pollution.

There are several types of microwave extraction techniques such as microwave assisted extraction (MAE), microwave hydrodiffusion and gravity (MHG), microwave steam distillation (MSD), microwave steam diffusion (MSDf) and most recently discovered technique which is solvent free microwave extraction (SFME). Table 2.1 comprises the summary of advance extraction techniques.

Table 2.1
Comparison among advance extraction techniques

| | **Advance Extraction Techniques** | | |
|---|---|---|---|
| | **Microwave Assisted Extraction (MAE)** | **Supercritical Fluid Extraction (SFE)** | **Ultrasound Assisted Extraction (UAE)** |
| Description | • Plant material sample in microwave absorbing solvent in a reactor and irradiate microwave | • Plant material sample is insert in high pressure vessel and extracted with supercritical fluid (commonly used solvent is carbon dioxide at pressure of 150 to 450 bar and temperature of 40 $^0$C to 150 $^0$C). The extracts are collected in a small volume of solvent, in a separator or onto a solid-phase trap, which will be rinse with solvent in later path. | • Sample is completely immersed in solvent in a vessel and placed in ultrasonic using ultrasound probe or ultrasound bath |
| Sample size | 1–10 g | 1–5 g | 1–30 g |
| Extraction period | 3–30 min | 10–60 min | 10–60 min |
| Volume of solvent used | 10–40 mL | 2–5 mL (solid trap) | 30-200 mL |
| Costing | Moderate | High | Low |
| Advantages | • Fast extraction<br>• Moderate solvent usage<br>• Easy to handle and process<br>• Elevated temperature | • Fast extraction<br>• Low solvent consumption<br>• Possible high selectivity<br>• Low temperature<br>• No usage of toxic solvent<br>• Automated system | • Easy to handle<br>• Multiple extraction |

Table 2.1 continued.

| | Advance Extraction Techniques | | |
|---|---|---|---|
| | **Microwave Assisted Extraction (MAE)** | **Supercritical Fluid Extraction (SFE)** | **Ultrasound Assisted Extraction (UAE)** |
| Disadvantages | • Selective extraction solvent (must absorb microwave energy)<br>• Filtration step is needed<br>• Wait for the vessel to cool down before collect the crude extracts | • Many parameters to optimize | • Large amount of solvent usage<br>• Filtration step required<br>• Wait for the vessel to cool down before collect the crude extracts |

Adapted from: Veggi et al. (2012)

### a) Microwave Assisted Extraction

Microwave assisted extraction (MAE) is an advance and innovative extraction technique which is a combination of compressed air microwave distillation (CAMD) and vacuum microwave hydrodistillation (VMHD) techniques. Although dry spices are being subjected to extraction during MAE, the materials are often being soaked with solvents that have high heating efficiency under microwave phenomena. The higher temperature obtain by microwave radiation may hydrolyse the cellulose (main component in the plant cell wall), and turn into soluble fraction within few minutes. The higher temperature gained by the plant cell wall in MAE method, accelerate the dehydration process of cellulose and a subsequent mechanical constraint is developed. As a result, the solvent used is easily absorbed by the plant cell wall (Latha, 2007). The efficiency of the MAE technique was investigated by Zhou and Liu (2006) in their research through examining tobacco leaf sample which have been subjected to MAE. He examined the surface through scanning electron microscopy (Zhou and Liu, 2006). The scanning electron micrographs of the untreated, heat reflux extraction and MAE samples were studied. Both untreated sample and heat reflux extraction sample shows that no structural difference except for few slight rupture was visible on the surface of the sample. On the other hand, the MAE samples' surface was observed to be greatly destroyed and this could be related with the sudden temperature rise and subsequent pressure increased in the plant cell wall. It was reported that microwave energy caused the surface of the MAE sample to be aggressively destroyed. The rupture of the plant cell wall release the essential oil from the oil gland and permits the volatile oils to flow into organic solvent (Garcia Ayuso et al., 2000). The essential oil may be then be recovered from the organic solvent using rotary evaporator.

Improvement to the MAE method began to arise due to the urge of the technology revolution on the green extraction concept and the requirement for new energy saving, cost reduction and technically feasible methods. These improvements were brought about by taking advantage of the dielectric heating properties of MAE especially for essential oils extraction from aroma plant materials. Thus, the concept of MAE further evolved into the development of a various other innovative extraction techniques using microwave like microwave assisted hydrodistillation (MAHD) which incorporates microwave (advance technology) with hydrodistillation (conventional method) (Ferhat et al., 2006; Golmakani and Rezaei, 2008 and Stashenko et al., 2004), microwave accelerated steam distillation (MASD) (Chemat et al., 2006), microwave hydrodiffusion and gravity (MHG) (Vian et al., 2008), microwave steam distillation (Naima Sahraoui et al., 2008), solvent free microwave extraction (SFME) (Lucchesi et al., 2007) and portable microwave assisted extraction (PMAE). These MAE techniques have been observed to offer promising and excellent extraction products. They produce high yield of essential oils with shorter extraction time, the methods are easy to handle, helps to reduced consumption of organic solvent and they are energy saving.

### b) Microwave Hydrodiffusion and Gravity

Microwave hydrodiffusion and gravity (MHG) is an emerging technique in the field of extraction. This technique combines the concept of microwave heating and earth gravity at atmospheric pressure. The principle of this technique is extraction of essential oil from plant materials without using any organic solvent or water. The microwave intensity directly penetrates the fresh plant materials with a minimum initial moisture content of 60 % and heats it up. The moisture contained in the fresh material is converted into steam and this steam helps the essential oils trapped inside the secretory oil gland to rupture and it is released due to high temperature and pressure effect of microwave energy. Then a mixture of hot crude extract (essential oils and steam (in situ water)) travels downwards by earth gravity on a spiral condenser which is fitted outside the microwave cavity. When the hot mixture passes through a condenser, the hot crude extract in steam form is condensed into room temperature crude extract. The condensed crude extract is collected using a collector flask (Bousbia et al., 2009). It is noteworthy that in this method, the distillation and evaporation process are not taking place, in another words, this process is a not a time consuming method (Chemat et al., 2006). Therefore, the major advantages of this extraction technique compared to hydrodistillation and other conventional method is reduced extraction time (only 20 min for MHG but it took 90 min for hydrodistillation to fully recover the essential oil from plant materials like spearmint and pennyroyal). This technique also has low energy consumption, it is efficient and it is environmental friendly (Vian et al., 2008).

In another vein, a similar technique with minor modification was conceived by (Farhat et al., 2010) where microwave dry-diffusion and gravity process (MDG) was introduced to extract essential oils of dried caraway seed as illustrated in Figure 2.2. The principle of the MGD is similar to MHG except that the extraction is carried out on dry plant materials without necessity for initial moisture content. MDG technique performs better than MHG on dry spices. It enables rapid extraction of essential oils (45 mins versus 300 min in the case of hydrodistillation). In addition to being fast, this technique is clean, power saving and its extraction processes are efficient.

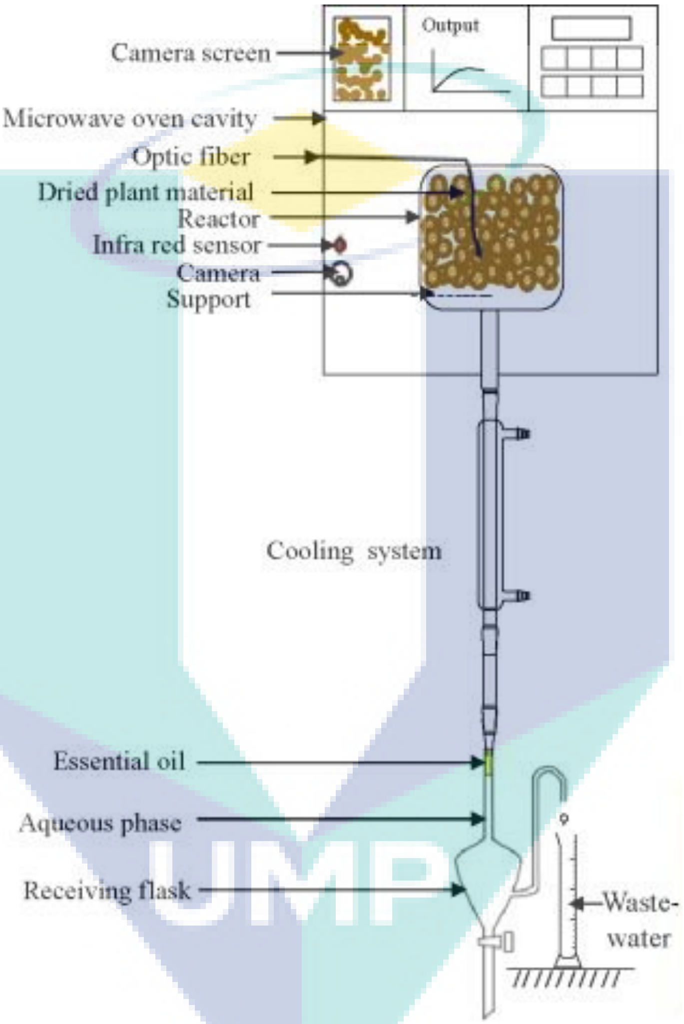


Figure 2.2: Setup for microwave dry-diffusion and gravity process.
Source: Farhat et al. (2010)

### c) Microwave Steam Distillation and Microwave Steam Diffusion

Microwave steam distillation (MSD) is a new and improved extraction method which is a modification to the conventional steam distillation technique. It is used to extract essential oil from aromatic natural products. This extraction technique involves placing the material to be extracted into an extractor reactor also known as cartridge of plant material. The cartridge and plant material is then placed in the microwave cavity. Microwave irradiation is then applied directly to the plant materials resulting in selective heating. Since the essential oils of plant material (e.g. lavender flower) obtain higher dielectric loss compared to the surrounding steam, the essential oils may directly absorb all the microwave irradiation. Figure 2.3 shows the schematic diagram of MSD. It is noteworthy that although water (liquid) has the highest dielectric properties which could absorb more microwave energy compared to other solvents. However water in the form of steam (gas) or ice (solid) cannot absorb microwave energy. This is because when water (steam) in gaseous state the distance between two molecules are far apart from each other to generate frictions whereas in water (ice) in solid form the arrangement of molecules are well packed and thus there is no space for the molecules to rotate and move when heat is applied.

The MSD method has been studied by some researchers such as for the extraction of essential oil from orange peel (Sahraoui et al., 2011) and dry lavender flower (Naima Sahraoui et al., 2008). The MSD technique exhibits better effective performance than steam distillation in term of shorter extraction time (same yield obtained at 6 min for MSD and 30 min in the case of steam distillation), less energy consumption and provide cleaner environment. In addition the volatile oils that were extracted through MSD did not experience structural changes in their chemical composition.

On the other hand, microwave steam diffusion (MSDf) is one of the cleaner and innovative extraction techniques that incorporates the principle of conventional steam diffusion. MSDf exhibits similar principle like MSD except for the saturated steam flow through the aroma plant materials down. Therefore, the plant crude extract may diffuse outside the plant material and flow down by earth gravity out the microwave gravity. The schematic diagram of MSDf setup is depicted in Figure 2.4. Some of the important researches which have been conducted using this technique incudes extraction of essential oils from lavender (Farhat et al., 2009), tomato (Xiao et al., 2016), orange peel (Farhat et al., 2011) and Lavendin flowers (Perino Issartier et al., 2013). Interestingly, this method was observed to show better efficiency in terms of extraction kinetics (3 min for MSDf versus 6 min for MSD and 20 min for conventional steam diffusion required extraction time to obtain similar yield). Other benefits of this technique is that it is environmental friendly, clean, produces better quality of crude extract, saves power and reduce wastage. In essence, this method may be referred to as a green technology, cleaner and economically feasible extraction technique.

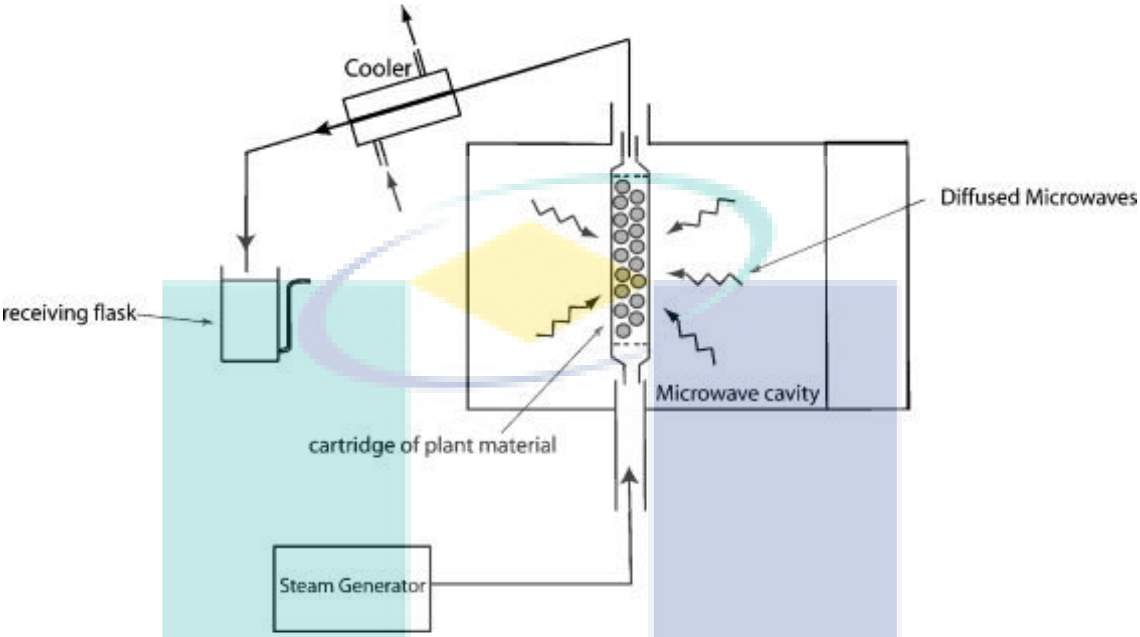


Figure 2.3: Setup for microwave steam distillation (MSD).

Source: Naima Sahraoui et al. (2008)

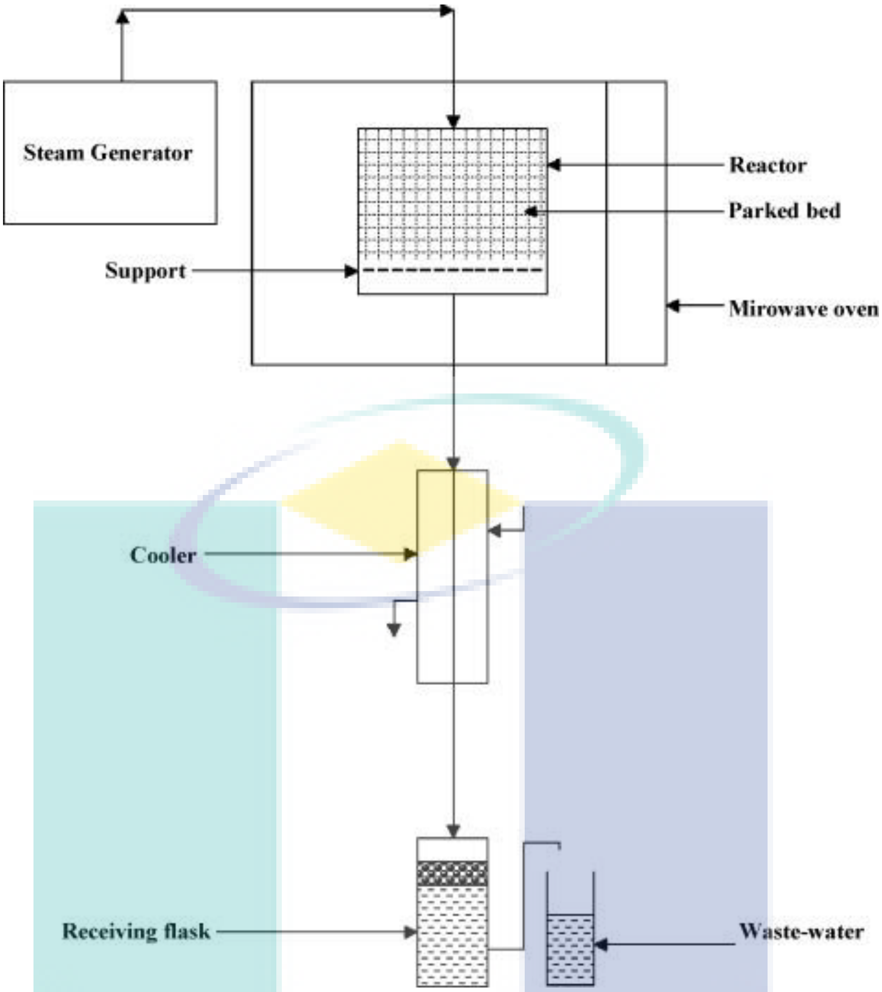


Figure 2.4: Schematic diagram of principle of microwave steam diffusion (MSDf).
Source: Farhat et al. (2009)

### d) Solvent Free Microwave Extraction

Solvent free microwave extraction (SFME) is a very recent, new emerging extraction technique which is a combination of dry distillation and microwave heating energy. It exhibits the mechanism of microwave dry-distillation technique at atmospheric pressure which extract essential oils from fresh plant materials without any addition of organic solvents or water (Filly et al., 2014). This method was initiated by Chemat and co-workers in the year 2004 (Chemat et al., 2014). Since microwave obtain selective heating property, the microwave irradiation subjected to in situ moisture (water) content of fresh plant materials causes the secretory oil glands to expand. Due to high temperature there is a subsequent formation of high internal pressure which burst the oil gland. As a result, the oil glands release the essential oils, which are immediately evaporated by azeotropic distillation process (Li et al., 2013a). Literature reveals that lab scale SFME technique has the potential to be upgraded into pilot scale due to its feasibility in terms of technicality and economy (Filly et al., 2014). This technique has therefore been used for the extraction of essential oil laboratory scale (Filly et al., 2014). The speciality of this technique is that isolation and concentration of crude extract may be carried out within just 30 min whereas conventional hydrodistillation method requires about 2 h or more.

## 2.6 Microwave Assisted Hydrodistillation

Microwave assisted hydrodistillation (MAHD) is an emerging and innovative advanced technique which is been used for extracting volatile oils from plant materials. Extraction through MAHD has been observed to hold numerous potentials for producing good quality and high yield of essential oils.

### 2.6.1 Theory of Microwave

Microwave refers to non-ionizing radiation waves with frequency range of 300 MHz to 300 GHz and wavelengths from 1 cm to 1 m. It is situated between X-ray and infrared rays in the electromagnetic spectrum (Letellier and Budzinski, 1999a). Initially, the two main applications of microwaves are as energy vectors and for communication purposes. However, it was later developed to be use in applications of food technology especially for the purpose for heating. Microwave is formed through two perpendicular fields which are magnetic field and electric field (responsible for heating) (Letellier and Budzinski, 1999a). Generally, conventional heating depends on convection and conduction processes in which case most of the energy is being lost or release to the environment. However, in the case MAHD, heating specifically takes places in selective and targeted materials. There is therefore no significant heat loss to the environment as the process occurs in a closed system. This excellent mechanism may practically shorten the extraction period (Huie, 2002).

The basic scientific principle of heating using MAHD depends on its direct interaction with polar solvents. During microwave heating, two phenomena are called to play which are ionic migration and dipole rotation. Most often, these phenomena occurs simultaneously (Letellier and Budzinski, 1999a and Letellier et al., 1999b). Ionic migration is the electrophoretic conduction of ions by the influence of changing electric field. This phenomenon occurs when a solution offers resistance to the migration of ions thereby producing friction which may bring about the heating up of the solution. On the other hand, dipole rotation stands for the realignment of dipoles of a molecule to an oscillating electric field. Heating is generally effective at a microwave frequency of 2450 MHz with wavelength of 12.2 cm and the domestic microwave is operated at this conditions. The electric component of the wave changes rapidly at 4.9 x $10^4$ times per sec at this frequency (Mirza et al., 2003). At this frequency, the solvent molecule tries to orientate itself with the electric field in order to maintain itself in the same phase. Unfortunately, rapidly changing electric component of the wave causes the molecules to fail its reorientation thereby causing it to vibrate vigorously and produce heat through frictional force.

On the other hand, when the microwave frequency is higher than 2450 MHz, the electrical component of the waves increases its speed. Due to this, the solvent molecules do not get the opportunity or enough time to initiate orientation with the external electric field. Thus there is no occurrence of friction for the creation of any heating process. In another vein, if the frequency is lower than 2450 MHz, the electrical component of the waves decreases its speed and as a result, the molecules get excessive time to align itself with the electric field and therefore become slowed down. Thus there would be no occurrence of heat at all. This indicates that only the dielectric solvents or materials that have permanent dipoles can be heated up via microwave. The heating efficiency of the solvents in microwave is based on the dissipation factor (tan δ). Dissipation factor refers to the capability of the solvent to absorb microwave energy and converted it to heat energy which is transferred to the surrounding molecules (Mirza et al., 2003). The dissipation factor is illustrated in Eq. 2.3.

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \tag{2.3}$$

where,

tan δ is the dissipation energy
$\varepsilon''$ is the dielectric loss
$\varepsilon'$ is the dielectric constant

The dielectric loss refers to the ability to convert microwave energy to heat energy whereas dielectric constant stands for measure of the ability to absorb microwave energy. The dielectric properties of some commonly used solvents in extraction are tabulated in Table 2.2. From the table, it can be seen that solvents like ethanol and methanol exhibit lower ability to absorb microwave energy compared to water as depicted by their lower value of ε'. However, the overall heating efficiency for both solvents are higher than for water as depicted by their greater tan δ values. On the other hand, hexane and other less polar solvents such as chloroform are transparent to microwave and therefore do not generate heat.

Table 2.2
Dielectric properties of commonly used solvents in extraction

| **Solvents** | **Dissipation factor (tan δ) at 2.45 GHz** | **Dielectric constant[a] (ε')** | **Dipole moment[a] (debye)** |
|---|---|---|---|
| Acetone | - | 21.4 | - |
| Ethyl acetate | - | 6.02 | 1.88 |
| 1-Butanol | 0.571 | - | 1.66 |
| Ethanol | 0.941 | 25.7 | 1.69 |
| Methanol | 0.659 | 33.7 | 1.70 |
| Diethyl ether | - | 4.39* | - |
| Hexane | - | 1.88 | <0.10 |
| Chloroform | - | 4.80 | - |
| Water | 0.123 | 80.4 | 1.84 |

[a] represents determined at 20 $^{0}$C and * at 18 $^{0}$C

Adapted from: Desai et al. (2010)

### 2.6.2 Principle of MAHD Extraction

When heat is applied via microwave to the moisture (water) inside the cells of plant materials, there is evaporation, expansion and subsequent generation of high pressure on the oil gland cell wall (Wang and Weller, 2006). The generated internal pressure pushes out the oil gland cell wall, and intensively stretches out the wall to the extent that it might become ruptured. This would ease the process of release or leach out of essential oil from the plant material to the surrounding solvent (water). This phenomenon can be enhanced if the plant material is soaked in solvents like water with high microwave heating efficiency (higher dissipation factor value). Cellulose which is the active constituents in most of the plants may therefore be transformed into soluble fractions within few minutes. The high temperature absorbed by the plant cell wall may enhance the dehydration process of cellulose and decrease the mechanical strength of the plant. As a result, the solvent from the surrounding can easily enter into the cell wall (Latha, 2007).

A number of researches have been conducted to investigate the morphology of oil gland cells of some plants before and after MAHD extraction. These glands were observed with the help of scanning electron microscopy (SEM) techniques. In a particular research changes to the oil glands of *Thymus vulgaris* L. was studied (Golmakani and Rezaei, 2008). Similarly, surface of orange peel oil gland after MAHD and HD extraction was investigated (Ferhat et al., 2006). These studies shows that the oil cell glands after MAHD extraction shows mild disruption of the oil cell gland which was associated with the nature of heat distribution during MAHD extraction.

During MAHD extraction, the microwave energy is being highly absorbed by water (high dielectric properties). This energy is then converted into heat energy which is thereafter transferred to the plant material (Desai et al., 2010). Transfer of this heat energy can bring about a localization of heat at desired parts of the plant material especially the portion containing the oil gland. The oil glands can therefore conveniently release its oil without experiencing much damaging rupture as shown in Figure 2.5. This is not the case with conventional methods such as HD in which case higher levels of damaging rupture are often experienced by the glands of the plant material as can be seen in Figure 2.6. This have been attributed to the nature of heat distribution by HD in which case heat energy first reaches the solvent surface before heating the targeted plant material (Desai et al., 2010). This can therefore lead to explosive rupture of the glands in order to retrieve the oil (Ferhat et al., 2006 and Golmakani and Rezaei, 2008).

Generally, the effect of microwave irradiation is strongly influence by the dielectric properties of both the plant matrix and solvent (Zuloaga et al., 1999). In most cases, the plant material is soaked into single solvent or a mixture of solvents that have high dielectric properties prior to the extraction in order to strongly absorb the microwave energy (Zuloaga et al., 1999). As the microwave irradiation intensity increases, the temperature rises alongside with it thereby easing the process of penetration of the solvent into the plant matrix. Essential oils are therefore released to the surrounding solvent.

However, in some special cases, where the crude extract contains highly thermolabile components, the plant material is usually soaked into solvents which are transparent to microwave. These solvents such as hexane and chloroform can therefore help to prevent degradation of the bioactive constituents (Letellier and Budzinski, 1999a; Letellier et al., 1999b; Wang and Weller, 2006 and Zuloaga et al., 1999).

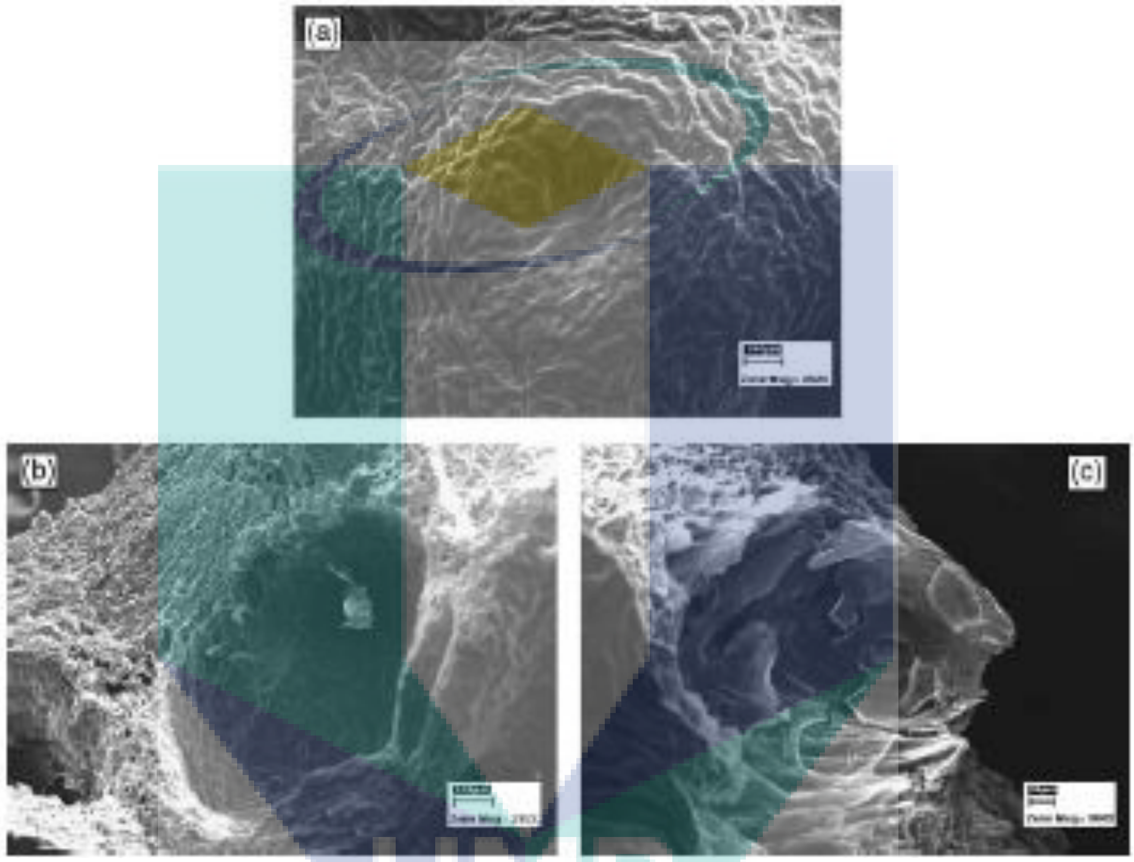


Figure 2.5: Scanning electron micrographs of orange peel (a) untreated, (b) after MAD for 30 min and (c) after HD for 3h.

Source: Ferhat et al. (2006)

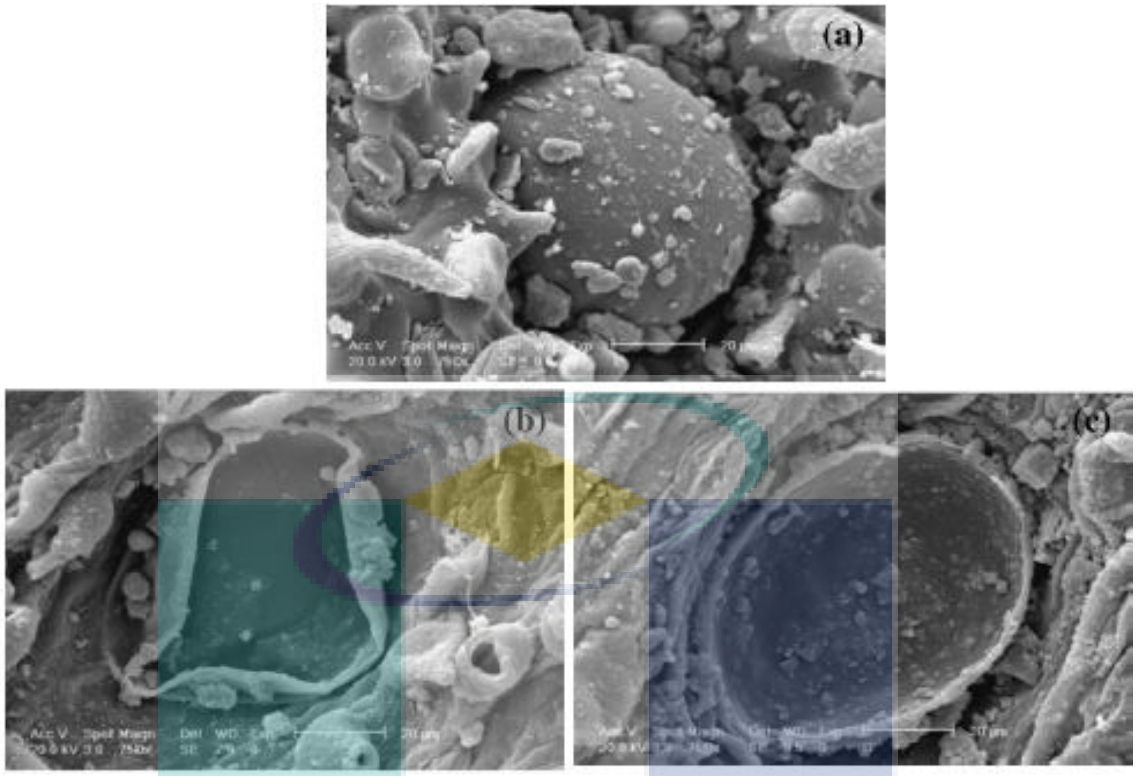


Figure 2.6: Scanning electron micrographs of thyme leaves for (a) untreated, (b) after HD (60 min) and (c) after MAHD (30 min).

Source: Golmakani and Rezaei (2008)

### 2.6.3 Mechanism of Microwave Assisted Hydrodistillation

The basic scientific mechanism of the MAHD method is different from those of conventional methods. Extraction in MAHD takes places with the aid of electromagnetic waves which changes the structure of oil gland cells. There is a synergistic effect of two transport phenomena such as heat and mass transfer which are acting in the same direction. This may therefore result in rapid production of extracts from the plant material (Chemat et al., 2006). In the case of conventional method, both mass and heat moves in opposite direction. Thus, the mass transfer takes place from inside to outside whereas heat is transferred from the surrounding (outside) to inside of the substrate as illustrated in Figure 2.7. Furthermore, in conventional extraction heat is being supplied from the heating source to the interior of plant material sample, whereas, in MAHD heat is volumetrically distributed inside the irradiated medium.

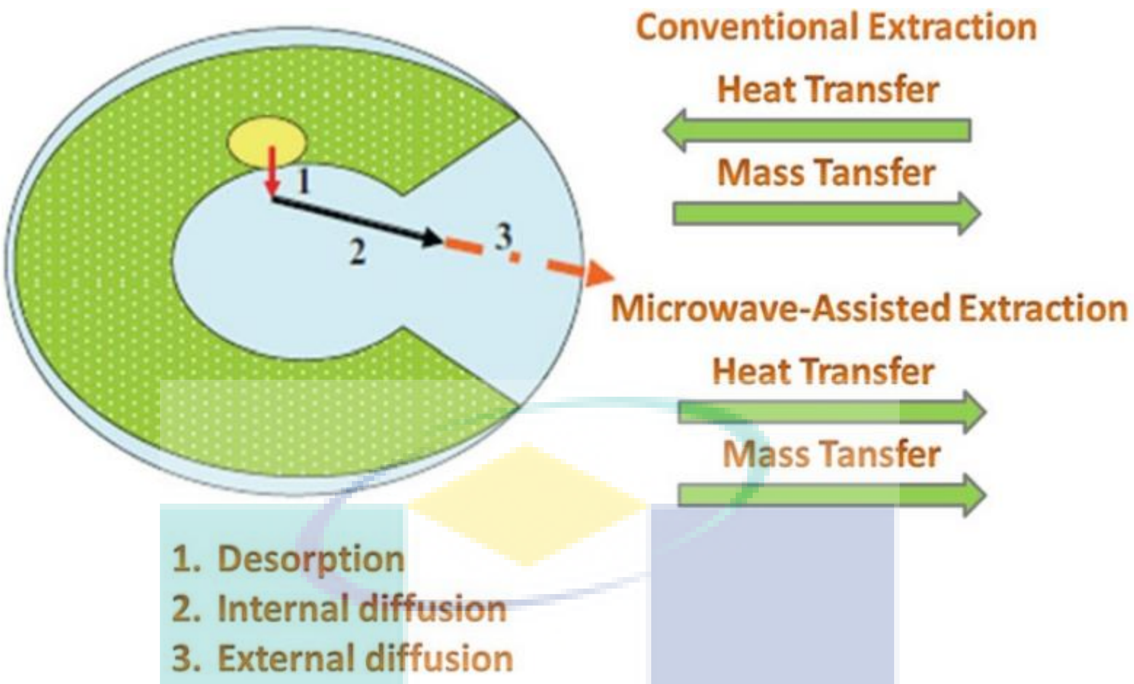


Figure 2.7: Fundamental mechanism of mass and heat transfer in conventional and microwave extraction.

Source: Veggi et al. (2012)

### 2.6.4 Heat Transfer in Microwave Assisted Hydrodistillation

Generally, due to the water content in plant material, they may be termed as poor electrical insulators. In essence, they can store and distribute energy when microwave is irradiated on them. However, the microwave energy radiated is in itself not a thermal energy. Rather, the heating is the combination of electromagnetic radiation formed with the dielectric properties of the materials when it is subjected to electromagnetic field. Dielectric properties therefore act as an essential parameter in determining the interaction between plant matrix and the electric field (Wang and Weller, 2006). The rate of conversion of electrical energy into heat energy in plant materials is described in the following Eq. 2.4 (Latha, 2007).

$$P = K.f\varepsilon' E^2 tan\delta \tag{2.4}$$

where,

P is the microwave power distribution per volume unit, W/m$^3$

| | |
|---|---|
| K | is the constant |
| $f$ | is the frequency applied, Hz |
| $\varepsilon'$ | is the dielectric constant of material used |
| $E$ | is the electric field strength, V/m |
| $tan\delta$ | is the dielectric loss tangent |

It is assumed that heat transferred from the plant material to the surrounding solvent (water) is a rapid process which considers water and the plant matrix to have the same temperature (Fang and Lai, 1995). Hence, the volume rate of heat generation of plant matrix is calculated using energy balance equation illustrated in Eq. 2.5.

$$q_{MW} = \frac{hA}{V}(T - T_a) + \frac{\varepsilon\sigma A}{V}[(T + 273.16)^4 - (T_a + 273.16)^4] + \rho C_p\left(\frac{dT}{dt}\right) \qquad (2.5)$$

where,

$\frac{hA}{V}(T - T_a)$ is the heat loss by convective heat transfer to the surrounding

$\frac{\varepsilon\sigma A}{V}[(T + 273.16)^4 - (T_a + 273.16)^4]$ is the radiative heat loss from the wall surface

$\rho C_p\left(\frac{dT}{dt}\right)$ is the heat accumulation in the cinnamon matrix

The contribution of the first and second terms from the Eq. 2.5 is very much smaller compared to the third term. Besides that, the heat produced by the glass reactor is assumed to be negligible as it has only minimum mass at the same low dielectric constant. Therefore, in calculating the volume of heat generation, $q_{mw}$, the density (ρ) and heat capacity ($C_p$) of the plant matrix were calculated based on the mixing rule.

After eliminating the heat loss by convective and radiative heat loss, the volume rate of heat generation can be derived. Volume rate of heat generation is a critical term for analysing the efficiency of microwave heating properties. This term interrelates temperature rise and dielectric properties of the plant matrix. Volume rate of heat generation of the matrix can therefore be calculated using the following formula.

$$Q_{mw} = \rho_{mix}\ x\ C_{p\ mix}\ x\ \frac{dT}{dt} \qquad (2.6)$$

where,

$Q_{MW}$ is the volume rate of heat generation, cal/ $cm^3$.sec

$\rho_{mix}$ is the density of plant matrix mixture, g/$cm^3$

$C_{p\ mix}$ is the heat capacity of plant matrix, cal/ $^0$C.g

$\frac{dT}{dt}$ is the rate of temperature increase, $^0$C/ sec

To apply Eq. 2.6, the density (ρ) and specific heat capacity ($C_p$) of the matrix mixture was calculated by the following simple mixing rules:

$$P_{mix} = \rho_W \emptyset + \rho_s (1 - \emptyset) \tag{2.7}$$

$$C_{p\ mix} = C_{p,W} \emptyset + C_{p,s} (1 - \emptyset) \tag{2.8}$$

where,

Φ is the volume fraction

Generally, the rate of heat generation is influenced by the dielectric properties. Three dielectric properties that are predominantly used in microwave heating are tangent loss, dielectric constant and dielectric loss. Tangent loss can be measured by dividing dielectric loss by dielectric constant. The dielectric constant and dielectric loss of water was calculated by using the following models illustrated in Eqs. 2.9 and 2.10 respectively. The value of dielectric constant and dielectric loss of the plant material (cinnamon bark powder) was reported as 2.8 and 0.38 respectively by (Khan and Chandel, 2011) at 2450 MHz.

Simple mixing rule was then applied to calculate the total dielectric properties.

$$\varepsilon_w' = 85.215 - 0.33583T \tag{2.9}$$

$$\varepsilon_w'' = 320.685T^{-1.0268} \tag{2.10}$$

where,

$\varepsilon'_w$ is the dielectric constant of water,

$\varepsilon''_w$ is the dielectric loss of water

T is the temperature ($^0$C)

On the other hand, penetration depth, $D_p$ at a particular frequency is influenced by the dielectric properties of material matrix in an inverse manner. The depth of penetration is defined as the depth into the plant matrix at which the power density has decrease to 1/e (about 37 %) of its surface value. Eq. 2.11 illustrated the penetration depth:

$$D_p = \frac{\lambda_{\circ}\, x \sqrt{\varepsilon\prime}}{2\pi\varepsilon\prime\prime} \tag{2.11}$$

where $\lambda_{\circ}$ is the wavelength of microwave energy at 12.24cm for 2450 MHz.

### 2.6.5 Types of MAHD

Two types of MAHD systems are commercially available which are closed vessel microwave extraction and open vessel microwave extraction (Luque Garcıa and de Castro, 2004). The closed vessel microwave extraction operates under controlled temperature and pressure. On the other hand, the open vessel microwave extraction is also known as focused microwave assisted oven or focused microwave assisted Soxhlet or solvent extraction (FMASE). In this case, the reactor (extraction vessel) that contains the plant material is irradiated with microwave and the Clevenger apparatus is exposed to the atmosphere. This system may either be in single or multimode (Luque Garcıa and de Castro, 2003). A multimode system permits random dissipation of microwave radiation within the microwave cavity such that every part in the cavity including the plant materials sample is evenly radiated. Whereas in single mode otherwise known as focused system the microwave radiation is localised at particular points. Thus the plant sample can be exposed to greater electric field compared to multimode system. Modified multimode domestic microwave oven works as an open vessel extraction. The microwave heating equipment for either multimode or single mode consists of four major components. These components and their functions are tabulated in Table 2.3

Table 2.3
Major microwave parts and functions

| **Microwave parts** | **Function** |
|---|---|
| Microwave generator | Also known as magnetron which generates microwave energy |
| Wave guide | Used to transmits the microwave from the source to the microwave cavity |
| The applicator | Also known as reactor where plant sample is placed |
| Circulator | Permits the microwave to move only in the forward direction |

There are few benefits which may be obtained by using the closed vessel system which includes the fact that this system can attain higher temperature compared to open vessel systems. This is because the internally risen pressure might increase the boiling rate of the solvents used. Also, this temperature increase may tend to reduce the extraction time in microwave applications. Furthermore, this system may ensure less possibility for loss of volatile constituents during microwave extraction. Less solvent is being used in this system since no evaporation process occurs during extraction. This system can also help to avoid the risk of airborne contamination on the extracted extracts. The fumes produced during closed microwave extraction using chemical solvents are trapped within the vessel. Thus, there is no requirement for fume hood to be customized for this process.

However, this system also has its drawbacks such as the use of high pressure which may lead to possible explosion thereby making it a highly risky process. Also, limited amount of plant sample can be subjected for extraction purpose at a time. Furthermore, the commonly used PTFE (polytetraflouro ethylene) type of reactor cannot withstand very high temperatures.

Usually open vessel microwave extraction has more advantages than close vessel extraction, especially in the effective sample preparation. Open vessel extraction operates under atmospheric pressure whereas close vessel extraction operates under induced pressure. Thus the open vessel considered as safe primarily because it operates at atmospheric pressure with openly attached apparatus.  In this system, solvents can be added any time during the extraction process. Apart from that, various types of vessels could be used in this system such as glass, quartz and PTFE. In fact, this system has the ability to extract large amount of samples without any aid of cooling down and depressurization equipment. Therefore, this system ensures low cost of devices. This process goes through leaching cycles until it attains the required amount of targeted extract. It is a fully automated and it is more suitable for thermolabile substances as it uses lower temperature than closed vessel system. Despite these advantages, the open vessel extraction system also has few drawbacks.

## 2.7 Effects of Operating Factors on Efficiency of MAHD

There are several factors which may influence the efficiency of extraction using MAHD as well as the quality and quantity of oils which may be obtained through this technique. These include extraction time, microwave power level and the nature and volume of solvents used in the extraction process.

### 2.7.1 Nature and Volume of Solvent

Selection of suitable solvent for extraction is essential in order to obtain the desired extraction yield. Usually the type of solvent used in extraction process depends on the solubility of the target analyte, the interaction between solvent and plant material and also the dielectric properties of the solvent (Letellier et al., 1999b). For isolation purpose the choice of solvent is preferable to have great selectivity on the target compound instead of other matrix compounds. In another vein, the extracting solvent must be compatible with the chromatographic analytical procedure. The solvent used in conventional extraction method could be used in microwave assisted extraction. However, the extraction yield obtained from both processes may differ. For instance, ginger extracted via microwave assisted extraction using hexane produced lesser yield compared to Soxhlet extraction method (Alfaro et al., 2003). On the other hand extraction of oil from different plant material using ethanol via microwave assisted extraction produced significantly higher yield than Soxhlet ethanol extraction. This can be associated with the differences in dielectric properties of the solvent. Hexane is a solvent which is transparent to microwave and does not heats up the solvent in microwave environment, but ethanol exhibits excellent microwave absorbing ability and therefore heats up the solvent rapidly and improve the extraction process. In essence, dielectric properties play an essential part and influence the selectivity and separation efficiency in microwave assisted extraction.

In some cases, two solvents with different dielectric properties may be mixed so as to produce a synergistic dielectric property needed for the microwave extraction solvent. For instance, ethanol is a good microwave absorber (ε' = 25.7) but not a good extraction solvent for particular constituent like solanesol (Zhou and Liu, 2006). On the contrary, hexane is a good extraction solvent but unfortunately transparent solvent for microwave extraction. Therefore, hexane and ethanol may be mixed in different ratios in order to make them suitable for microwave assisted extraction process. The extraction of solanesol from tobacco leaves have been carried out by using ratio 1 to 3 of hexane to ethanol respectively and the yield obtained was reasonably high. However, for the same extraction of solanesol from tobacco by using ethanol alone solvent a low yield was obtained.

Presently, instead of using chemical solvents, most researchers are considering the use of water which is more environmentally friendly. For MAHD, water is the solvent of preference and that explains the name the process i.e. microwave is being used to assist hydro (water) distillation. Water is a suitable solvent to use in microwave extraction because it has high dielectric properties (ε' = 80.4) which makes it highly susceptible to microwave irradiation (Wang et al., 2006). Recently, solvent free microwave assisted extraction has been developed in order to extract volatile oils from the aroma plants in which the natural moisture content present in the plant material acts as the heating source and no additional extraction solvent are used for this techniques (Wang, et al., 2006). Apart from the solvent selection, the volume of solvent used for MAHD extraction is also very important.

The fundamental principle of solvent in microwave extraction is that the volume of solvent used to immerse the plant material in microwave extraction must be sufficient throughout the entire irradiation period. A lot of varying reports exist regarding the ratio of volume of solvent to plant material that should be used for MAHD extraction (Pan et al., 2000; Xuejun Pan et al., 2003 and Ranitha et al., 2014). Generally, a higher volume of solvent to plant material may be effective to the conventional extraction methods. However, for the case of microwave it is the reverse as confirmed by a particular research (Wang and Weller, 2006). In another research on the optimization of pectin extraction through microwave assisted extraction method, the effects solvent volume was investigated (Wang et al., 2007). It was found that at lower volume of solvent higher yield was obtained compared to higher volume. Conversely, in the research on microwave assisted extraction of artemisnin from Aretimisia annua L., it was reported that higher extraction rate was obtained by using larger volume of solvent (Hao et al., 2002). Likewise, microwave assisted extraction of flavonoids from S. Medusa dried cell cultures was carried out. It was found that as the solvent to solid ratio increases from 25:1 (mL/g) to 100:1 (mL/g), the yield also increased (Gao et al., 2006). However there was the caution that excess solvent may lead to unwanted energy consumption and more time will be required to condense the extraction solution during the purification process. Hence, the liquid to solid ratio of 50:1 (mL/g) was found to be the optimum ratio for extracting high yield of flavonoids from the S. Medusa dried cell cultures. In several other researches, a ratio of about 10:1 (mL:mg) to 20:1 (mL:mg) was reported to be the optimum (Li et al., 2004; Pan et al., 2001; Xuejun Pan et al., 2003 and Talebi et al., 2004). Hence, due to these irregularities in the values presented, there is need for meticulous pre extraction studies in order to deduce the actual volume of solvent necessary for each extraction process

### 2.7.2 Extraction Time

In MAHD extraction, extraction time is another critical factor. Basically, the extraction time is directly proportional for the quantity of analytes extracted. As the extraction time increases, the amount of extracted analytes increases, although there is a potential risk for the plant material to be thermally degraded. Usually 15 to 20 min of extraction period is sufficient to recover full yield of volatile oils, but even 40 sec have been reported to produce good recovery (Li et al., 2004 and Wang et al., 2007). The extraction of caffeine and polyphenols via microwave assisted extraction reported to attain maximum yield at 4 min and then later reduce with increasing time (Xuejun Pan et al., 2003). Also in the extraction of artemisnin from Artemisia annua L. the highest yield of 92 % was attained at 12 min and later the extraction yield decreased (Hao et al., 2002). Reason for the decreased yield at increasing extraction time have been accrued to possible thermal degradation of active constituents from volatile oils over prolonged extraction process. Similar observation has been reported in extraction of phytocompounds from *Clinacanthus nutans* Lindau via microwave assisted extraction (Mustapa et al., 2015).

However, some extraction reports show that extraction time does not really influence the yield obtained. One of such research is the extraction of active components from pepper samples, various length of time were examined. Extraction period from 5 to 20 min with an increment of 5 min were studied. The results demonstrated that after 5 min, there is was no significant increment in the yield of capsaicinoids (Barbero et al., 2006). Thus a deliberate pre-extraction investigated is necessary to determine the optimum extraction time. This is because the extraction time may vary with different plant materials and parts used for extraction. It should be noted however that microwave irradiation time is influenced by the dielectric properties of the solvents used in extraction. For instance, solvents like water, methanol and ethanol may heat up the plant matrix more rapidly under microwave and shorten the induction time compared to other solvents which are transparent to microwave.

### 2.7.3 Microwave Irradiation Power or Temperature

In MAHD technique, microwave power is an essential factor to be taken into account. Increase the microwave power level may cause the plant matrix immersed in water to absorb great amount of microwave irradiation. This would enhance the rapid heating up of the plant materials, thereby enhancing the extraction rate. In fact, the irradiation power often influence the extraction time. Generally, at higher irradiation power, the solvent (water) will absorb more microwave energy and turns it into thermal energy. This can therefore help in rapid rupture of oil gland in plant materials such that the volatile oils are leached out to the bulk solution. Conversely, at lower irradiation power, longer time may be required for rupture of the oil gland cells to occur due to short wave length.

## 2.8 Characterization of Essential Oil

After extraction of volatile oil from plant material, the oil needs to undergo some analysis in order to evaluate the efficiency of the extraction methods as well as the quality of the oil extracted. Generally, the most commonly used analysis method is gas chromatography-mass spectrometry (GC-MS) analysis. Other analysis techniques include scanning electron microscope (SEM) and Fourier transform infrared spectroscopy

(FTIR) among others. The purpose of the equipment used for these analyses are tabulated in Table 2.4.

Table 2.4
Functions of equipment used for essential oil analysis

| Equipment used for analysis | Function |
|---|---|
| Gas chromatography-mass spectrometry | Examine the quality of cassia oil extracted through HD and MAHD and analyse the chemical composition present in the oils. |
| Scanning electron microscope | Examine physical changes of cinnamon bark oil glands before and after extraction |
| Fourier transform infrared spectroscopy | Analyse the functional groups present in HD and MAHD extracted cassia oil |

### 2.8.1 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is an instrumental analytical technique which combines gas chromatography (GC) and mass spectrometry (MS). It is used to analyse complex biochemical and organic mixtures (Skoog et al., 2007). GC is capable of separating volatile and semi-volatile chemical compounds but cannot identify them. On the other hand, MS provides detailed chemical structural information on most of the compounds such that they can be identified and quantified. Therefore, GC and MS analytical techniques are coupled to obtain an analytical technique which can separate, identified and quantified complex chemical compound mixtures. This technique provides easy analysis for many relatively low molecular weight compounds especially essential oil from aroma plants or herbs. As stated, this technique is one of the most applied analytical techniques used for identification of the chemical compounds in volatile oils.

This method has been able to obtain viable information on chemical structures and their percentage composition in essential oil samples. There have been reports on analysis of the chemical composition of compounds present in essential oil obtained from *C. cassia* barks (Geng et al., 2011 and Li et al., 2013b). The oil was extracted through HD method and the efficiency of the extraction technique on compositional quality of the oil was investigated by using GC-MS. Report from both studies revealed that transcinnamaldehdye is the active compound in the oil. Total of 41 compounds were identified with a compositional value of 92.10–99.60 % (Geng et al., 2011). Similarly, 37 chemical compounds were identified with a range of 87.72–97.86 % essential oil content (Li et al., 2013b). Thus GC-MS is considered a suitable technique for investigating the composition of chemical compounds present in essential oils (Geng et al., 2011 and Li et al., 2013b).

### a) Principle of Gas Chromatography-Mass Spectrometry

Two types of phases are present in GC which is mobile and stationary phase. The mobile phase is a carrier gas like inert gas (helium or nitrogen) while stationary phase is a polymer or microscopic layer of liquid on an inert solid support or metal tubing. The stationary phase is commonly referred to as the column. The capillary column has a stationary phase with a solid support coated with non-volatile liquid. As sample pass through the column via steam of helium gas, chemical compounds present in the sample will be separated from each other. This is made possible due to difference in physicochemical properties of chemical compounds. This difference may cause them to take different time to pass through the column.

Thus the chromatography column separates the sample into pulse of pure chemicals according to their volatility by allowing an unreactive gas such as helium (mobile phase) to pass through them. This gas would therefore carry the sample through a stationary phase that is fixed in the column (Skoog et al., 2007). When the sample exits the end of the GC column, it is fragmented by ionization. The fragments are then identified, quantified and classified based on their mass-to-charge ratio (m/z) leading to the generation of a fragmentation pattern. Generally, the fragmentation pattern for each chemical compound is unique like the retention time (RT). Hence, one can easily identify a chemical compound based on their mass and specific retention time. The spectra obtained are saved and further analysed using some computer software.

### 2.8.2 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) uses a focused beam of high energy electrons rather than light to create many different signals at the surface of a solid sample. The signal obtained from electron to sample interactions give characteristic information on topography (surface features of a sample and its texture), external morphology (shape and size of the particles making up the sample), crystallographic information (orientation of atoms arrangement in a sample) and composition (relative amount of elements and compounds present in a sample). The data may be collected over a particular selected area of the surface of the test specimen. Test specimen surface area in the range of 1 cm to 5 µm in width can be imaged under SEM with magnification ranging from 20X to 30,000X with spatial resolution of 50 to 100 nm.

### a) Principle of Scanning Electron Microscopy

The principle of SEM is that electrons are created at the top of the microscope by a metallic filament also known as the electron gun. The emitted electrons are then turned into a beam and accelerated down the column toward the test sample. The beam is further focused and directed by electromagnetic lenses as it moves down the column. As the beam reaches the test sample, electrons are knocked loose from the surface of the test sample. These released electrons otherwise known as secondary electrons detected or visualised by a detector which amplifies the signal and send signals to a monitor. The electron beam scans top and bottom across the test sample developing an image from the variety number of electrons emitted from each spot on the sample. This whole process occurs inside a vacuum and ensures that the electrons beams do not interact with air but only on test samples. Therefore, the test sample should have the ability to withstand a vacuum and need to be conductive. Samples that are not conductive may be coated with a thin layer of conductive material like gold by a process known as sputter coating. The technique has been used to examine the morphological structure of plant materials like orange peels (Ferhat et al., 2006) and thyme (Golmakani and Rezaei, 2008). These studies were conducted to provide rupture evidence of essential oil glands after extraction by MAHD and HD.

### 2.8.3 Fourier Transforms Infrared Spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) has widely been used in structure analysis of either synthesized chemicals or natural origin materials. Also, its applicability spread in qualitative as well as quantitative analysis in most fields of science (Sawant et al., 2011). Generally, infrared spectroscopy is the analysis of interactions between test samples and electromagnetic (EM) field in the IR frequency range. In this spectral phase, the EM waves majorly combine with molecular vibrations. The probability of a specific IR frequency being absorbed is based on the actual interaction between the molecules and this frequency. Commonly, a frequency can be easily absorbed if its photon energy correlates with the molecules vibrational energy levels. Therefore, IR spectroscopy is an important technique which gives fingerprint information on the composition of a test specimen. FTIR give detailed and specific information on the rotation and vibration of the chemical bonding and molecular structure which become useful tool in analysing organic and certain inorganic materials. Researches have been conducted which takes advantage of this technique to analyse the chemical structure of several material products including essential oil (Li et al., 2013a,b). For instance, the chemical structures of cassia bark (Li et al., 2013b) and leaves (Li et al., 2013a) were investigated through FTIR. The spectra obtained were reported to correlate with and support the data obtained via GC-MS analysis.

### a) Principle of FTIR Spectrometer

The FTIR spectrometer operates through the emission of an infrared energy from a black body source. The beam then travels through a hole which helps to control the quantity of energy which is passed on to the test specimen. Spectral encoding thereafter takes place at a point called interferometer as the beam arrives the point. After encoding, a signal called interferogram leaves the interferometer and approaches the test sample. On arrival to the test sample, the signal can either be transmitted or reflected off the surface. At this stage, energy frequencies which are specific characteristic of the test sample are absorbed. After this, the beam travels to a detector which has been customized to measure the interferogram. After measurement, the signal is then digitized and sent to the computer Fourier transformation occurs.

### b) Infrared Spectra

The interpretation of infrared spectra is carried out by correlating the absorption bands in the spectrum of an unknown compound with the known absorption frequencies for specific types of bonds. Table 2.5 comprises some examples of absorption band, the characteristic chemical compound they represent and the specific chemical bonds.

Table 2.5
Some examples of infrared absorption frequencies

| **Bond** | **Type of compounds** | **Frequency range (cm$^{-1}$)** |
|---|---|---|
| C-H | Alkynes | 700-610 ($^{\wedge}$b) bend<br>3333-3267 (s) stretch |
| C-H | Phenyl ring substitution bands | 870-675 (s) bend |
| | Aromatic rings | 3100-3000 (m) stretch |
| | Phenyl ring substitution overtones | 2000-1600 (w) – fingerprint region |
| C-H | Alkenes | 1000-675 (s) bend<br>3080-3020 (m) stretch |
| C-H | Alkanes | 1470-1350 ($^{\#}$v) scissoring and bending<br>2960-2850 (*s) stretch |
| | CH3 Umbrella Deformation | 1380 ($^{x}$m-$^{+}$w) – Doublet-isopropyl, t-butyl |
| C=C | Alkenes | 1680-1640 (m, w) stretch |
| C=C | Aromatic rings | 1600, 1500 (w) stretch |
| C=C | Alkynes | 2260-2100 (w) stretch |
| C-O | Alcohols, carboxylic acids, esters, ethers | 1260-1000 (s) stretch |
| C=O | Aldehydes, carboxylic acids, esters, ketones | 1760-1670 (s) stretch |
| N-H | Amines | 1650-1580 (m) bend<br>3500-3300 (m) stretch |
| C-N | Amines | 1340-1020 (m) stretch |
| C≡N | Nitriles | 2260-2220 (v) stretch |
| NO2 | Nitro compounds | 1390-1260 (s) symmetrical stretch<br>1660-1500 (s) symmetrical stretch |

$^{*}$s – strong, $^{\#}$v – variables, $^{x}$m – medium, $^{+}$w – weak, $^{\wedge}$b – broad

The advantages of using FTIR include its high sensitivity due to its ability to measure all the frequencies simultaneously. Also FTIR technique is less energy consuming, and has high resolution and accuracy in collecting the data. However, there are some shortcomings exhibit such as provide minimal elemental information for some samples, the molecules should be active in the IR phase and background solvent or solid matrix required to be transparent.

## 2.9 Bioassay

There are several bioassays which are commonly conducted in order to detect the toxicity of plant extracts like insecticidal bioassay against larvae of Aedes aegypti (Vera et al., 2014), disk diffusion test (Sharifi Rad et al., 2015) and larvicidal bioassay on Aedes albopictus Skuse (Liu et al., 2014) and brine shrimp lethality assay (Sandanasamy et al., 2014). The brine shrimp lethality assay is a simple and useful tool for preliminary assessment of toxicity. The results obtained from this bioassay may be used for screening the potential pharmacological activities of plant extracts (Sandanasamy et al., 2014). Also, this method enhances the substitution of laboratory animals in bioassay thereby protecting the existence of these on one hand and reducing cost on the other hand.

The brine shrimp lethality bioassay was initiated by (Michael et al., 1956) and later was modified and developed by (Vanhaecke et al., 1981), then by (Sleet and Brendel, 1983). This assay has been widely applied in detection of plant extract toxicity (Ullah et al., 2013), heavy metals (Ates et al., 2013), pesticides (Zubaira and Oladosu, 2015), cyanobacteria toxins (Guidi Rontani et al., 2014) and cytotoxicity testing of dental materials (Pelka et al., 2000). While most of these researches were conducted using the hatched nauplii, few others have been carried out using dormant cyst (with embryos that are metabolically not active) (Migliore et al., 1997).

### 2.9.1 Growth of *Artemia salina*

Brine shrimp, *Artemia salina* is a very small aquatic animal that lives in high salinity environment. The life cycle of brine shrimp begins with a cyst which develops to nauplii, then to sub-adult (larvae) and at last fully grown adult. Schematic diagram of the life cycle of *A. salina* is depicted in Figure 2.8. Cysts are inhibition of brine shrimp babies encased in embryos that are metabolically inactive (Kanwar, 2007). Usually, cysts are spherical in shape with average diameter in the range of 250-350 µm. During the hatching process, the cysts break and free swimming larvae is release to the hyper salinity environment. Nauplii are the first stage in the life cycle of brine shrimp just after they have been hatched from cysts. Nauplii reach its maturity within 18–21 days at temperature around 20 $^0$C (Montague, 1977). The rate of hatching as well as maturation is influenced by temperature. *A. salina* exhibits two types of reproduction modes based on the environmental conditions. When the environmental conditions such as temperature and salinity are suitable, *A. salina* engages in ovoviviparous reproduction. On the other hand, if the environmental conditions are not too favourable such as low oxygen level and hyper salinity, *A. salina*, would engage oviparous reproduction. During oviparous, *A. salina* develops embryos which are later released as eggs (Nimura, 1967 and Sorgeloos and Persoone, 1975). Hence, environmental condition is a very essential factor in reproduction, hatching and maturity process of *A. salina*. Table 2.6 comprises few studies of brine shrimp lethality test on *Cinnamomum* species. From the table, it can be observed that there has so far not been any study on *C. cassia* bark oil extracted through MAHD. This makes it a potential area for research investigation.

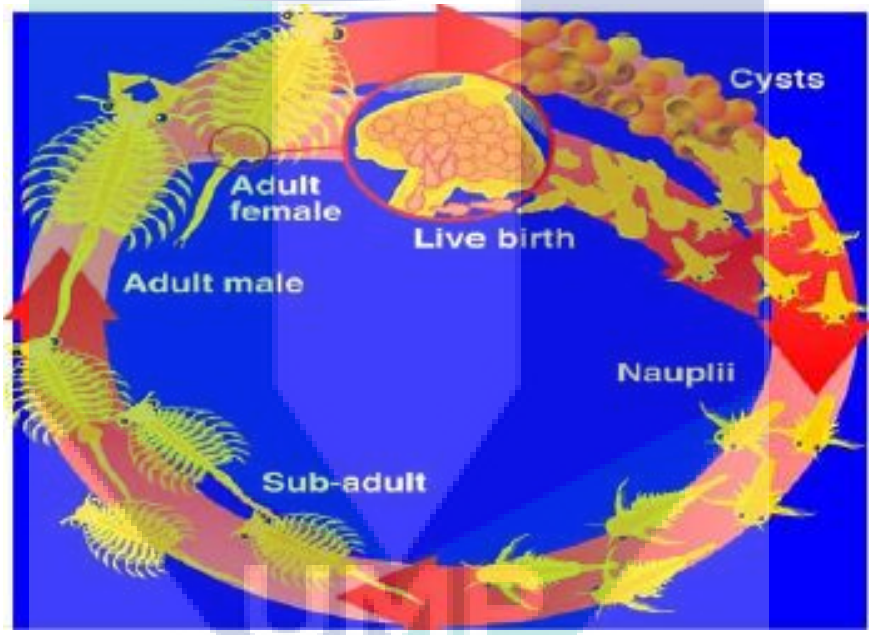


Figure 2.8: Life cycle of *Artemia salina* brine shrimp.
Source: Sorgeloos and Persoone (1975)

Table 2.6
Summary of brine shrimp lethality test on *Cinnamomum* species

| **Plant Species** | **Plant Parts** | **Extraction technique** | **Biological activity** | **Findings** | **References** |
|---|---|---|---|---|---|
| *C. zeylanicum* Blume<br>*Zingiber officinale* Rose | Bark<br>Rhizome | Hydrodistillation<br>Percolation (chloroform, petroleum ether, methanol) | Brine shrimp lethality assay | • Test sample examined at 10, 100 and 1000 µg/mL.<br>• The $LC_{50}$ values follow the order from chloroform, essential oil and petroleum ether extracts of cinnamon of 9, 10 and 18 µg/mL respectively.<br>• The $LC_{50}$ values follow the order from essential oil, petroleum ether, methanol and chloroform extracts of ginger of 0.03, 4.03, 7.90 and 8.89 µg/mL respectively.<br>• The cytotoxicity activity works better in cinnamon and ginger extracts compared to potassium dichromate ($LC_{50}$ = 27.75 µg/mL).<br>• All the fractions from cinnamon and ginger concluded to possess high cytotoxicity.<br>• Recommendation: Separation and identification of particular active components that in line with cytotoxicity. | Sharififar et al., 2009 |
| *C. osmophleum*<br>*Taiwania cryptomerioides*<br>*Cunninghamia lanceolate*<br>*Cryptomeria Japonica*<br>*Calocedrus formosana* | Heartwood<br>Sapwood<br>Bark<br>Leaf | Hydrodistillation | Brine shrimp lethality assay | • The $LC_{50}$ value of all the extracted essential oil recorded lesser than 200 µg/mL.<br>• The high $LC_{50}$ values recorded was 3.9, 6.0 and 6.9 ig/mL which were extracted from bark of C. formosana, heartwood and bark of C. japonica respectively.<br>• Recommendation: Isolation of chemical compounds for specific bioactivity are required. | Cheng et al., 2006 |

Table 2.6 continued.

| **Plant Species** | **Plant Parts** | **Extraction technique** | **Biological activity** | **Findings** | **References** |
|---|---|---|---|---|---|
| *Trachyspermum ammi*<br>*Ferula asafoetida*<br>*Amomum subalatum*<br>*Elettaria cardamomum*<br>*Cinnamomum zeylanicum*<br>*Cinnamomum tamal*<br>*Syzygium aromaticum*<br>*Coriandrum sativum*<br>*Litsea cubeba*<br>*Cuminum cyminum*<br>*Anethum sowa*<br>*Foeniculum vulgare*<br>*Zingiber officinale*<br>*Myristica fragrans*<br>*Myristica fragrans*<br>*Piper nigrum*<br>*Piper longum*<br>*Rosmarinus officinalis*<br>*Artemisia dracunculus*<br>*Thymus serpyllum*<br>*Zanthoxylum armatum* | Seed<br>Rhizome exudation<br>Fruit<br>Bark<br>Leaf<br>Bud<br>Berry<br>Peeled rhizome<br>Kernel | Hydrodistillation | Brine shrimp lethality assay | • The $LC_{50}$ value less than 1 consider as extremely toxic and suggested in anticancer agents. Asafoetida oil and cumin oil recorded the values less than 1.<br>• The $LC_{50}$ value falls in the range of 1–100 classified as very high toxic. Essential oil extracted from cinnamon bark, cinnamon, ajowan, small cardamom, cubeb, clove, coriander, fennel, dill, mace, ginger, long pepper, nutmeg, tarragon, rosemary, thyme and zanthoxylum falls in the range of very high toxic which also might has the potential for cancer prevention.<br>• The $LC_{50}$ value in the range of 100–200, known as highly toxic essential oil. Large cardamom and black pepper falls in the range.<br>• Since, all of the spices falls in the range of above highly toxic region, it's all has the anticancer property.<br>• Recommendation: The quality of spices required to be standardize and further studies on their potent cytotoxicity compounds. | Bajracharya and Tuladhar, 2012 |

Table 2.6 continued.

| Plant Species | Plant Parts | Extraction technique | Biological activity | Findings | References |
|---|---|---|---|---|---|
| *C. pubescens*<br>*C. javanicum*<br>*C. iners*<br>*C. impressicostatum*<br>*C. mollissimum*<br>*C. porrectum*<br>*C. camphora* | Leaf<br>Bark<br>Root | Hydrodistillation | Brine shrimp lethality assay | • The essential oil extracted from leaves of *C. iners*, *C. impressicostatum* and *C. mollissimum* indicated very high toxicity.<br>• The reason for the high toxicity might be because of the present of high percentage of benzyl benzoate followed by combination of other major constituents like benzoyl alcohol, geraniol and (E)-methyl cinnamate.<br>• High toxicity in essential oil may advised that the combination of compounds like nonterpenes, monoterpenoids and sesquiterpenoids might be the attributions.<br>• The essential oil contains high toxicity in the range of 0–12, suggested to serve as bioactive materials.<br>• Recommendation: Reliable brine shrimp lethality assay may acts as initiator for pharmaceutical application. However, further specific studies requires for specific pharmacological application. | Jantan et al., 2008 |

## 2.10 Statistical Model

Many factors may influence the efficiency any extraction method. For MAHD extraction method, factors such as water to raw material ratio, extraction time and microwave power level often influence the extraction process. Most often, these factors interacts with one another making it difficult to solely justify the efficiency of the extraction method based on one single factor. Therefore, an optimization technique needs to be applied for determination of optimum operating conditions. Recently, response surface methodology (RSM) optimization technique by using Design Expert software has attracted much attention in the field of numerical analysis. The technique helps to provides surface contours that give the best way to visualize parameter interactions (Cochran and Cox, 1992). The principle of RSM was explained by Khuri and Cornell (1996) and its aim is to optimize the response according to factors analysed by Montgomery (2001).

Generally, RSM uses a series of design experiments to obtain an optimal response. In another words, RSM investigate the relationship between few explanatory factors or variables and single or multiple response/s. RSM method has been observed to be efficient and requires less data compared to the classical or convention manual methods. It is therefore being widely used for optimization purposes (Began et al., 2000). Recently, a number of researches exploit the RSM method for optimization purposes. Some of these researches include optimization of extraction parameters for ultrasound-assisted extraction of natural pigment (Yolmeh et al., 2014), ultrasound-assisted extraction of volatile oil from papaya seed (Samaram et al., 2015), supercritical carbon dioxide extraction of essential oil from Cyperus rotundus Linn (Wang et al., 2012) and microwave assisted extraction of antioxidant from tomato (Pinela et al., 2016). All of these researches attest to the efficiency of this technique for optimization purpose.

### 2.10.1 Factorial Design

A factorial experiment otherwise known as fractional factorial design is a simple method to estimate the first degree polynomial model. This model is appropriate to determine the significant variables or factors that have a great influence on the response. In another words, this design can be used for screening many factors to find the significant few. The reason for the choosing few of the best and most influential variables is to avoid confusion and complexity in optimising of a process. Therefore, generally an experiment may be designed to analyse the variables with an aim of eliminating the less contributing or less significant ones. This type of experiment is known as screening experiment. Initially, in a response surface study, there could be many variables that might be involved in defining the response. The purpose of factor screening is to reduce the total number of variables involved such that subsequent experiments would consist of fewer runs (Khuri and Cornell, 1996 and Meyers and Montgomery, 2002).

Under the coverage of RSM, there are two types of smaller and efficient designs which are often exploited. They are central composite design (CCD) and Box-Behken design (BBD). The most frequent and well known method that is being used in extraction of essential oils is CCD. This technique has been implored for optimization studies during the extraction of volatile oils from *Jatropha curcas* (Subroto et al., 2015) and Myrtus communis (Ghasemi et al., 2011) Therefore, factorial design method and CCD can be conveniently applied to extraction of essential oils from other plant materials using different extraction techniques.

### 2.10.2 Central Composite Design

The CCD design was established by Box and Wilson (Box and Wilson, 1951). The CCD is being carried out in order to build a second order experimental model (Meyers and Montgomery, 2002). This technique consists of a factorial design, central point and axial point that have equal distance to the centre point. The factorial design elements of CCD represent the class of $2^k$ factorial where k is the total numbers of relevant factors used in an experiment. Each of the factors is considered at two levels which define as high and low numeric value. A coded numeric value of +1 and -1 is assigned which represents the high and low values respectively. This factorial design for 3 processing factors is like a geometric of cube where each of the corners represents an interaction of the variables or factors. When connecting all the points that are having equal distance from centre point, a spherical design is obtained. Generally, there are two types of axial values which are positive axial ($+\alpha$) and negative axial ($-\alpha$). Therefore, these axial values add two extra levels in the variables. The $\alpha$ value is measured from the equation of $\alpha = (n_i)^{1/4}$, where $n_i$ is the number of interactions based on the experimental factorial design, In a case where 3 factors are involved, 8 interaction points may be chosen as the interaction. Hence, for 8 interactions α value is equal to 1.682 (Meyers and Montgomery, 2002). However, the central point element in CCD represents the average of high and low values determined in the factorial design analysis. The central point is usually the point or region where the optimal conditions are expected to start declining. Thus CCD is one of the most common methods which is frequently being used in optimization of extraction processes. This is because of its ability to propose optimum extraction conditions even with a minimum number of experimental data. Therefore, CCD is suitable for the researches on extractions of nature origin products which most often are conducted in small scale (Galadima et al., 2013).

## 2.11 Summary

*C. cassia* bark is an abundant plant material in nature which exhibits great potential values for medicinal applications. Therefore, suitable extraction technique is required to obtain essential oil from these barks and preserve the active and essential constituents present in the oils. Though the conventional method, HD, is well established and still being used in industries till today, they have some shortcomings related to energy consumption and environmental impact. The nature of heating mechanism of this method is inconsistent. Also, its elevating temperature trend for prolonged extraction have been observed to cause alterations to chemical compounds of the volatile oil especially monoterpenes compounds. This can also increase the potential for loss or thermal degradation of these volatile compounds. In order to overcome this challenge, newly emerging green technology, MAHD is perceived to be a suitable alternative. This method not only ensures low energy consumption but it is also an environmental friendly technique. The idea of incorporation of microwave with conventional HD is thus developed to reduce the extraction time and minimize the solvent consumption. The reduced extraction time associated with this technique makes the extracted oil safer in terms of reduced risk of potential loss of volatile compounds. Therefore, the essential oils extracted through MAHD have more potential to be used in pharmaceutical industries.

Based on the information gathered from this literature review, the critical observations which necessitated the need for further research on this field are as follow:

1. Hydrodistillation (HD) is the most commonly used conventional extraction method and it has also been used to obtain essential oils from *Cinnamomum cassia*. Unfortunately, use of HD has been reported to alter the chemical compounds mainly the monoterpene compounds on the extracted oil. In addition to chemical alteration of the oil in form of total loss or thermal degradation of the volatile compounds, longer extraction time and high energy consumption of HD makes it less desirable.

2. There are several improvements on extraction techniques to meet with the present day domestic and industrial demand for essential oils. Ultrasound assisted extraction (UAE) is one advanced technique which have proven to be effective and reliable with many highlighted advantages. Notwithstanding, UAE has the disadvantage of organic solvent usage in some cases. Furthermore, certain report has indicated the damage to some bioactive constituents of medicinal plants extracted through UAE. This often occurs via formation of free radicals due to high ultrasound frequency (20 kHz) of the technique.

3. Similarly solvent free extraction (SFE) is another important advanced technique but the high operating cost and need to optimize many operating parameters often restrict the use of this technique. Also, the high pressure and temperature which accompanies this extraction technique often leads to less possibility for full recovery of the essential oil. Although the extracts obtained via this method sometimes possesses high yield with good oil quality, economical non-feasibility and complicated operating methods are usually the limiting factors.

4. Microwave extraction can offer several potential benefits and this method is constantly being improved to meet the emerging demands from industrial sectors. Improvement to this technique gave birth to microwave assisted hydrodistillation (MAHD). The urge of the technology revolution on the green extraction concept and the requirement for new energy saving, cost reduction and technically feasible methods makes this method highly desirable. Besides other important advantages which may be obtained through the use of MAHD, environmental friendliness of MAHD due to non-consumption of organic solvents makes it the method of preference.

5. The use of MAHD is fast becoming a household due to its many advantages. It has also been employed for obtaining essential oil from *Cinnamomum cassia*. However, properties of cassia oil obtained from this method have not been extensively studied. Notably, cytotoxicity of *C. cassia* bark oil extracted through MAHD is an area that requires orchestrated research.

# CHAPTER 3

# MATERIALS AND METHODS

## 3.1 Introduction

In this research, extraction of essential oil from cinnamon barks was done. Two different extraction techniques which are advanced microwave assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD) technique were employed for the extraction process. For MAHD, the effects of operation parameters such as microwave power level, water to raw material ratio and extraction time on the extraction yield and its major constituent were studied. Optimization was carried out on these MAHD parameters using Design Expert Software in order to obtain the optimum extraction condition. Based on the optimum condition, comparison was carried out on the effectiveness of both MAHD and HD, techniques for the extraction process. This was done with respect to extraction yield as well as the desired major component (trans-cinnamaldehyde) and other chemical compounds, and evaluation was done.

This chapter provides a clear description of the different materials used for this research as well as the experimental procedures. Raw material preparation, extraction steps and analysis of the essential oil are also discussed herein. Furthermore, some details of the important equipment used are also presented. Detailed flow process of the experimental design is illustrated in Figure 3.1.

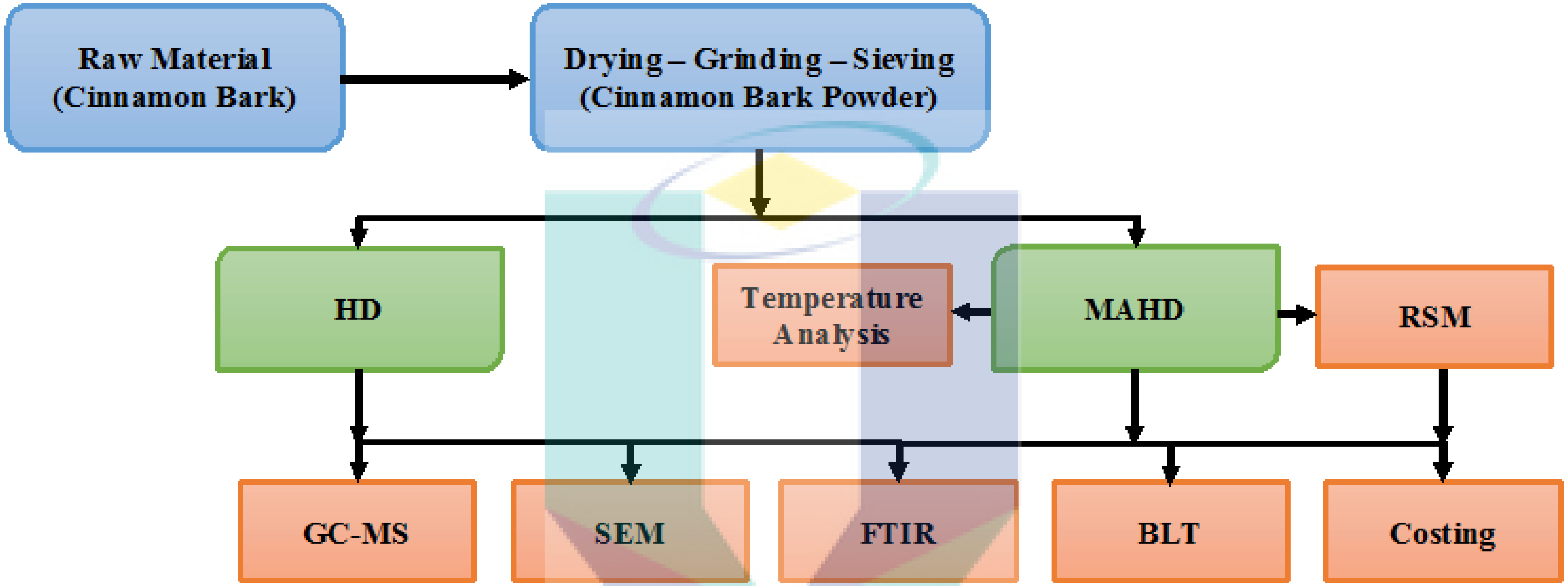


Figure 3.1: Flow process of the general experimental procedure.

## 3.2 Materials

### 3.2.1 Raw Material

*Cinnamomum cassia* barks were used as experimental raw materials for this study. The material was collected as experimental raw materials from Munisamy and Sons Sdn. Bhd. in Southern Peninsular Malaysia (Johor Bahru, Johor). The fresh cassia barks collected is included in Figure 3.2.

Figure 3.2: Fresh *Cinnamomum cassia* (cinnamon) barks.

### 3.2.2 Chemicals

In this studies, chemicals usage had been limited only for analysis purposes. The reason for this was to ensure the extracted essential oil is environmental friendly. The important chemicals used and the reasons for their usage is included on Table 3.1.

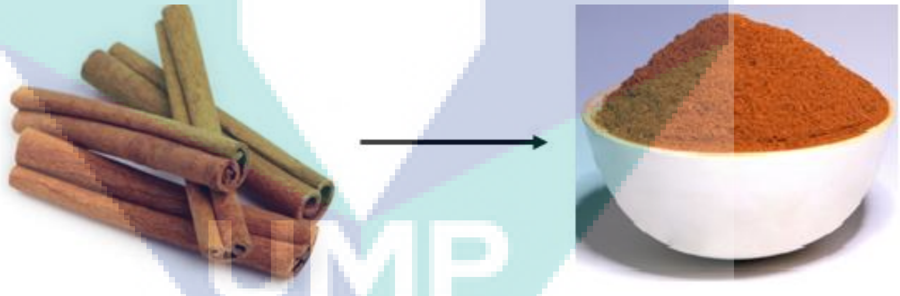
Table 3.1
List of chemicals

| Chemical | Supplier | Reasons for use |
|---|---|---|
| Anhydrous sodium sulphate ($Na_2SO_4$) | Fisher Scientific | To speed up the process of water evaporation from extracted cassia oil |
| Dichloromethane (DCM) | Fisher Scientific | Dilution solvent for extracted cassia oil in the GC-MS sample preparation |
| Dimethyl sulfoxide (DMSO) | Fisher Scientific | Positive control in the Brine Shrimp Lethality Test (BLT) |
| Potassium Dichromate ($K_2Cr_2O_7$) | Fisher Scientific | Negative control in the Brine Shrimp Lethality Test (BLT) |

## 3.3 Methods

### 3.3.1 Preparation of Raw Material

Summary of the preparation process for *Cinnamomum cassia* (cinnamon) bark is illustrated in Figure 3.3.



Fresh *C. cassia* bark

Powdered cinnamon bark

Figure 3.3: Illustration of *C. cassia* bark sample preparation.

The first step in the sample preparation is pre-treatment. The raw *Cinnamomum cassia* barks contained some dirt particles and certain other debris. In order to remove these adhering substances, the raw material was washed in running distilled water and dried in open air at an ambient temperature of 25–30 $^{0}$C and relative humidity of about 30–40 %. The drying process, the raw materials were prepared for extraction as follows:

### a) Crushing, Grinding and Sieving

Approximately 100 g of dried cinnamon barks were crushed using laboratory mortar and pestle. The crushed cinnamon bark powder was further ground with a Germany Retsch Ultra Centrifugal Mill ZM 200 grinder machine to reduce the sample particle size, and increase the surface area of test sample exposed for distillation process. Grinding and sieving processes were carried out based on reports from literature which stated that as the particles size of cinnamon bark powder decreases, the extraction yield increases due to higher amount of essential oil released as the bark phloem are destroyed by grinding (Khajeh et al., 2004 and Reverchon and Marrone, 1997). The dimensions of the grinder machine used were 410 mm x 515 mm x 365 mm. The grinding machine was set at a speed of 10, 000 r.p.m for each batch of a 250 g feed.

After this, 1 kg of grounded fine cinnamon bark powder was sieved with vibratory sieve shaker (model- Retcsh AS 200 digit, Germany). Sieving was done at amplitude of 3 mm for 60 min, and 100 µm size of cinnamon bark powder was collected. This particle size was selected because a reasonable amount of the fine powder was obtained from 100 µm mesh size of the sieve shaker.

### b) Drying

The 100 µm size cinnamon bark powder was then dried in accordance to standard AACC, 1983 (44-19) by using hot air drying oven (Thermo Scientific Heraeus) at 105±5 $^0$C until constant weight was attained. The moisture content of cinnamon bark powder was calculated according to the Eq. 3.1.

$$Moisture\ Content\ \% = \frac{W_1 - W_2}{Weight\ of\ sample}\ x\ 100 \tag{3.1}$$

where,

$W_1$ is the weight of cinnamon powder before heating, g

$W_2$ is the weight of cinnamon powder after heating, g

The fully dried cinnamon bark powder was kept in dessicator filled with silica gel in order to prevent moisture absoprtion before the extraction process. Prior to extraction, 25 g of the powdered cinnamon bark was measured and pre-soaked for 30 min in distilled water. Different water to raw material ratios were used such as 6:1, 8:1 and 10:1 of water to fully dried cinnamon bark powder respectively. All values were reported in dry weight basis sample.

### 3.3.2 Extraction of *Cinnamomum cassia* Bark Oil through Microwave Assisted Hydrodistilation (MAHD)

Microwave assisted hydrodistillation (MAHD) is an advance extraction technique method that comprise the incorporation of microwave irradiation into the conventional method, hydrodistillation (HD). A domestically modified microwave oven (Samsung MW71E model) was fitted to the Clevenger-type apparatus as describe previously by (Golmakani and Rezaei, 2008 and Stashenko et al., 2004). The dimensions of the microwave oven are 557 mm x 329 mm x 418 mm with a total power consumption of 1150 W, maximum power output of 800 W and 240 V–50 Hz power source and two knobs for adjusting microwave power and extraction time respectively. This Samsung microwave oven model has inbuilt unique feature of triple distribution system (TDS) which helps to distribute heat evenly from all the direction to the cinnamon solution during extraction.

For MAHD extraction, 1 L sized reactor (round bottom flask) containing 25 g of powdered *Cinnamomum cassia* (cinnamon) bark pre-soaked distilled water at different ratio of 6:1, 8:1 and 10:1 of water to dried cinnamon bark powder was placed within the microwave oven cavity. The dimensions of the microwave oven cavity were 306 mm x 211 mm x 320 mm. A Clevenger apparatus which has been set on top, outside the microwave oven, was used to collect the extracted essential oils. Extraction was done at different time length (30-150 min) and at different microwave power level (200-250 W). Details of the MAHD extraction conditions are put together in Table 3.2. The maximum extraction time and microwave power level was selected based on justification from literature (Golmakani and Rezaei, 2008 and Ranitha et al., 2014). After extraction, the cassia oil was dehydrated over anhydrous sodium sulfate to remove excess water, then the concentrated cassia oil was weighed and stored in vial at 4 $^0$C for further analysis.

Table 3.2
Overall parameters and conditions of MAHD extraction process

| Parameters | Conditions |
|---|---|
| Microwave power (W) | 200, 225 & 250 |
| Water to Powdered cinnamon bark (w/w) | 6:1, 8:1 & 10:1 |
| Extraction time (min) | 30, 60, 90, 120 & 150 |

### 3.3.3 Extraction of *Cinnamomum cassia* bark oil by using Hydrodistilation (HD)

Extraction of essential oil from powdered *Cinnamomum cassia* (cinnamon) bark through hydrodistillation (HD) method was done using a Clevenger apparatus in accordance with the HD method described in the European Pharmacopoeia (Pharmacopoeia, 1998). For this extraction, an electromantle (model- Nahita, 655) was used as the heating source. The mechanism of HD extraction is similar to MAHD extraction process. However, the type of heat supplied is the distinguising factor for each techniques. During HD, the heat is directly transferred or supplied from the electromantle to the reactor by conduction and convection process. On the other hand, the heat transferred or supplied in MAHD is usually developed through the molecular motion within the ionic species inside the polar components after the absorption of microwave energy. In order to maintain the close uniformity in extraction process between HD and MAHD, same size of the reactor which is 1 L, 25 g of the powdered cinnamon bark as well as same water to powdered cinnamon bark ratio was maintained in both extraction methods. However, the extraction period in HD extended to 3 hours since conventional methods need a longer extraction time to obtain maximum oil recovery (Golmakani and Rezaei, 2008; Lucchesi et al., 2007 and Naima Sahraoui et al., 2008). The HD operating conditions and extraction parameters are included in Table 3.3.

Table 3.3
Overall parameters and conditions of HD extraction process

| Parameters | Conditions |
|---|---|
| Electromantle power (W) | 350 |
| Water to Powdered cinnamon bark (w/w) | 6:1, 8:1 & 10:1 |
| Extraction time (min) | 30, 60, 90, 120, 150 & 180 |

## 3.4 Optimization of Cassia Bark Oil Extraction

### 3.4.1 One Factor at a Time (OFAT)

One factor at a time (OFAT) is an experiment technique which varies only one factor or variables at a time while others are kept constant or fixed. This technique is mainly applied to evaluate the effect of each factor as a response. In this experimental work, there were three factors analysed in the extraction of cassia oil from powdered cassia bark which are microwave power level, water to powdered cinnamon bark ratio and extraction time. The condition ranges were set for water to raw material ratio of 6:1, 8:1 and 10:1, microwave power level of 200 W, 225 W and 250 W and extraction period from 30 min to 150 min. Generally, these three parameters are the main factors that might effects the extraction process by MAHD. The same observations had been reported by (Azmir et al., 2013). Besides that, for HD, two parameters are the major factors that might effects the extraction process which are extraction time from 30 min to 180 min and water to raw material ratio of 6:1, 8:1 and 10:1 at constant operating power of 350 W.

### 3.4.2 Screening of MAHD Extraction Parameters using Factorial Design

Factorial design was conducted to screen out the unnecessary factors. In present study, three factors such as water to raw material ratio, extraction time and irradiation microwave power were taken into account to examine the effects on yield of cassia bark oil (%). A total of 13 runs were performed according to a full factorial design with three factors (8 point to the factorial design and 5 centre point to validate the experimental errors). The variable factors which are actual, coded and results acquired for response variable (yield of cassia oil (%)) tabulated in Table 3.4. This was done with the help of a Design Expert Software Version 7.1.6 Stat-Ease Inc., USA.

Table 3.4
Two level factorial design experiment matrix with response variable

| **Std** | **Run** | **Water : raw material ratio (w/w)** | **Microwave power (W)** | **Extraction time (min)** | **Yield of cassia oil (%)** |
|---|---|---|---|---|---|
| 1 | 12 | 6 | 200 | 30 | 1.46 |
| 2 | 7 | 10 | 200 | 30 | 2.01 |
| 3 | 11 | 6 | 250 | 30 | 1.83 |
| 4 | 10 | 10 | 250 | 30 | 2.31 |
| 5 | 13 | 6 | 200 | 120 | 2.55 |
| 6 | 8 | 10 | 200 | 120 | 2.37 |
| 7 | 6 | 6 | 250 | 120 | 2.69 |
| 8 | 1 | 10 | 250 | 120 | 2.55 |
| 9 | 4 | 8 | 225 | 75 | 2.81 |
| 10 | 2 | 8 | 225 | 75 | 2.58 |
| 11 | 3 | 8 | 225 | 75 | 2.71 |
| 12 | 9 | 8 | 225 | 75 | 2.68 |
| 13 | 5 | 8 | 225 | 75 | 2.68 |

| **Factor** | **Level** | | |
|---|---|---|---|
| | **-1** | **0** | **+1** |
| Water : raw material ratio, $x_1$ | 6 | 8 | 10 |
| Microwave power, $x_2$ | 200 | 225 | 250 |
| Extraction time, $x_3$ | 30 | 75 | 120 |

### 3.4.3 Optimization of MAHD Extraction Parameters using Response Surface Methodology (RSM)

Optimization was done in order to obtain the optimum condition necessary to produce the desired yield of essential oil from cinnamon bark using MAHD extraction. This was done with the help of a Design Expert Software Version 7.1.6 Stat-Ease Inc., USA, Central Composite Design (CCD). Three independent variables (microwave power levels, water to powdered cinnamon bark ratio and extraction time) were evaluated for one response (cassia oil yield), which is the dependent variable. The range of values for the independent variables is put together in Table 3.5 while the experimental design for factor combination is shown in Table 3.6. An experimental design is a well-planned study that consists of a set of experimental runs, and each run is defined by a combination of factor levels.

Table 3.5
Design Summary

| Factor | Independent variable | Unit | Symbol | | Level | |
|---|---|---|---|---|---|---|
| | | | Actual | Coded | Actual | Coded |
| A | Extraction Time | min | T | $x_1$ | 67.50 | - 1 |
| | | | | | 75.00 | 0 |
| | | | | | 82.50 | 1 |
| B | Microwave Power | W | P | $x_2$ | 222.50 | - 1 |
| | | | | | 225.00 | 0 |
| | | | | | 227.50 | 1 |
| C | Water to Powdered cinnamon bark ratio | w/w | R | $x_3$ | 7.50 | - 1 |
| | | | | | 8.00 | 0 |
| | | | | | 8.50 | 1 |

Table 3.6
Experimental design plan and responses

| Standard order No. | Experimental Factors | | |
|---|---|---|---|
| | Extraction Time (min) A | Microwave Power (W) B | Water to cinnamon bark ratio (w/w) C |
| 1 | 67.50 | 222.50 | 7.50 |
| 2 | 67.50 | 227.50 | 7.50 |
| 3 | 67.50 | 222.50 | 8.50 |
| 4 | 67.50 | 227.50 | 8.50 |
| 5 | 82.50 | 222.50 | 7.50 |
| 6 | 82.50 | 227.50 | 7.50 |
| 7 | 82.50 | 222.50 | 8.50 |
| 8 | 82.50 | 227.50 | 8.50 |
| 9 | 75.00 | 220.00 | 8.00 |
| 10 | 75.00 | 230.00 | 8.00 |
| 11 | 75.00 | 225.00 | 7.00 |
| 12 | 75.00 | 225.00 | 9.00 |
| 13 | 60.00 | 225.00 | 8.00 |
| 14 | 90.00 | 225.00 | 8.00 |
| 15 | 75.00 | 225.00 | 8.00 |
| 16 | 75.00 | 225.00 | 8.00 |
| 17 | 75.00 | 225.00 | 8.00 |
| 18 | 75.00 | 225.00 | 8.00 |
| 19 | 75.00 | 225.00 | 8.00 |
| 20 | 75.00 | 225.00 | 8.00 |

## 3.5 Analysis

For the purpose of analysis, the condensate which consists of a mixture of water and cassia oil was collected in a vial. The condensate was dehydrated over anhydrous sodium sulphate to remove the excess water and then the concentrated cassia oil was weighed by using analytical weighing scale and stored in a vial at 4 $^0$C for analysis purpose.

### 3.5.1 Calculation of Extracted Cassia Oil Yield

The yield of the extracted cassia oil was calculated to analyse the performance of MAHD and HD in extraction of essential oil. The yield of the cassia oil was measured in triplicate, and the results were presented as a mean value. Extracted essential oil from cinnamon bark was calculated by using the following Eq. 3.2:

$$Yield\ of\ cassia\ oil\ \left(\%\frac{w}{w}\right) = \frac{Amount\ of\ cassia\ oil\ (g) obtained}{Amount\ of\ powdered\ cinnamon\ bark\ (g) used}\ x\ 100 \qquad (3.2)$$

### 3.5.2 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) analysis was conducted in order to analyse the constituents which are present in the extracted cassia oil. The extracted cassia oil samples were analysed with the aid of a gas chromatography/ mass spectrometry (GC-MS) instrument (Agilent 5975C inert, USA) coupled with a mass spectrometer. The GC system (model- Agilent 7890A) was paired with a DB-5MS column with the dimensions of 30 mm x 0.25 mm id x 0.25 µm film thickness). The mass selective detector was operated in the electron impact mode with an electron energy of 70 eV (source temperature 230 $^0$C) to evaluate the constituent of the cassia oil extracted from the cinnamon bark and analyse its quality. The oven temperature regime was initially held constant at 100 $^0$C for 4 min and the temperature increase to 130 $^0$C at 5 $^0$C min$^{-1}$ and held steady for 20 min at the same temperature. Lastly, 3 min allowance was given for a complete column clean-up. The test samples for the analysis were diluted with Dichloromethane (DCM) at a ratio of 1:10, v:v and a total volume of 1.0 mL sample prepared. Sample with volume of 1.0 µl was injected to the GC system with the injector in split mode (split ratio: 1:30). The carrier gas, helium (He) was used at rate of 1.0 mL $min^{-1}$ (Kim, Seo, and Park, 2011). The mass analyser was held constant for 1 s scan time where the scan ranged from 41 to 400 amu. The compounds found present in the extracted cassia oil were compared with the mass spectra data of essential oils of the National Institute of Standard and Technology NIST MS library (Adams, 2001 and Masada, 1976). Quantification of the cassia oil were performed based on the close similarities in the retention period of compounds that were present in both methods. Normalization method was performed during the calculation of each peak area where a total peak area was estimated to be 100 %. The percentage of each component present in the extracted cassia oil was calculated by using the area of each peak.

### 3.5.3 Fourier Transform Infrared Spectroscopy (FTIR)

Functional group analysis was conducted on the cassia bark oil obtained through MAHD and HD. The purpose of this test was carried out to analyse the bonding structures and possible changes to the chemical compositions of the volatile oil with respect to extraction methods. The analysis was carry out with the aid of an FTIR spectrometer (Nicolet iS5 iD7 ATR; Thermo Scientific, Germany), fitted with OMNIC software. The standard KBr technique was used to obtain the IR spectra in a scanning wavelength range of 500-4000 $cm^{-1}$.

### 3.5.4 Scanning Electron Microscopy (SEM) Analysis

Morphological changes to the *C. cassia* bark powder during its preparation for extraction was investigated through SEM analysis. SEM images were gathered for untreated dried *C. cassia* bark powder sample, pre-soaked *C. cassia* bark powder sample, and after extraction of *C. cassia* bark oil from *C. cassia* bark powder at 75 min and 150 min for MAHD and HD respectively. These samples were observed by a Zeiss EVO 50 SEM system. The test specimens were attached on adhesive tape and then air-dried in order to make them dirt-free and moisture-free after which they were sputter coated with a thin layer of gold prior to SEM observation. Sputter coating was done in order to enable or improve the imaging of test sample and eliminate electrical discharge during SEM observation. All the testing samples were examined under high vacuum condition at an accelerating voltage of 0.2 to 30 kV (40 and 500 magnification) and analytical working distance at 6 mm.

## 3.6 Brine Shrimp Lethality Bioassay

### 3.6.1 Brine Shrimp Hatching

Artificial sea water was prepared by dissolving 35 g of commercial sea salt in 1000 mL of distilled water. Appropriate amount of *Artemia salina* eggs was enter into the prepared artificial sea water. The eggs were exposed and leave to good aeration and illumination for about 1 day for hatching process. After 24 h, the aeration was stopped and the nauplii were separated from the embryos and transferred into another new container with similar concentration of artificial sea water, good aeration and illumination. The nauplii were fed with yeast for 2 days or almost 48 h prior to the bioassay study.

### 3.6.2 Sample Preparation

Stock solutions were prepared separately by dissolving appropriate amount of cassia bark crude extract obtained through HD and MAHD respectively in 1 % of dimethyl sulfoxide (DMSO) with artificial sea water. 25 mL of various concentration extract solutions (10, 50, 100, 500 and 1000 ppm) were prepared. The negative control was prepared by dissolving 1 % of DMSO in artificial sea water to obtain 25 mL solution whereas positive control was prepared by dissolving 0.1 % of potassium dichromate in artificial seawater to obtain 25 mL solution. 10 nauplii of *A. salina* were tested in negative control for 24 h prior to the experiment in order to check the surviving ability of the brine shrimp. Generally, if the brine shrimps are alive after 24 h, it indicates that the brine shrimp nauplii are eligible to be used for further experiment. On the other hand, positive control was used to obtain the desired outcome of mortality of brine shrimp and compare the result with crude extract obtained through HD and MAHD.

### 3.6.3 Brine Shrimp Bioassay

Brine shrimp lethality test was performed after minor modification from previous research by (Sandanasamy et al., 2014). The bioassay was conducted on test samples (10, 50, 100, 500 and 1000 ppm), negative and positive controls. 3 mL each of test sample and controls were transferred into small test tubes and ten active brine shrimps were placed into them. The test tube with brine shrimps was placed into an incubator at 27–29 $^0$C. After 24 h, the lethality of the brine shrimps was measured. The bioassay was performed in triplicate and the values were recorded in mean value. The brine shrimp lethality data was calculated according to Eq. 3.3 and corrected lethality in the function of negative control was calculated and corrected by Abbott's formula as illustrated in Eq. 3.4 (Abbott, 1987). Probit analysis was carried out to produce a linear function graph for the corrected percentage mortality (Finney, 2009). The brine shrimp lethality was reported as $LC_{50}$ which defines the concentration at which 50 % of the shrimp died.

$$Mortality\ (\%) = \frac{Dead\ shrimp}{Total\ tested\ shrimp}\ x\ 100 \tag{3.3}$$

$$Corrected\ Mortality\ (\%) = 1 - \frac{Shrimp\ in\ treated\ sample}{Shrimp\ in\ negative\ control}\ x\ 100 \tag{3.4}$$

where,

Dead shrimp is the amount of shrimp killed after 24 h

Total tested shrimp is the total amount of shrimp used for the bioassay (10 shrimps)

Shrimp in treated sample is the number of shrimp that was alive after 24 h

Shrimp in negative control is the number shrimp that was alive in negative control after 24 h

## 3.7 Temperature Analysis

Figure 3.4 illustrates the schematic diagram of a modified domestic microwave oven used for the extraction of cinnamon bark oil. For the temperature analysis of cinnamon matrix (water + cinnamon powder), the pre-soaked cinnamon bark powder was transferred into 1 L round bottom flask which was set up inside the cavity of the modified domestic microwave oven prior to the experiment. Pico log TC-08-USB model data collector was used in this experiment to collect the temperature of the cinnamon bark matrix until it reached the boiling point. Three temperature sensors of the Pico Log device was immersed inside the cinnamon bark according to the following position which is bottom< middle< top as illustrated in the Figure 3.4. Temperature per second (°C/s) was recorded for the all three microwave power level of 200 W, 225 W and 250 W.

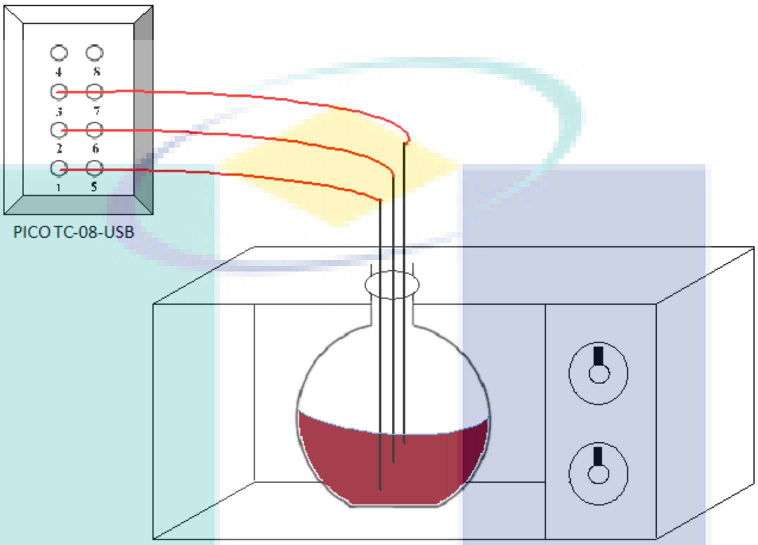


Figure 3.4: Modified domestic microwave oven with Pico TC-08-USB data collector.

# CHAPTER 4

# RESULTS AND DISCUSSION

## 4.1 Preliminary Study

Preliminary studies was conducted in order to determine the range of factors to be used in this experiments for the investigated parameters such as microwave power level, extraction time and water to raw material ratio.

### 4.1.1 Selection of Factor Range

Based on literature studies, some preliminary set of test experiments were carried out in order to identify the range of parameters to be investigated for the two extraction techniques used for this research. The two methods adopted are microwave assisted hydrodistillation (MAHD) and hydrodistillation (HD). It was found that microwave irradiation power is a critical factor in MAHD and considerations were made to determine the range for microwave power levels. Firstly, there is need to select the irradiation power which would suitably enhance the attainment of boiling point by the solvent (water) during the extraction process. The boiling point of water is 100 $^{0}$C and a minimum power of 200 W was required to achieve this temperature. It is important to use sufficient power supply to achieve the boiling point of water in the extraction process because water will act as the carrier of essential oil from the cinnamon bark. However, very high power supply in the extraction process may lead to loss of volatile components. Thus, 250 W was chosen to be the maximum microwave irradiation level in this study.

Another factor which was considered is the water to powdered cinnamon bark ratio which was chosen based on the reactor (round bottom flask) capacity. During the preliminary test, it was observed that the usage of less than 150 mL of solvent (raw material-to-water ratio of 1:6) burnt off the plant material in HD after some period of time. On the other hand, usage of too much of water in the extraction of MAHD led to spill out of the sample solution from the distillation column. As a result, the range of factors chosen for powdered cinnamon bark-to-water ratio was 1:6 as minimum and 1:10 as maximum.

The third factor investigated was the extraction period. The range for extraction period was selected by observing the full recovery of cinnamon bark oil through both methods. Based on the preliminary experiments, it was observed that maximum 2 h and 3 h of extraction time were sufficient to extract essential oil from the cinnamon bark by using MAHD and HD respectively. Fortunately, this observation was found to conform to reports made by similar researchers (Golmakani and Rezaei, 2008 and Ranitha et al., 2014).

After careful selection of the range for each factor, it was necessary to determine the best mix of parameters to combine the factors prior to the main experiment. Otherwise, a long extraction period with less powdered cinnamon bark-to-water ratio and maximum microwave power level for instance may burn off the cinnamon bark sample. Hence, a good combination of factors was achievable through preliminary study.

### 4.1.2 Pre-Extraction Processes

After determining the range of factors for extraction of cinnamon bark oil using MAHD and HD, pre-extraction process was carried out. The effect of particle size on yield of the extracted cassia oil was studied by comparing the yield from coarse cinnamon bark and 100 µm finely grinded fully dried cinnamon bark powder. Extraction of oil from these two different material sizes was conducted at microwave power range from 200 W to 250 W, water-to-raw material ratio of 150 mL to 250 mL of distilled water/ 25 g of cinnamon bark, and extraction period ranging between 30 min to 120 min. Average yield of 2.34 % was obtained from the sample which was finely grinded compared to the coarse sample which produced an average of 1.09 %. This suggests that there is more possibility to obtain higher yield from smaller sized particles than can be obtained from bigger size particles perhaps due to the larger surface area associated with small sized particles (Guan et al., 2007). Generally, grinding may destroy the bark phloem and enhance the release of essential oil from the oil gland of cinnamon bark as stated in literature (Khajeh et al., 2004 and Reverchon and Marrone, 1997). It was therefore noted that grinding is a vital stage which is required in the raw material preparation for effective essential oil extraction.

Another pre-extraction process that was carried out is soaking process. The effect of soaking on yield of the cinnamon bark oil was carried out by preparing two different matrixes (each consisting of 100 µm finely grinded bark powder and water) for extraction. The first sample was soaked for 30 min prior to extraction whereas the second matrix was immediately transferred to the extraction vessel after addition of water. The result shows that soaked cinnamon bark powder produced yield of 2.09 % whereas 1.98 % of yield was obtained from cinnamon bark powder that was not soaked. This indicates that the soaking process can enhanced the essential oil extraction because during the soaking period, the solvent (water) may enter into the bark phloem and expand the bark. When the phloem present on the surface of the barks gets expanded, the oil cell glands distributed on the bark also might be expanded. This would therefore make the oil glands susceptible to easy breakage when heat is applied during the extraction process. This may be associated with the fact that the internal pressure generated inside the oil gland cells, may rupture the oil glands so as to release the essential oil. Thus the total amount of essential oil released may increase even at short period of extraction (Veggi et al., 2012).

The other pre-extraction process that was investigated is the effect of drying process on yield of the extracted oil. Two separate batches were prepared for extraction out of which one batch was made up of oven dried cinnamon bark powder whereas the other batch contained cinnamon bark powder which was not oven dried. About 25 g each of these materials were weighed and soaked for 30 min prior to extraction. Sample from the dried category produced a yield of 2.15 % compared to sample which was not dried (1.89 %). This observation can be associated with possible rupture of the surface of bark phloem at high temperature during the drying process. This can increase the capacity for water absorption during soaking process with concurrent expansion of the oil gland cells. This would invariably enhance the production of yield as stated in the previous paragraph. Furthermore, drying the cinnamon bark powder would reduce the moisture content. Thus the weight measured for extraction would be more accurate and dependent on the actual powder weight whereas in the sample which contained moisture, the measured weight might not be accurate. This is because the measured weight will be dependent on both the actual weight of the powder as well as the weight of the moisture present. The implication of this is that less cinnamon powder will be extracted for the sample which was not dried and this may have influenced the yield. The results gathered from these preliminary set of pre-extraction processes demonstrated that the amount of yield and extraction efficiency during the extraction of essential oils from cinnamon bark can be influenced by the processes such as particle size, moisture content and soaking process.

## 4.2 Effects of Operating Parameters

One factor at a time (OFAT) method which is a manual technique was used to analyse the optimum condition for extraction of cinnamon bark oil through MAHD and HD methods. Three parameters were evaluated for the extraction process which are extraction time (30, 60, 90, 120 and 150 min), distilled water to cinnamon powder ratio (w/w) (6:1, 8:1 and 10:1) and irradiation power (200, 225, and 250 W) for MAHD. On the other hand, two parameters were evaluated in the extraction process for HD which are extraction period (30, 60, 90, 120, 150 and 180 min) and distilled water to raw material ratio (w/w) (6:1, 8:1 and 10:1) at fixed operating power of 350 W supplied by a heating mantle.

Based on separate preliminary studies of the individual parameters, it was found that the extraction time of 90 min, at an operating power of 225 W and water to raw material ratio of 8:1 were the optimum points for obtaining the maximum yield. The OFAT analysis was hereby used to investigate the interrelationship between these factors. This was done by varying one of the factors at a time while the other two are held constant.

### 4.2.1 Effect of Water-to-Cinnamon Powder Ratio on Yield

Figure 4.1 depicts the effect of different water-to-cinnamon powder ratio of 6:1, 8:1 and 10:1 on yield of essential oil obtained through MAHD and HD at fixed optimum irradiation power of 225 W and extraction period of 90 min. As can be seen, the cinnamon bark oil yield obtained for MAHD are 2.06 %, 2.55 %, and 2.07 % for water-to-raw material ratio of 6:1, 8:1 and 10:1 respectively. It can be seen that the yield increased from 2.06 % to 2.55 % when the water ratio (w/w) was raised from 6 to 8 but later decreased to 2.07 % when the water ratio was further increased to 10. Generally in extraction processes, the water (solvent) acts as the carrier of essential oil and prevent the plant matrix from being thermally degraded. The less yield obtained at 6 w/w solvent suggest that the small volume of solvent may result in incomplete extraction of the targeted compound as reported in literature (Gao et al., 2006 and Ma et al., 2009). On the other hand, the reduction in yield when the water ratio was raised to 10 w/w indicates that the use of large volume of solvent may lead to more rapid generation of heat to the cinnamon matrix due to the high dielectric constant of water. This might therefore lead to partial degradation of some components of the extracted oil and invariably reducing the overall yield (Dhobi et al., 2009). This also might lead to more energy and time consumption in the extraction process. For HD, at water-to-cinnamon powder ratio of 6:1, 8:1 and 10:1 the yield obtained are 1.68 %, 2.02 % and 2.10 % respectively. As can be seen, there is a progressing increase in the yield of cinnamon bark oil as the volume of solvent increase for the case of HD. However the increase of 2.02 % to 2.10 % when the ratio of water was raised from 8:1 to 10:1 does not seem large enough to justify the energy and time that would be consumed to obtain this increment.

Thus, the highest cassia oil yield through MAHD was obtained at water-to-cinnamon powder ratio of 8:1 meanwhile the maximum yield for HD was obtained at water-to-cinnamon powder ratio of 10:1. Based on these observations, MAHD requires less solvent to attain maximum yield of cinnamon bark oil compared to HD. Hence, 8:1 of water-to-cinnamon powder ratio was chosen to be the optimum ratio for subsequent extraction processes.

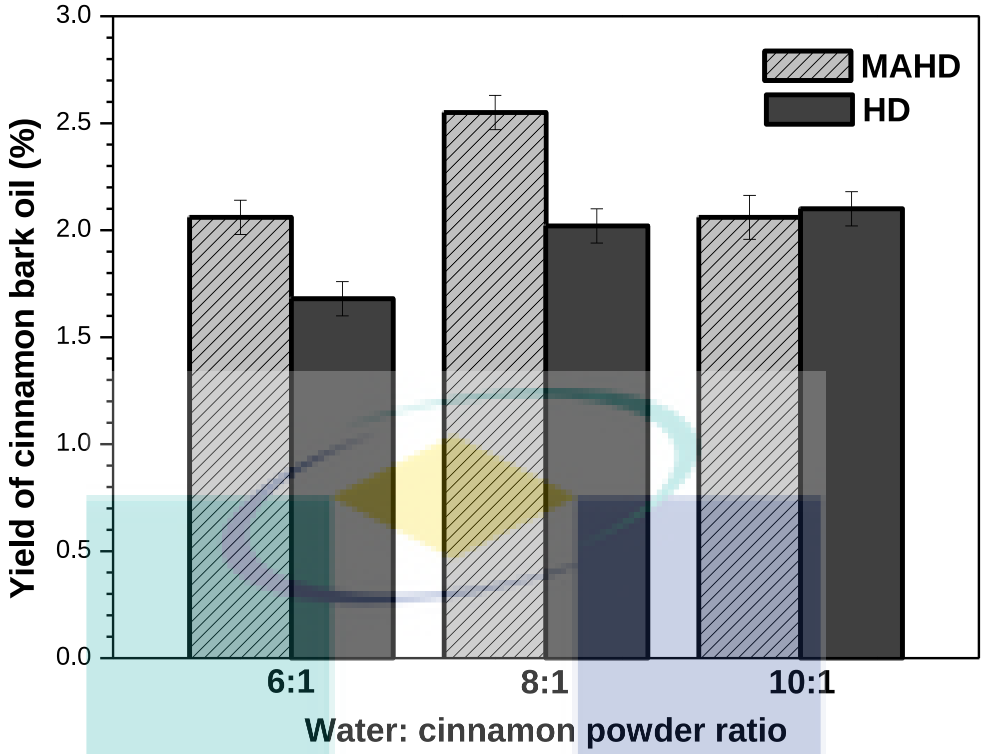


Figure 4.1: Effect of different water-to-raw material ratio on extraction yield of MAHD and HD at fixed irradiation power of 225 W and extraction time of 90 min.

### 4.2.2 Effect of Extraction Period on Yield

The effect of various extraction times from 30, 60, 90, 120, 150 and 180 min on the cinnamon bark oil yield for MAHD and HD at fixed irradiation power of 225 W and water-to-cinnamon powder ratio of 8:1 is illustrated in Figure 4.2. As can be seen from the graph, there was an initial increase in the yield of cassia oil as the extraction period was increased from 30 to 90 min for MAHD. The bark oil yield obtained at the extraction time of 30, 60 and 90 min were 1.82 %, 2.48 % and 2.55 % respectively. However, when the extraction time was prolonged from 90 to 150 min, there is an obvious decrease in the bark oil yield. Similar observation was also reported in literature for the extraction of *Radix Astragali* (Xiao et al., 2008). The initial rise in the yield of oil obtained via MAHD in the range of 30 to 90 min extraction period could be due to the high dielectric properties possessed by water which tends to enhance the heating rate inside the microwave as the temperature increases. However, extending the extraction period beyond 90 min could lead to possible cinnamon bark degradation or evaporation of volatile compounds from the cassia oil. This might be responsible for the decrease in the bark oil yield as at extraction period greater than 90 min as reported by other researchers (Gao et al., 2006 and Rezvanpanah et al., 2008). Based on this observation, 90 min was chosen as the optimum extraction time in extracting cinnamon bark oil through MAHD.

For HD technique, an increasing trend of the bark oil yield as the extraction period increase from 30 to 150 min can be seen, followed by an observable decrease when the extraction period was further increase from 150 min to 180 min. Prolonged extraction period using HD can lead to partial or full natural constituents degradation. This is notably for monoterpene compounds which can easily experience changes in chemical structure under high temperature for long period (Gavahian et al., 2012). The reduction in yield after 150 min of HD extraction therefore suggest that some degradation might have occurred to some components of the extracted oil thereby reducing the yield.

Generally from the graph, it can be seen that MAHD produced its maximum yield at 90 min whereas HD attained maximum yield at 150 min extraction period. This indicates that it requires more time to obtain the maximum yield in HD compared to MAHD as observed elsewhere (Lucchesi et al., 2007). Although water is being used as solvent to extract essential oil in the both methods, the longer extraction period required for HD to attain maximum yield can be related to the heat distribution system of the conventional HD method. The heat is often supplied specifically at one particular portion (bottom of reactor). Therefore, the water molecules at the bottom of the reactor would get heat energy first and converted it into kinetic energy that vibrate and rotate the cinnamon particles so as to tranfer it to other parts of the reactor. This transfer of heat from one point to another might be responsible for the longer time required for HD to produce the maximum yield compare to MAHD which is an indication that MAHD is more energy and time saving.

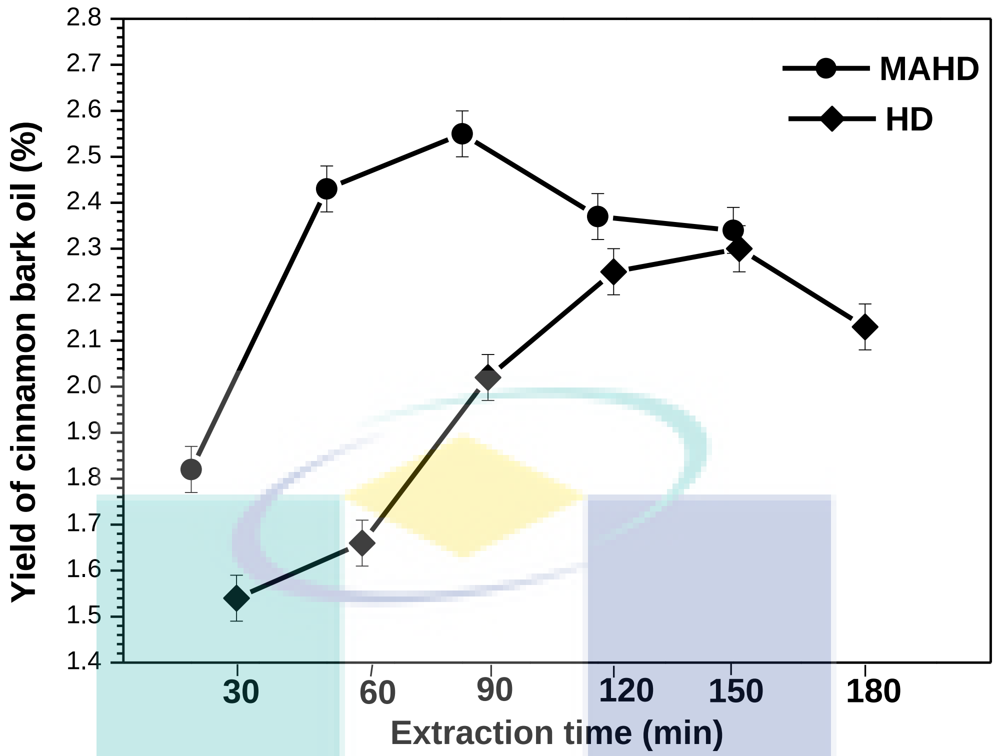


Figure 4.2: Effect of different extraction time on yield of cinnamon bark oil by MAHD and HD at fixed irradiation power of 225 W and water to cinnamon powder ratio of 8:1.

### 4.2.3 Effect of Irradiation Power on Yield and Induction Time

The effect of microwave irradiation power on cinnamon bark oil yield and induction time for MAHD was compared with yield and induction time for HD method at fixed distilled water-to-cinnamon powder ratio of 8:1 and constant extraction period of 90 min. The irradiation power for MAHD was varied from 200-250 W, as illustrated in Figure 4.3. It can be seen that as the irradiation power increases from 200 W to 225 W, the yield of cassia oil increase from 1.89 % to 2.55 %. When the power was further increased to 250 W, the yield was reduced to 2.42 %. Similar observation was reported by Hu et al. (2008) in their research work on extraction of saikosaponins from Radix Bupleuri (Hu et al., 2008). The initial increase in yield can be attributed to the fact that for microwave heating system, higher irradiation power would produce higher energy supply to the cinnamon matrix with a subsequent generation of rapid heat. This would also lead to the formation of high pressure gradient inside the cinnamon matrix which may enhanced and accelerate the extraction of cinnamon bark oil. However, with further increase in irradiation power above 225 W, the yield of cassia oil decreased. Reason for this could possibly be that the higher energy supply might overheat the plant matrix through undesirable increase in temperature. The resultant effect of this could be in form of breakdown or decomposition of certain chemical compounds contained in the essential oil as reported elsewhere (Dhobi et al., 2009 and Rezvanpanah et al., 2008). This bores down to the fact that essential oils are volatile compound which are highly fragile and unstable in the chemical structure especially at elevated temperatures. At high temperatures, the essential oil might oxidize, volatize or degrade easily (Asbahani et al., 2014).

The highest yield from MAHD was obtained at irradiation power of 225 W and comparison between this yield and the maximum yield obtained from HD (350 W) is illustrated in Figure 4.4. As it can be seen from this graph, the highest yield for HD is 2.02 % which shows that MAHD produced 26 % more yield than HD. The observable difference in yield from these two techniques can be attributed to the nature of their heat distributions. In MAHD, heat is distributed by radiation to the cinnamon matrix through electromagnetic waves via dipole reorientation and induced polarization. In addition, conduction and convection are also involved in the heat distribution of MAHD. For HD, heat is distributed by conduction and convection methods only. Therefore, the effective heat distribution from MAHD can accelerates the process of extraction and production of more yield.

On the other hand, the effect of irradiation power on induction time (time required to reach boiling point of the mixture) for MAHD is included in Figure 4.3. It can be seen that the induction time reduces as the irradiation power increases. The induction time recorded for 200 W, 225 W and 250 W were 15 min, 9 min and 7 min respectively. Since the dielectric properties of the solvent determines the rate at which boiling is accelerated, higher irradiation power means that higher wave would be transferred to the mixture based on the high dielectric properties of the solvent and vice versa (Rezvanpanah et al., 2008). There is therefore higher tendency for the mixture to attain boiling point faster at higher temperature compared to lower temperature. This suggests the reason for decrease in induction time as irradiation power increased.

Meanwhile for HD, the induction time recorded was 23 min. Comparison between the induction time for MAHD at optimum irradiation power (225 W) and HD (350 W) is included in Figure 4.4. As can be seen, MAHD requires shorter time to reach induction time (9 min) and began to produce essential oil earlier, producing almost about 60 % of total extracted oil even before HD (23 min) started the distillation process as illustrated in the Figure 4.4. This can be attributed to effective heat distribution in MAHD as discussed in previous paragraph compared to HD. Therefore, 225 W was chosen as the optimum microwave power for further experiment in evaluating other effect of parameters.

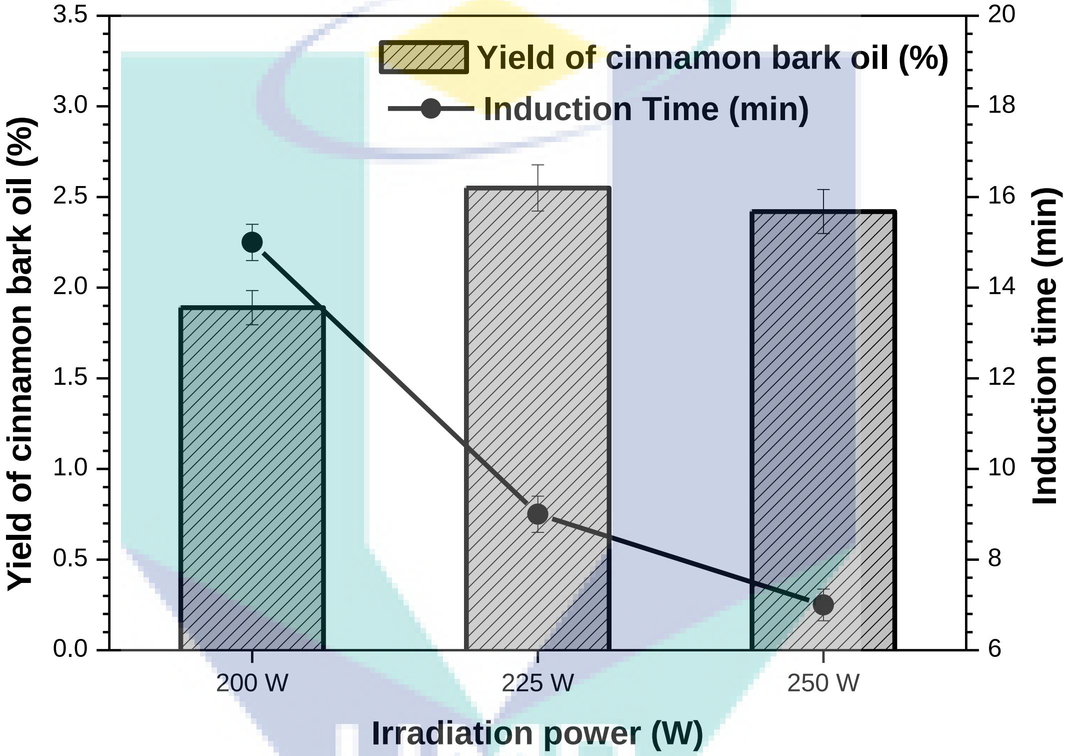


Figure 4.3: Effect of various irradiation power on cinnamon bark oil yield (%) and induction time (min) of MAHD and HD at fixed extraction time of 90 min and water-to-cinnamon powder ratio of 8:1.

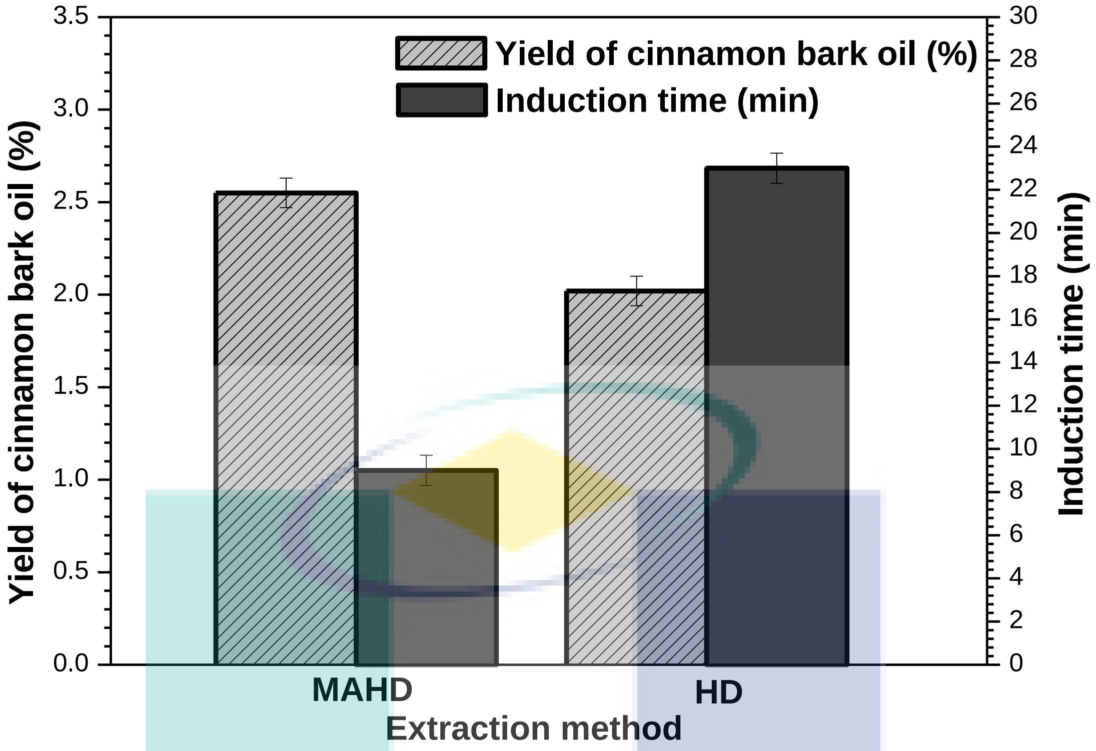


Figure 4.4: Effect of extraction methods on cinnamon bark oil yield (%) and induction time (min) of MAHD (225 W) and HD (350 W) at fixed extraction time of 90 min and water-to-cinnamon powder ratio of 8:1.

## 4.3 Optimization of MAHD Parameters

In this section, MAHD parameters were analysed and optimized with the aid of Design Expert software. The factors (water/ raw material ratio, microwave power and extraction time) used in MAHD were analysed to obtain the best operating parameters for MAHD and narrow down the operating parameters' range in order to find the best optimum condition point in extracting cinnamon bark oil. In order to analyse the best operating parameters in MAHD, two level factorial analysis via Design Expert software was used. On the other hand, the best optimum condition for MAHD was evaluated using Central Composite Design via Design Expert software.

### 4.3.1 Factorial Analysis of MAHD Parameters

A total of 13 experimental runs were performed based on the two level factorial design with three factors (8 points of the factorial design and 5 centre points to determine the experimental errors). Table 4.1 shows the test of significance for yield of cinnamon bark oil. The *p* values were used to verify the significance of each coefficients, and to determine the possible interaction between the variables. Generally, as the value of *p* decreases, the corresponding coefficients will be more significant (Haber and Runyon, 1980). The effect of linear coefficients ($x_1, x_2, x_3$) and the interaction coefficients ($x_1 x_2, x_2x_3, x_1x_3$) were investigated for their significance. From the table, linear coefficients ($x_1, x_2, x_3$) and interaction coefficient ($x_1x_3$) were significant on the yield of cinnamon bark oil. However, the other interaction coefficients ($x_1 x_2$ and$x_2x_3$) were not significant as the *p*-values were more than 0.01.

Table 4.1
Test of significant for the extracted cinnamon bark oil

| **Model Term** | **Cinnamon bark oil yield (%)** | | |
|---|---|---|---|
| | ***p*- values** | **% Contribution** | **Coefficient Estimate** |
| $x_1$, w/w | 0.0192 | 3.2300 | 0.0890 |
| $x_2$, W | 0.0051 | 6.2800 | 0.1200 |
| $x_3$, min | <0.0001 | 41.640 | 0.3200 |
| $x_1 x_2$ | 0.6205$^*$ | 0.0058 | -0.0037 |
| $x_2x_3$ | 0.1542$^*$ | 0.7800 | -0.0440 |
| $x_1x_3$ | 0.0013 | 11.670 | -0.1700 |

$^*$Coefficients with p-values more than 0.01, are not significant.

The contribution of the main effect of factors were studied and presented in Pareto chart in Figure 4.5. According to the chart, the factors above the t-value limit (2.57058) were accepted to be the contributing variables on the response (cinnamon bark oil yield). The higher the value of the factors than the t-value limit, the more the contribution of the variables on the cinnamon bark oil yield and for the lower value of the factors are otherwise. The least contribution variables indicate that the range of the factors does not affect the response. In essence, either the range of the least contribution factor is increased or decreased; the cinnamon bark oil yield would not change. On the other hand, there were two types of effects (positive and negative) that influences the yield of cinnamon bark oil. As for positive effect factors, there is a direct proportionality to the corresponding responses whereas for the negative effect factors the proportionality is inverse. In any case, contributions of the positive effects and negative effects in a process are dependent on the particular goal set for the responses. From the Pareto chart in Figure 4.5, it was observed that the extraction time (C) is the most significant factor that contributes in MAHD extraction, followed by the interaction of water-to-raw material ratio and extraction time (AC), linear effects like microwave power (B) and water-to-plant material ratio (A). However, the interaction effects of water-to-raw material ratio and extraction time (AC) were the negative effects contribution which might reduce the cinnamon bark oil yield. On the other hand, linear effects (A, B, C) appeared to be the positive effects which may enhance the yield of cinnamon bark oil independently. The interaction effects of microwave power and extraction time (BC), water-to-raw material ratio and microwave power (AB), and water-to-raw material ratio, microwave power and extraction time (ABC) on cinnamon bark oil yield were not significant.

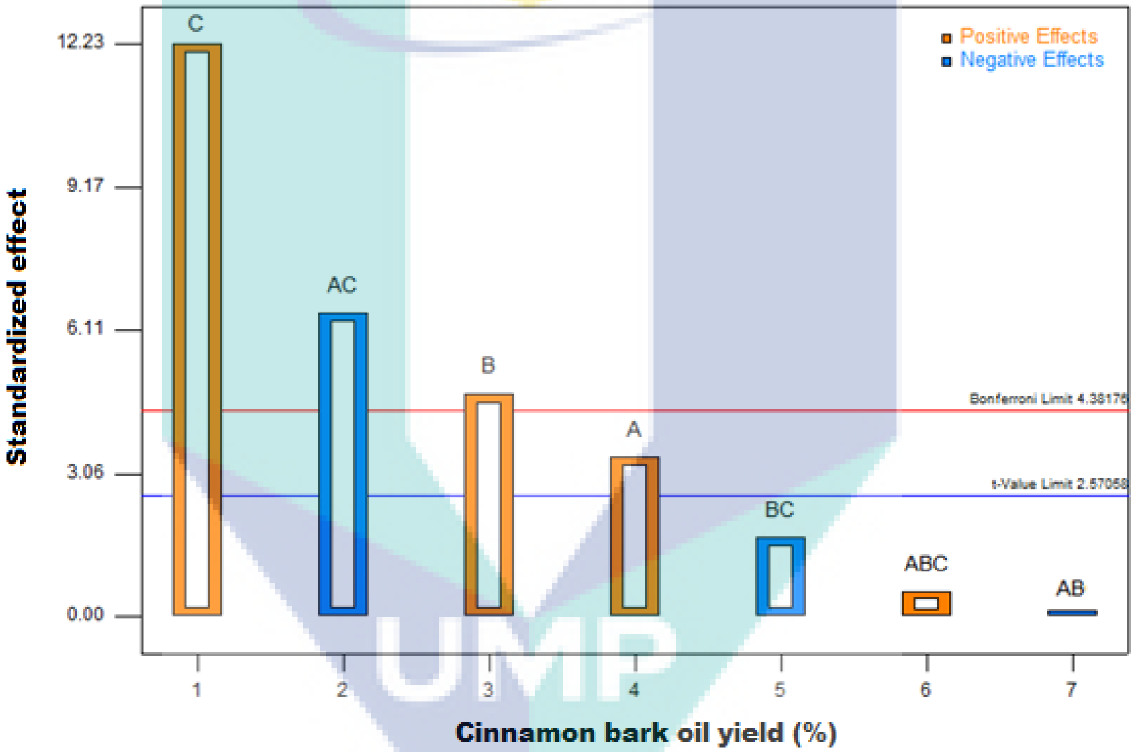


Figure 4.5: Pareto’s chart of standardize effects of factors using the cinnamon bark oil yield (A: Water: raw material ratio, B: Microwave power, C: Extraction time).

Based on the analysis results obtained, Eq. 4.1 represents the best description after the elimination of less contribution (< 0.1 %) factors from the results summarized in Table 4.1. Following is the final equation in terms of actual factors:

Final equation in terms of actual factors:

$$\text{Yield of Cinnamon Bark Oil (\%)} = (-2.52) + 0.31A + 0.012B + 0.042C - 0.0033AC - 0.000088BC + 0.0000061ABC \quad (4.1)$$

where,
A is the water: cinnamon bark powder ratio,
B is the microwave power level and
C is the extraction time.

Table 4.2 summarizes the statistical data used to adequate the model. The goodness of fit was evaluated by the coefficient of determination, $R^2$, adjusted $R^2$, predicted $R^2$, coefficient of variance (CV), prediction residual error sum of squares (PRESS), adequate precision and the lack of the fit test for the model from the ANOVA results.

Table 4.2
Results for the test goodness of fit

| | **Cinnamon bark oil yield (%)** |
|---|---|
| *p*-Value of model | 0.0005 |
| $R^2$ | 0.9786 |
| Adjusted- $R^2$ | 0.9529 |
| Predicted- $R^2$ | 0.9610 |
| CV | 3.0700 |
| PRESS | 0.0500 |
| *p*-Value of lack of fit | 0.9037 |

Based on the results obtained, the *p*-values of the model is <0.01 which implies that the variables in the model have significant effects on the yield of cinnamon bark oil. $R^2$ is the portion of the variation in the response that contributed to the experimental model. It is advised that $R^2$ should be very close to 1 for a good fit model (Xiangli et al., 2008). However, the value of $R^2$ being close to 1, does not implies that the model is a good one. This is because, $R^2$ value may increase with the input of new variables in the model regardless of whether the variables are significant or not. Therefore, it is necessary to consider the adjusted-$R^2$ in the evaluation of the model adequacy. The adjusted-$R^2$ preferably should be more than 90 % in order to signify a very high correlation between measured and predicted values. It is noteworthy that in this model the $R^2$ and adjusted-$R^2$

values were more than 90 % due to omission of the insignificance variable which is interactive factor of water: cinnamon bark powder ratio and microwave power level. Besides that, the predicted-$R^2$ describes the amount of differences in the new data measured by the model. As the $R^2$ and adjusted $R^2$, the predicted-$R^2$ also should be more than 90 % to fit the model adequacy. The predicted-$R^2$ of 0.9610 obtianed in this model is in reasonable agreement with the adjusted-$R^2$ of 0.9529.

Coefficient of variance (CV) is the standard deviation of the variables demonstrated in the percentage of mean. Generally, CV should not be more than 10 % for a good fit of a model and for this model the value obtained is 3.07 for cinnamon bark oil yield (%). PRESS usually measured the overall measurement of the inconsistency between the real data and estimation model. In order to get the better model's estimation, the PRESS value should be small. Adequate precision test is performed in order to measure the signal to noise ratio. The large value of adequate precision which is more than 4, indicates that the model can be used to navigate the design space. The ratio of 21.2310 shows an adequate signal. Finally, it is advisable for the lack of fit to be not significant because it is a measure of the failure of a model to the pure error which points were not in the experimental range. For this model, the lack of fit was found to be not significant which is a good conformity with the model expectations. Thus, the results summarized in Table 4.2 demonstrates that all the terms agreed for a good fit of the model for extraction of cinnamon bark oil yield.

Figure 4.6 illustrates the 3D response surface plot of main and interaction effect of factors on extracted cinnamon bark oil yield (%). 3D surface plot of yield at variation of water to cinnamon bark powder ratio and microwave power at fixed extraction period of 75 min is depicted in Figure 4.6 (a). The effect of microwave power is more prominent than water-to-raw material ratio. The increase in the microwave power level, increase gradually the yield of essential oil from cinnamon bark. Similar observation was reported in previous research work for *Radix Bupleuri* (Hu et al., 2008). The reason for this may be related to the rapid microwave heat generation inside the cinnamon matrix solution where the water which used as solvent for extraction has high dielectric properties. Therefore, water may obstruct the electromagnetic wave produced by microwave and convert it into heat energy which is distributed inside the cinnamon matrix (Kormin et al., 2013). As a result, higher microwave power may produce more microwave energy which absorbed by high dielectric properties of solvent (water), and it may immediately form a high internal pressure gradient inside the cinnamon matrix (Dhobi et al., 2009 and Rezvanpanah et al., 2008). Thus, when the irradiation power increases, the yield of cinnamon bark oil also increases. Similarly, as the water-to-cinnamon bark powder ratio increases, the yield of cinnamon bark oil also increases slightly. Generally, the purpose of solvent in extraction process is to protect the plant from being thermally degraded and also to act as the essential oil carrier during the distillation process. In microwave environment, solvent plays an important role in extracting essential oil. This is because the properties of water which has high dielectric constant and dielectric loss may increase the capacity of solvent (water) to absorb more microwave energy. This would also lead to fast heating rate and highly enhanced extraction process. Similar observation have also been reported in literature by Letellier and Budzinski (1999a) that in an open-vessel microwave extraction method, the rate of heating is faster when more volume of solvent used (Letellier and Budzinski, 1999a and Shaharuddin et al., 2014)

However, using large amount of solvent may offer negative impact to the environment. Reason for this is because more energy may be required to reach the boiling point of the large amount of solvent. Hence, more fossil fuel or gas may need to be burnt in order to provide required energy to reach the boiling point of the large volume of solvent and to maintain the boiling point temperature. This may result in the emission of more carbon dioxide to the environment. It is therefore advisable to minimize the total consumption of solvent in the extraction of essential oil (Chen et al., 2007). From the Pareto chart in Figure 4.5, the contribution of solvent: raw material ratio factor produced the least percentage of contribution compared to the other two main factors. Therefore, increasing or decreasing the amount of solvent might not really affect the extraction yield. This indicates that just the sufficient amount of solvent (water) is good for enhancement of extraction yield. Figure 4.6 (b) illustrates the 3D surface plot of yield at water: raw material ratio and extraction time at fixed microwave power level of 225 W. The extraction time demonstrated the most significant effect on the extraction yield. Extraction yield increased drastically with the increment of extraction time. Similar trend was observed for the cinnamon bark oil yield from the effects of the extraction time and microwave power which is depicted in Figure 4.6 (c).

These results are in agreement with the previous studies on the extraction of saikosaponins from *Radix Bupleuri* (Hu et al., 2008). From that research, it was observed that the extraction yield increases correspondingly to the increase in extraction time. Also according to Lucchesi et al. (2007), it was reported that the extraction time generally presents a positive effect on the extraction yield of essential oil. Similarly, from the studies by Xiao et al. (2008), it was suggested that the yield of extracted product may be enhanced by the usage of larger volume of solvent and longer irradiation time on the plant matrix.

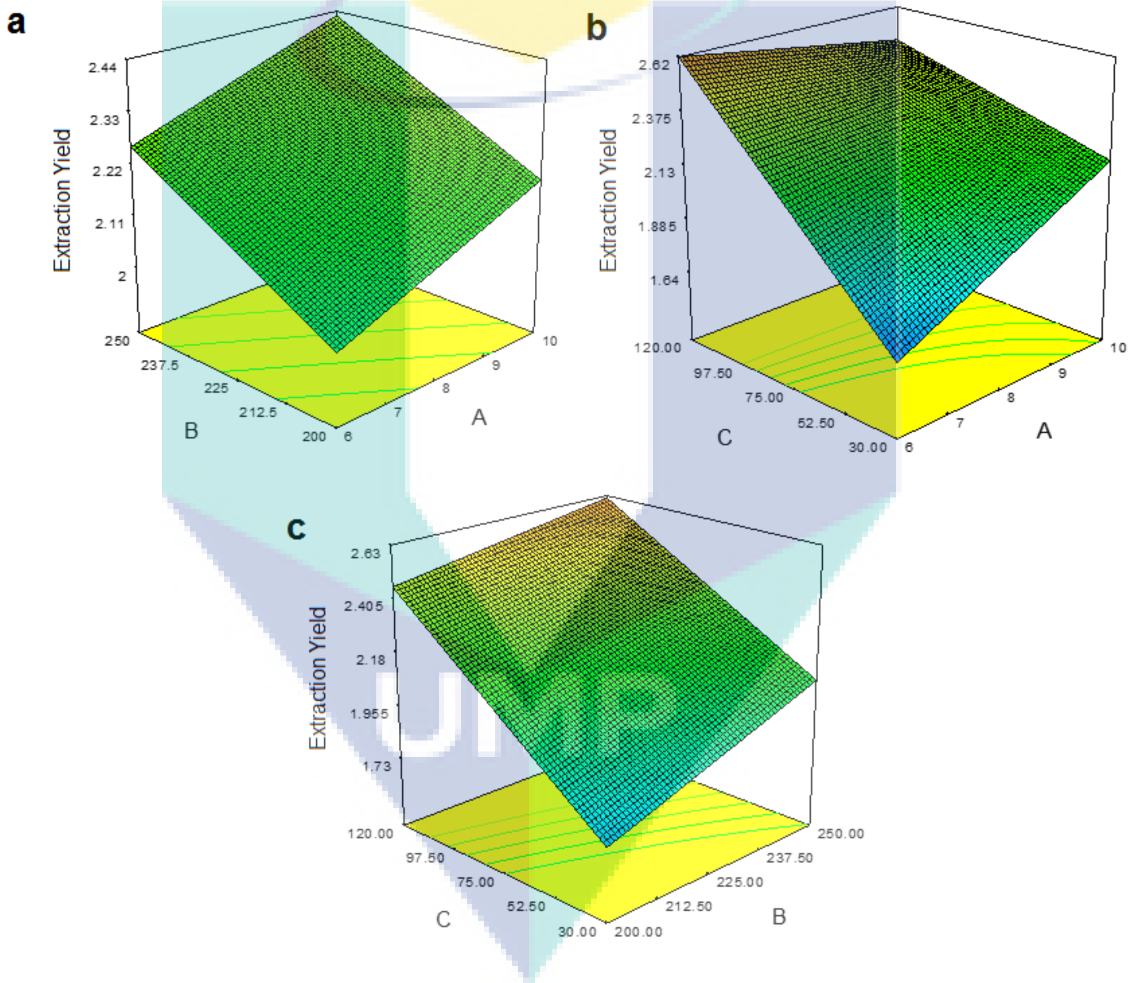


Figure 4.6: Response surface plot of extracted cinnamon bark oil yield as a function of: (a) water: raw material ratio (A) and microwave power (B) at fixed extraction time of 75 min; (b) water: raw material ratio and extraction time (C) at fixed microwave power of 225W; (c) microwave power and extraction time at fixed water: raw material ratio of 8:1.

Twelve additional experimental runs were performed in order to experimentally verify the adequacy of the developed empirical model. Figure 4.7 depicts the comparison of predicted and experimental (actual) responses. The results demonstrated that the predicted values were in reasonable agreement with the experimental data, further indicating the good prediction ability of the model. As a result, the best conditions were chosen from factorial analysis to further evaluate the optimum conditions for MAHD by using Response Surface Methodology (RSM), Design Expert. The best operating conditions are irradiation power of 225 W, extraction time of 75 min and water: cinnamon powder ratio of 8:1. The optimization of MAHD process parameters is further discussed in the following section.

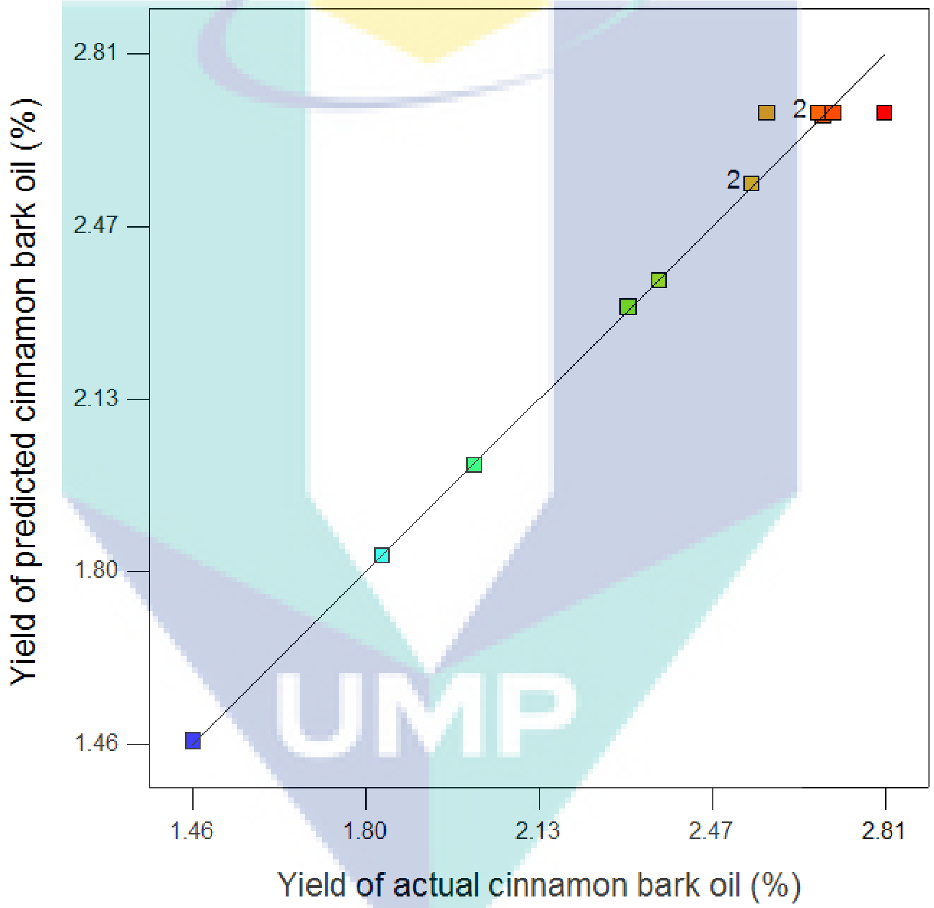


Figure 4.7: Comparison on response of the yield of predicted and experimental cinnamon bark oil.

### 4.3.2 Optimization of Cinnamon Bark Oil Extraction through MAHD by using Response Surface Method

Based on results obtained from the two level factorial analyses, all the three linear factors were significant and chosen for optimization process. The range for factors like water: raw material ratio, microwave power levels and extraction time were narrowed down to find the best optimum process conditions for extraction of cinnamon bark oil with maximum extraction yield. Experiments were designed according to Central Composite Design (CCD) in three numeric factors under the Response Surface Methodology (RSM), Design Expert software. The experimental design and cinnamon bark oil yield (%) are summarized in Table 4.3.

Table 4.3
Experimental layout of Central Composite Design (CCD) in extraction of cinnamon bark oil yield

| **Std** | **Run** | **Factor 1** | **Factor 2** | **Factor 3** | **Response 1** |
|---|---|---|---|---|---|
| | | **A: Microwave Power (W)** | **B: Water-to-Raw Material Ratio (w/w)** | **C: Extraction Time (min)** | **Cinnamon bark oil yield (%)** |
| 1 | 16 | 222.50 | 7.50 | 67.50 | 2.59 |
| 2 | 17 | 227.50 | 7.50 | 67.50 | 2.61 |
| 3 | 20 | 222.50 | 8.50 | 67.50 | 2.54 |
| 4 | 3 | 227.50 | 8.50 | 67.50 | 2.60 |
| 5 | 12 | 222.50 | 7.50 | 82.50 | 2.55 |
| 6 | 4 | 227.50 | 7.50 | 82.50 | 2.59 |
| 7 | 8 | 222.50 | 8.50 | 82.50 | 2.51 |
| 8 | 18 | 227.50 | 8.50 | 82.50 | 2.58 |
| 9 | 10 | 220.00 | 8.00 | 75.00 | 2.50 |
| 10 | 9 | 230.00 | 8.00 | 75.00 | 2.59 |
| 11 | 1 | 225.00 | 7.00 | 75.00 | 2.57 |
| 12 | 6 | 225.00 | 9.00 | 75.00 | 2.53 |
| 13 | 11 | 225.00 | 8.00 | 60.00 | 2.52 |
| 14 | 2 | 225.00 | 8.00 | 90.00 | 2.47 |
| 15 | 13 | 225.00 | 8.00 | 75.00 | 2.69 |
| 16 | 7 | 225.00 | 8.00 | 75.00 | 2.73 |
| 17 | 19 | 225.00 | 8.00 | 75.00 | 2.70 |
| 18 | 14 | 225.00 | 8.00 | 75.00 | 2.69 |
| 19 | 5 | 225.00 | 8.00 | 75.00 | 2.70 |
| 20 | 15 | 225.00 | 8.00 | 75.00 | 2.75 |

From the collected results, it can be observed that the highest and lowest yields of cinnamon bark oil were obtained at same irradiation power (225 W) and water: cinnamon powder ratio (8:1). The only difference is the extraction time which is 75 min and 90 min and the yield obtained from them are 2.75 % (75 min) and 2.47 % (90 min) respectively. These observations suggest that extraction time gives major impact on the extracted yield of cinnamon bark oil. Initially as the extraction time increases from 60 min to 75 min, the extraction yield increases at the fixed irradiation power (225 W) and water: raw material ratio (8:1), and then further increases of the extraction time to 90 min, reduction in yield of cinnamon bark oil was observed. Similar observations has been reported by Rezvanpanah et al. (2008) and Xiao et al. (2008) in extraction of essential oil from *Satureja hortensis* and *Satureja montana* and *Radix astragali* by using MAHD respectively. The reason for the rise in yield of cinnamon bark oil at the early parts of the extraction may be attributed to effective heat distribution to the cinnamon matrix. This was made possible due to the high dielectric properties of water which has the tendency to generate efficient heat to the matrix after it might have absorbed the microwave energy. This is because solvents that have high dielectric properties tend to absorb more microwave energy. Meanwhile, decrease in the yield of cinnamon bark oil at 90 min of extraction time could be associated with possible degradation of cinnamon samples in the solution inside the microwave, as the extraction period becomes prolonged perhaps due to overheating of the plant sample (Kaufmann et al., 2001 and Xiao et al., 2008). Also, prolonged application of microwave energy on the cinnamon solution might lead to undesirable evaporation of the volatile component in the cinnamon bark oil which thereby resulting into decrement in the extraction yield.

### 4.3.3 Fit Summary and Analysis of Variance (ANOVA)

The 4 models which were suggested in the fit summary are linear, two factorial level, quadratic and cubic. According to the model summary statistics, the suggested model by the design expert software was quadratic polynomial model. The ANOVA showed that the adjusted $R^2$ value for the quadratic model (0.9540) was higher than that of linear (-0.0633) and two factorial (-0.3003) models. The cubic model was found to be aliased. The extracted cinnamon bark oil yield (Y) was expressed as a function of independent variables shown in Eqs. 4.2 and 4.3 in terms of coded and actual levels.

$$\text{Coded : Yield of cinnamon bark oil (\%)} = 2.71 + 0.023X_1 - 0.012X_2 - 0.013X_3 + 0.0088X_1X_2 + 0.0038X_1X_3 + 0.0013X_2X_3 - 0.042X_1^2 - 0.040X_2^2 - 0.054X_3^2 \quad (4.2)$$

Actual : Yield of cinnamon bark oil (%) = $-335.50 + 2.93X_1 + 0.96X_2 + 0.095\,X_3 + 0.0070X_1X_2 + 0.00020X_1X_3 + 0.00033X_2X_3 - 0.0067X_1^2 - 0.16X_2^2 - 0.00096X_3^2$ (4.3)

where,

$X_1$ is the microwave power,

$X_2$ is the water: raw material ratio

$X_3$ is the extraction time

The results for ANOVA response surface quadratic model is illustrated in Table 4.4. From the ANOVA summary, p-value was used to estimate the coefficients of this model and to check the significance and interaction influence of each parameter. The values of p-value <0.01 indicates the model terms are significant. Based on the table, it can be seen that p-value of the model is <0.0001 which implies that the model is significant. In this model, the main factors, $X_1$, $X_2$, $X_3$ are significant model terms. The lack of fit value is 0.9977 calculated based on p-value and indicates that the lack of fit is not significant. In order for the model to be fit, the lack of fit p-value should not be significant as in this model. The value of coefficient of determination $R^2$ and adjusted coefficient of determination ($R^2$adj) were calculated as 0.9758 and 0.9540 respectively. According to literature it was reported that for a good fit of a model, the $R^2$ value should be more than 0.8000 for biological process and 0.9000 for chemical process (Lee et al., 2010). It is noteworthy that the $R^2$ value of this model was more than 0.9000 which indicates that the estimated model fits the experimental data adequately. This is further certified through the regression of model.

Table 4.4
ANOVA for response surface quadratic model

| **Source** | **Degree of freedom, df** | **Sum of squares** | **Mean square** | **F-value** | **p-value probability** |
|---|---|---|---|---|---|
| Model | 9 | 0.1300 | 0.0140 | 44.740 | <0.0001 |
| Microwave power, $X_1$ | 1 | 0.0086 | 0.0086 | 27.230 | 0.0004 |
| Water: raw material ratio, $X_2$ | 1 | 0.0023 | 0.0023 | 7.1800 | 0.0231 |
| Extraction time, $X_3$ | 1 | 0.0028 | 0.0028 | 8.7700 | 0.0143 |
| $X_1X_2$ | 1 | 0.0061 | 0.0061 | 1.9500 | 0.1929 |
| $X_1X_3$ | 1 | 0.0011 | 0.0011 | 0.3600 | 0.5629 |
| $X_2X_3$ | 1 | 0.0013 | 0.0013 | 0.0400 | 0.8459 |
| $X_1^2$ | 1 | 0.0430 | 0.0430 | 138.40 | <0.0001 |
| $X_2^2$ | 1 | 0.0410 | 0.0410 | 130.20 | <0.0001 |
| $X_3^2$ | 1 | 0.0740 | 0.0740 | 234.08 | <0.0001 |
| Residual | 10 | 0.0003 | 0.0003 | | |
| Lack of fit | 5 | 0.0001 | <0.0001 | 0.0480 | 0.9977 |
| Pure error | 5 | 0.0030 | 0.0006 | | |
| Correction total | 19 | 0.1300 | | | |
| $R^2$ | 0.9758 | | | | |
| Adj $R^2$ | 0.9540 | | | | |

Figure 4.8(a) depicts the experimental versus predicted cinnamon bark oil yield calculated based on Eq. 4.3. A linear distribution is observed which suggests a well-fitted model. The values predicted from Eq. 4.3 were close to the actual values of cinnamon bark oil yield that were obtained by running the experiment. The normal probability plot is also presented in Figure 4.8(b). The plot demonstrated that the residuals (variation between actual and predicted values) follow the normal distribution with a straight line.

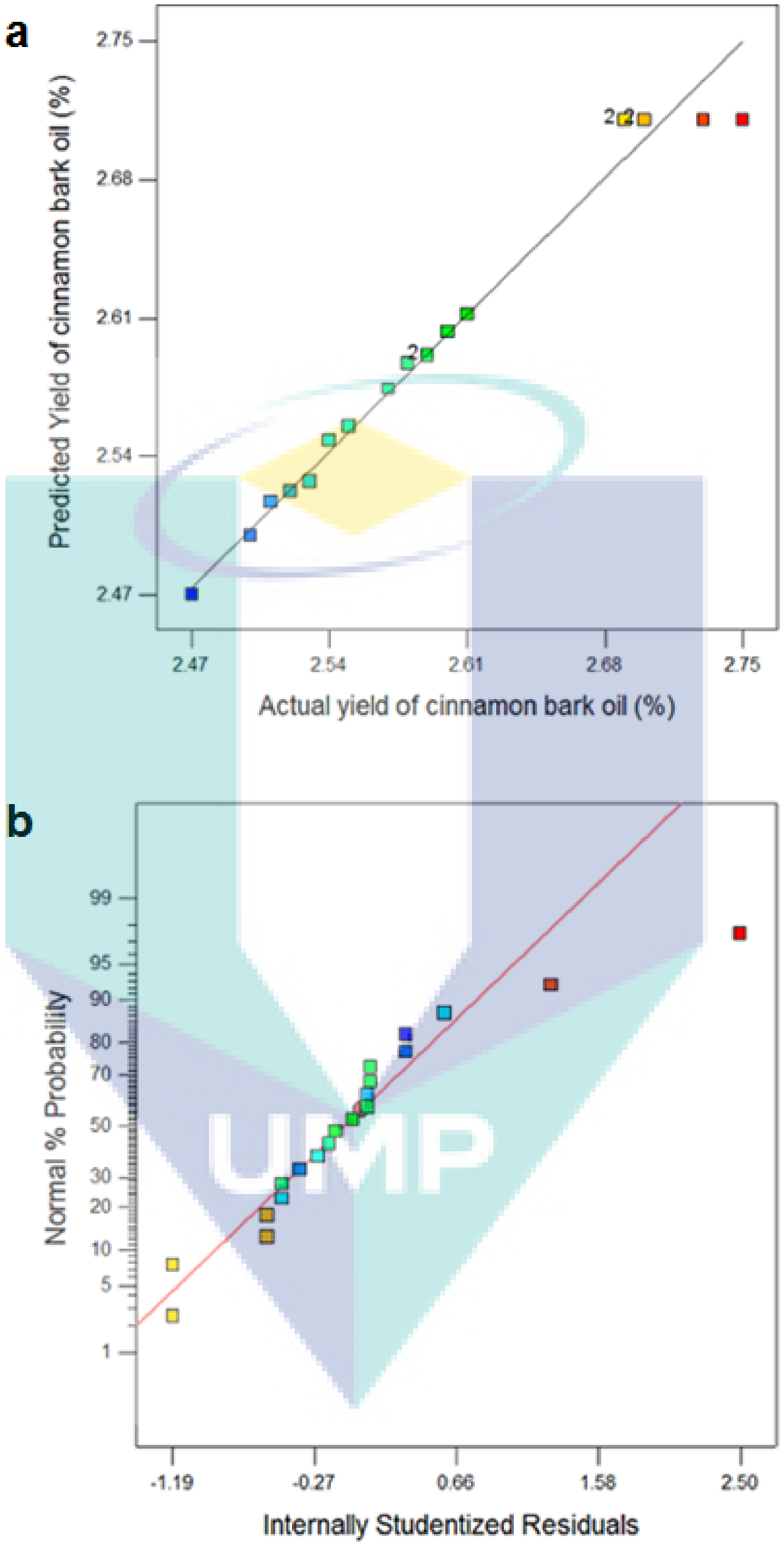


Figure 4.8: (a) Correlation of actual and predicted values by the models and (b) normal probability of residuals.

### 4.3.4 Effect of Independent Processing Parameters

There were three independent variables (microwave power, water: raw material ratio and extraction time) which effect was investigated on the yield of cinnamon bark oil as depicted in Figure 4.9. From Figure 4.9(a), it can be seen that the extracted cinnamon oil yield kept on increasing as the irradiation power increases from 222.5 W to 225.0 W and began to decrease after 225.0 W. Noteworthy is fact that the highest yield was obtained at 225.0 W, at fixed water: cinnamon powder ratio of 8:1 and extraction time of 75 min. The extraction yield can be seen to increase from 2.64 % (222.5 W) to 2.71 % (225.0 W) and then level off to 2.69 % (227.5 W). The initial yield increase when the microwave irradiation power was raised from 222.5 W to 225.00 W (1.12 % power increase) is about 2.65 %. However, upon the same increment range of the microwave power from 225.0 W to 227.5 W, 0.74 % yield decrease was observed. The initial increase in yield with increasing microwave power indicates that the rapid generation of heat was effective at producing the necessary pressure gradient for the immersed cinnamon powder to absorb sufficient microwave energy. In effect, this could enhance the quick release of essential oil from the glands of the material as reported in literature (Ferhat et al., 2006). However the decrease in yield when extraction was done at higher irradiation power of 227.50 W suggests possible degradation of the plant materials at elevated microwave irradiation power which might have led to undesirable escape of volatile component from cinnamon bark oil. As a result, decrease in bark oil yield was obtained. This conforms to the observations reported by Ma et al. (2009) for the case of *Rubia cordifolia* and Asghari et al. (2012) for the case of *Echinophora platyloba DC.*

The effect of water: cinnamon powder ratio on cinnamon oil yield is illustrated in Figure 4.9(b). At a fixed irradiation power of 225.0 W and at extraction time of 75 min, extraction yield increase from 2.68 % (7.5) to 2.70 % (8.0) but then decrease to 2.66 % (8.50). Generally, solvent (water) acts as the carrier of essential oil during the distillation process before condensation takes place and it thereby prevents the cinnamon sample from being thermally degraded. As the ratio of solvent increased from 7.5:1 to 8:1, the yield of essential oil was increased perhaps due to increase of the driving force of the mass transfer (Lee et al., 2013). However, the yield obtained began to decline when the water: raw material ratio was further increased to 8.5:1. This suggests that large amount of solvent (water) may lead to thermal stress to the cinnamon sample due to high absorption of microwave energy by the high dielectric constant solvent (water) during the rapid heat generation as reported elsewhere (Dhobi et al., 2009). Similarly, it had been reported that high temperature and large amount of solvent for a prolonged extraction of essential oil process may lead to undesirable yield loss via decomposition, oxidization or hydrolysis of components present in essential oils (Maria et al., 2010). Therefore, by using the sufficient amount of solvent ratio in an open system operation, it is possible to increase the yield of essential oil (Fathi and Sefidkon, 2012). As a result, it is important identify the optimum solvent ratio in the extraction of essential oil process in order to prevent the reverse act of the water. Hence, the results identify 8:1 as the optimum water-to-cinnamon powder ratio.

On the other hand, the relationship between yield of cinnamon bark oil and extraction period is illustrated in Figure 4.9(c). The yield can be seen to increase with increasing extraction period, particularly when the extraction time was increased from 67.5 to 75.0 min. However, further increase in the extraction time from 75.0 to 82.5 min made the yield of bark oil to visibly level off. For instance, at microwave power level of 225.0 W and water: cinnamon powder ratio of 8:1, the amount of yield increase about 1.88 % from 2.66 % to 2.71 % by raising the extraction time from 67.5 to 75.0 min. However, the cinnamon oil yield decreased more by 2.65 % to 2.64 % when the time was prolonged to 82.5 min. In general, the extraction rate was high at the beginning of the extraction but decline gradually over time as observed in similar research (Wang et al., 2008). A large number of researchers studied the effect of extraction time on various plant materials Asghari et al. (2012), Chen et al. (2007), Golmakani and Rezaei (2008) and Xiao et al. (2008), and almost all of them reported similar findings as depicted in Figure 4.9(c). This result also agrees with the Fick's second law of diffusion regarding the attainment of final equilibrium between the concentrations of solute in cinnamon powder and solvent (water) after a certain time. Therefore, when the extraction period was prolonged, it did not lead to significance effect on cinnamon oil yield. However, the initial raise in the extraction yield of bark oil can be accrued to the high absorption of microwave energy by polar solvent (water) that has high dielectric constant and helps to increase the heating rate of the matrix. Prolonged extraction period may lead to undesirable loss of volatile component in the cinnamon bark oil, due to absorption of excessive microwave energy by the solvent. This is because the amount of solvent (water) in the cinnamon solution may start to reduce due to evaporation process. The cinnamon powder may therefore be thermally degraded thereby leading to decrease in extraction yield. Hence, 75.0 min was chosen as the optimum extraction time for extraction of essential oil from cinnamon bark.

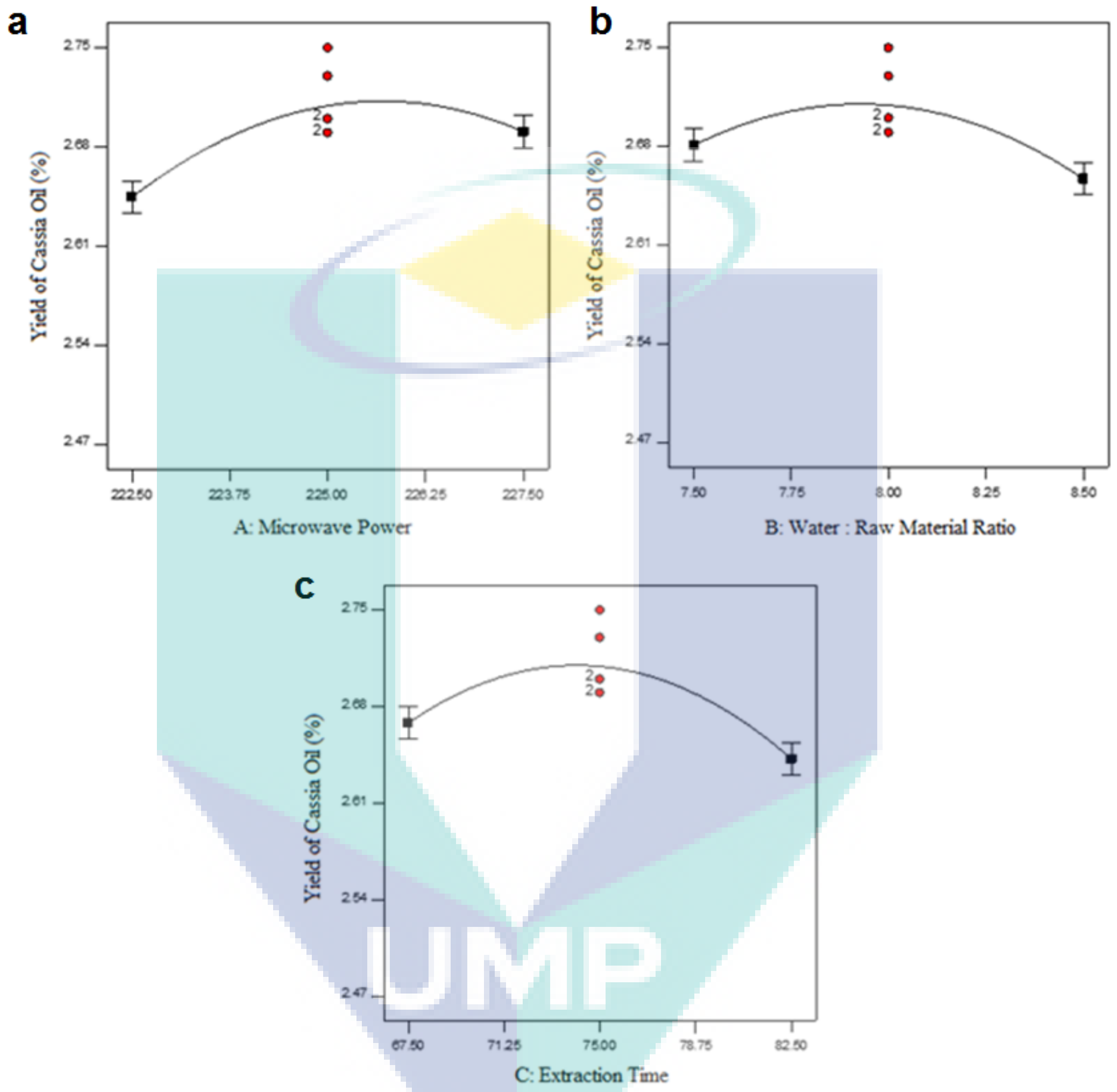


Figure 4.9: Effect of independent factors: (a) microwave power, W, (b) water: raw material ratio, and (c) extraction time (min) on the yield of cinnamon bark oil.

### 4.3.5 Effects of Interactive Factors

Figures 4.10 and 4.11 depict the effects of interaction between the factors such as microwave power level, water: cinnamon powder ratio and extraction time. The effect of interaction between microwave power and water-to-raw material ratio on the cinnamon bark oil yield extracted for 75 min is illustrated in Figure 4.10(a). The contour plot is illustrated in Figure 4.11(a). The effect of microwave power can be seen to be more significant at higher solvent ratio. For instance at 7.5:1 and 8.5:1 of water: cinnamon powder ratio, the yield of cinnamon oil rose from 2.62 % to 2.66 % and from 2.58 % to 2.66 %, respectively at fixed extraction period of 75 min. The reason for the more significant contribution of microwave power at higher solvent ratio is associated with the tendency for larger amount of solvent (water) to absorb more microwave energy. This owes to high dielectric constant of the solvent. Therefore, larger solvent (water) is capable of withstanding high irradiation power for prolonged extraction until the cinnamon oil is fully recovered from the cinnamon sample. Thus, the yield of cinnamon bark oil was higher at large volume of solvent. However, the extraction at lower volume of solvent (water) may lead to incomplete extraction of the cinnamon matrix due to the insufficient solvent (water) inside the cinnamon matrix which could not withstand high microwave energy for longer extraction period. As a result, the lower usage of solvent could thermally degrade the cinnamon matrix in the reactor. Thus, the yield of cinnamon bark oil might be less (Dhobi et al., 2009 and Gao et al., 2006). This indicates that the effect of solvent is one of the essential factor that influence the interaction between irradiation power as demonstrated in the work of Hu et al. (2008) on the extraction of saikosaponins from *Radix Bupleuri*.

Figures 4.10(b) and Figure 4.11(b) illustrated the effect of microwave power and extraction time on the extracted bark oil yield at constant water: cinnamon powder ratio of 8:1. An increase in yield of cinnamon oil was observed when the extraction time increased from 67.5 to 75.0 min. However, the yield of cinnamon bark oil began to decrease at prolonged extraction period from 75.0 to 82.5 min. This effect was observed for all levels of extraction time used in this research. For instance, when cinnamon powder was extracted at water: cinnamon powder ratio of 8:1 for 67.5 min, the yield of bark oil increased from 2.60 % to 2.67 % upon increasing irradiation power from 222.5 to 225.0 W. It however decreased slightly to 2.65 % when the power was increase further to 227.5

W. A similar observation was obtained at extraction period of 82.5 min. The yield of cinnamon oil increased from 2.57 % to 2.66 % by increasing microwave power from 222.5 to 225.0 W and the slightly drops to 2.64 % towards a power of 227.50 W. Based on the results obtained, the effect of irradiation power seem to be more significant at medium level of extraction period of 75 min. Similar observation has been reported by (Hu et al., 2008 and Lianfu and Zelong, 2008) in their research work on extraction of *Radix Bupleuri* and tomato paste respectively. This also suggests that the interaction between irradiation power and extraction period at higher levels may tend to become counteractive. This is because rise in irradiation power of microwave may increase the temperature inside the cinnamon matrix thereby decreasing the yield of cinnamon bark oil. Prolonged extraction may lead to unwanted evaporation of volatile component especially monoterpenes compounds that are present in the bark oil at the elevated temperature (Gavahian et al., 2012).

The effect of interaction between water: raw material ratio and extraction time at fixed irradiation power of 225.0 W is illustrated in Figures 4.10(c) and 4.11(c). As can be seen from the figures, at water: cinnamon powder ratio of 7.5:1 the yield of cinnamon oil was increased from 2.64 % to 2.68 % by raising the extraction period from 67.5 to 75.0 min and then drop to 2.61 % at 82.5 min. Similar trend was obtained for the cinnamon oil yield at water: cinnamon powder ratio of 8.5:1. The yield of cassia oil was increased from 2.61 % to 2.67 % by raising the extraction time from 67.5 to 75.0 min and decreased to 2.64 % at 82.5 min. Longer extraction time at large water: cinnamon powder ratio presents more noticeable effect on yield of cinnamon oil than at lower solvent ratio. This observation was also reported by Yang and Zhai (2010) in their research on extracting the anthocyanins from purple corn (*Zea mays* L.) cob.

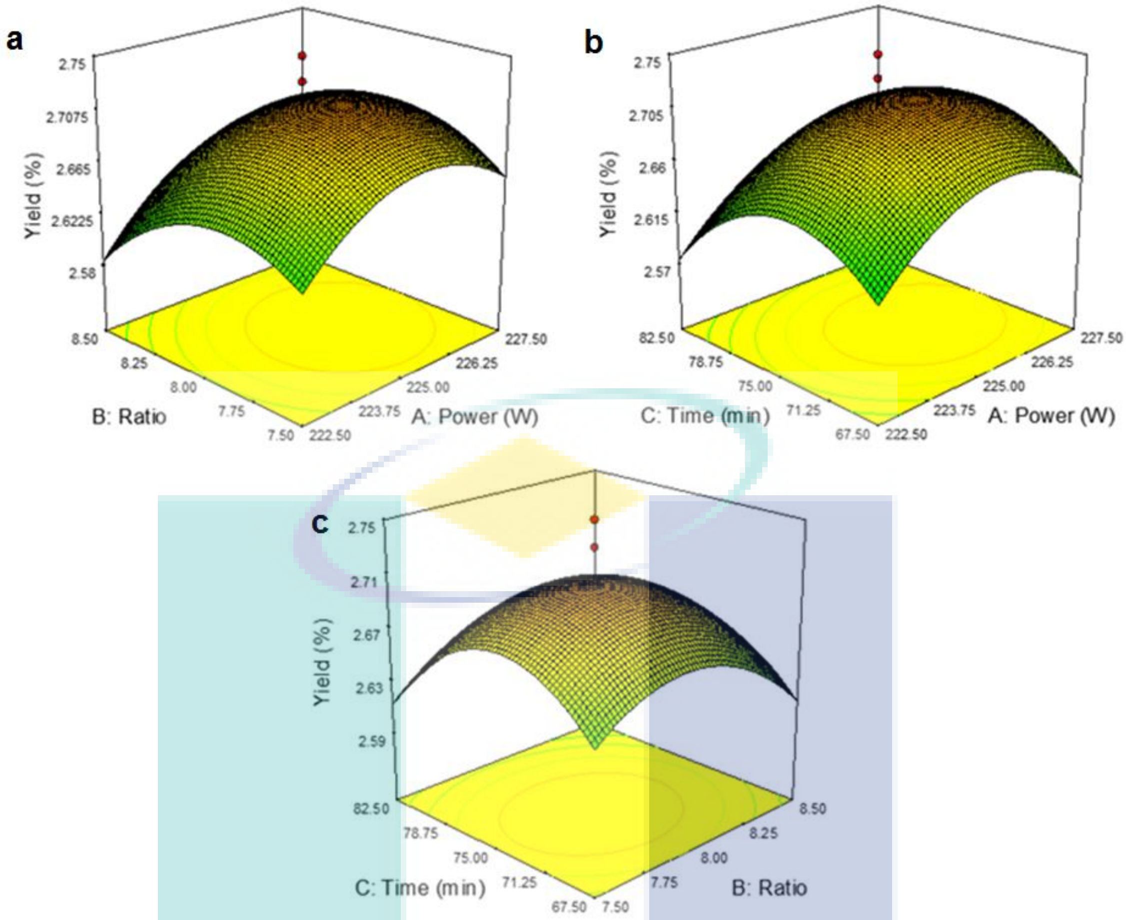


Figure 4.10: Response surface of cinnamon bark oil yield as function of (a) microwave power and water: cinnamon powder ratio at extraction time of 75.00 min, (b) microwave power and extraction time at water: cinnamon powder ratio of 8:1 and (c) water: raw material ratio and extraction time at microwave power of 225.00 W.

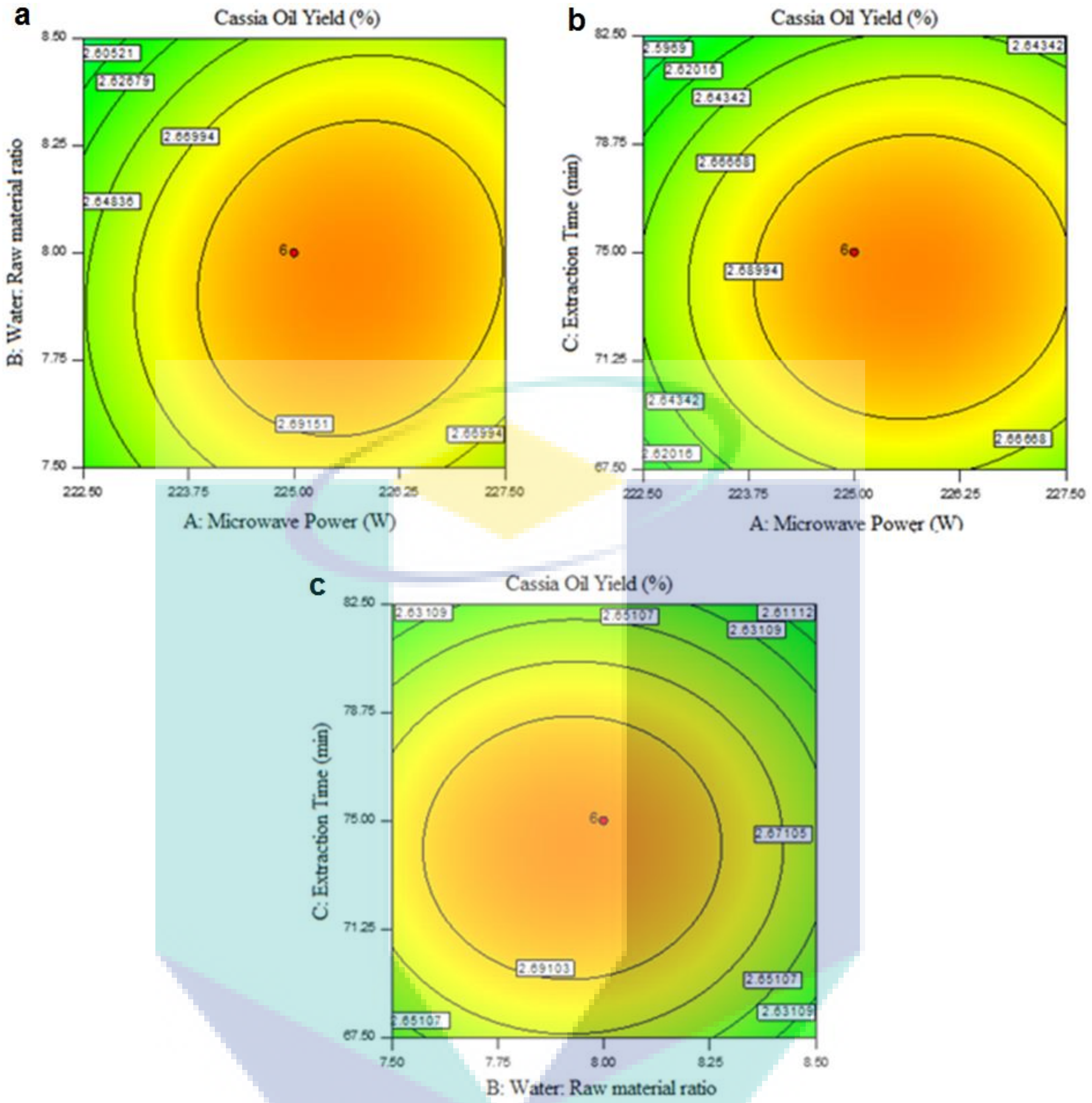


Figure 4.11: Contour plot of cinnamon bark oil yield as function of (a) microwave power and water: cinnamon powder ratio at extraction time of 75.00 min, (b) microwave power and extraction time at water: cinnamon powder ratio of 8:1 and (c) water: raw material ratio and extraction time at microwave power of 225.00 W.

### 4.3.6 Model Validation

The adequacy of the model equation was verified by running 5 confirmation tests and compare the results with the predicted optimum response values as illustrated in Table 4.5. The processing conditions for maximum cinnamon bark oil yield were used to experimentally validate and predict the values of the responses using the model equation. There was found a close agreement between the values calculated by using the model equation and experimental values of the response variables at the point of interest. The validity of the model of this process was calculated by using the $X^2$ goodness-of-fit test (Mooney and Swift, 1999). The $X^2$ value for 95 % confidence level for 3 degrees of freedom should be less than 7.81. This indicates that the model is valid and significant at 95 % confidence level.

From the model equation, the maximum cinnamon bark oil yield predicted is 2.71 % at the process parameters of 225 W of irradiation power, 8:1 of water: cinnamon powder ratio and 75 min of extraction time. The actual cinnamon oil yield obtained is 2.73 %. The result of validity was calculated as 0.0042 of $X^2$ value which means that this model is valid and significant.

Table 4.5
Validation results

| **Run** | **Variable Properties** | | | **Yield of cinnamon bark oil (%)** | | **Validity, *$X^2$** |
|---|---|---|---|---|---|---|
| | **Power (W)** | **Ratio** | **Time (min)** | **Experimental (E)** | **Predicted (P)** | |
| 1 | 225.00 | 8.00:1 | 75 | 2.73 | 2.71 | 0.0001 |
| 2 | 222.70 | 7.88:1 | 74 | 2.60 | 2.66 | 0.0014 |
| 3 | 225.00 | 8.50:1 | 74 | 2.58 | 2.66 | 0.0024 |
| 4 | 225.00 | 7.95:1 | 81 | 2.64 | 2.66 | 0.0002 |
| 5 | 227.50 | 8.00:1 | 81 | 2.66 | 2.64 | 0.0002 |

$*X^2 = \Sigma\frac{((E-P)^2)}{P} = 0.0042$

### 4.3.7 Comparison between OFAT Method and Response Surface Method Effects on Yield of Cinnamon Bark Oil

In Section 4.2, OFAT method was described as carried out to obtain the optimum conditions of MAHD and HD. However, in Section 4.3, two level factorial analysis, Design Expert software was used to analyse the best parameters to extract cinnamon bark oil through MAHD. The obtained optimum parameters were used to find the best conditions via central composite design, response surface method. It should be noted that only MAHD extraction method was evaluated by using Design Expert software in order to obtain the best optimum conditions in the extraction of cinnamon bark oil. This is because for this study, MAHD method is of particular interest. HD is a well-established conventional method in extraction field which has some drawbacks including long extraction period and loss of volatile compounds especially light weight monoterpene compounds with heavy consumption of energy (Gavahian et al., 2015). Therefore, efforts to replace the existing conventional HD method with upcoming advance MAHD extraction technique is being analysed meticulously. Hence, optimization process was conducted to obtain the best conditions of MAHD in extracting the maximum yield of cinnamon bark oil. This was evaluated in details so as to provide a platform for industries to consider the development of MAHD technique for large scale production.

Based on the results obtained from the OFAT method, the optimum parameters in the extraction of the cinnamon bark oil were irradiation power of 225 W, water: raw material ratio of 8:1 and extraction period of 90 min. Whereas for HD the optimum conditions obtained were water: raw material ratio of 8:1 and extraction period of 150 min at constant operating power supply of 350 W. In another vein, the optimum conditions obtained through design expert software were microwave power of 225 W, water: cinnamon powder ratio of 8:1 and extraction time of 75 min for MAHD. Looking at the results obtained through these two different methods, two out of the three factors analysed were obtained to be same. These are irradiation power of 225 W and solvent ratio of 8:1. However, extraction time was found to be different in both methods. It is noteworthy that lower extraction time value was obtained via response surface method compared to OFAT method. As discussed in this studies and mentioned in several literatures, prolonged extraction time has the possibility to offer losses of volatile compounds from the essential oil. Furthermore, the cinnamon matrix may be thermally degraded if the extraction time is too long (Asbahani et al., 2014; Gao et al., 2006; Gavahian et al., 2015 and Rezvanpanah et al., 2008). Based on these inferences, the optimum conditions chosen for further analysis in this research were microwave power of 225 W, water: cinnamon powder ratio of 8:1 and extraction time of 75 min for MAHD. On the other hand, the parameters adopted for conventional HD method are water: raw material ratio of 8:1 and extraction period of 150 min at constant operating power supply of 350 W.

## 4.4 Morphological Studies of Cinnamon Powder

In this section, the morphological changes to cinnamon powder before and after extraction will be evaluated at the optimum conditions of MAHD and HD. Furthermore, additional study was conducted on the effect of pre-treatment (soaking). To the best of our knowledge, there have been no studies on the effects of pre-treatment (soaking) on cinnamon bark powder.

### 4.4.1 Surface Morphology of Untreated and Pre-Treated Cinnamon Bark Powder

The surface morphologies of untreated dried powdered cinnamon bark and pre-treated powdered cinnamon bark (after soaking for 30 min) are shown in Figures 4.12a and 4.12b. From Figure 4.12a, it can be seen that the raw powdered bark is dry and lean. On the other hand, after pre-treatment of the powdered cinnamon bark, visible physical changes can be observed. The powdered cinnamon bark appears swollen and expanded as can be seen in Figure 4.12b. This indicates that the plant material (cinnamon bark) had absorbed solvent (water) during soaking for some period and the plant material began to expand. This would enhance the swelling and subsequent rupture of the tissue, making it easy for the volatile oils to flow into the water (Filly et al., 2014).

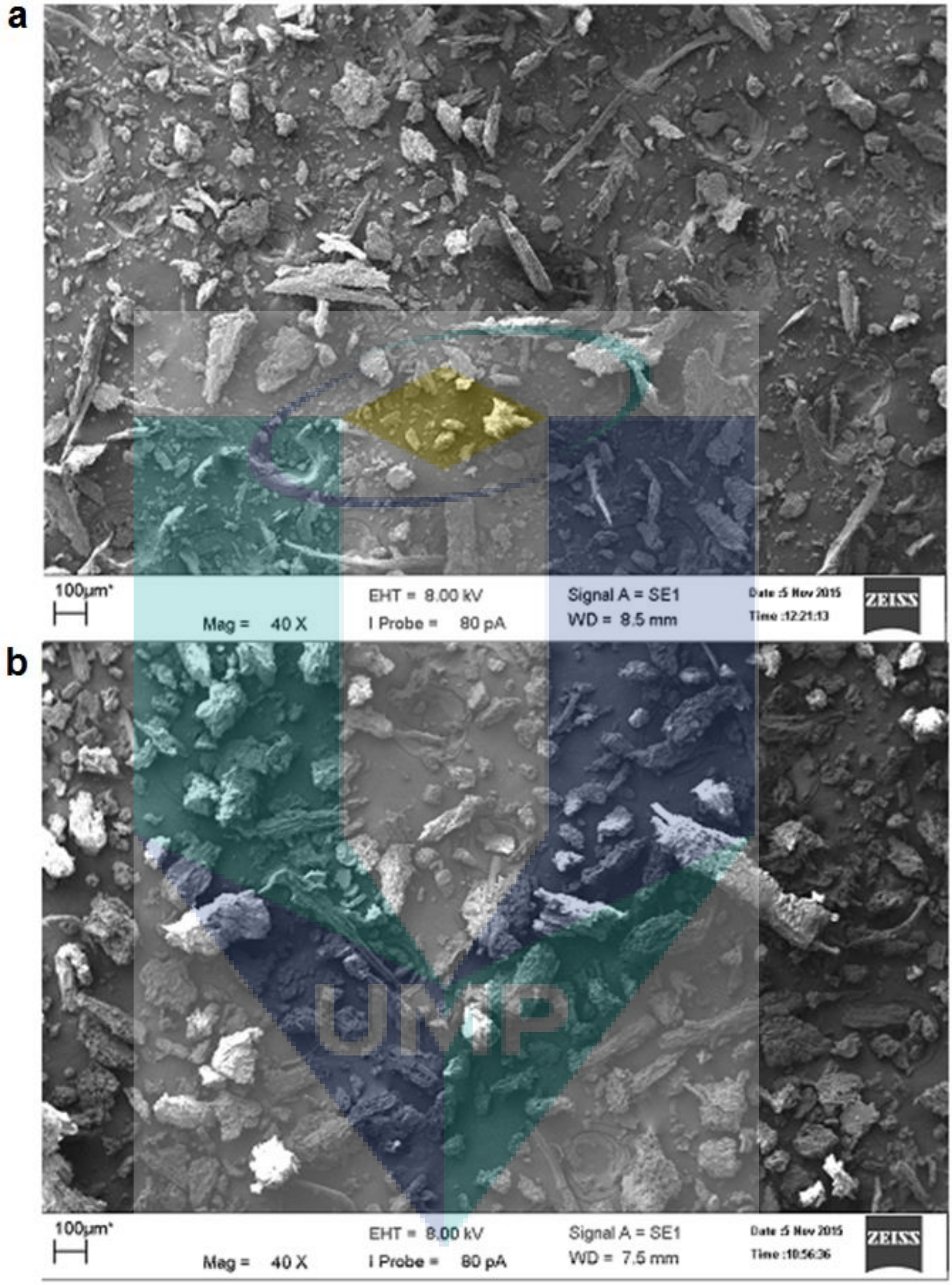


Figure 4.12: SEM images of (a) untreated raw cinnamon bark powder and (b) pre-treated cinamon bark powder after soaking for 30 min.

### 4.4.2 Morphological Changes to Cinnamon Bark After Extraction with MAHD and HD

During soaking, there is high tendency for the oil glands in the powder to become swollen and expanded as can be seen in Figure 4.13a. This would be helpful in enhancing the smooth and quick release of oil from the material during extraction. On the other hand, if extraction is carried out without soaking of the material, the oil glands which might have been contracted and shrunken would have to undergo a higher stress to release its oil. Invariably, higher amount of energy would be needed to raise the internal pressure of the oil glands before rupture and oil release could be possible. It is therefore suggested that plant materials should be pre-treated (soaking process) prior to extraction of essential oils from them in order to reduce the induction time, as well as overall energy consumption.

Figure 4.13b represents the SEM image of the oil gland after MAHD extraction of 75 min whereas Figure 4.13c represents SEM image of the oil gland after HD extraction of 150 min. As can be seen from these two images, the nature of disruption (highlighted in red) experienced by the oil gland differs between the two techniques. In the case of MAHD, there is a mild disruption of the oil gland which can be associated with the nature of heat distribution during MAHD extraction. Efficient heat distribution would allow the oil gland to conveniently release the oil without necessarily causing a damaging rupture to the glands (Figure 4.13b). However, in the case of HD, a high level of damaging rupture can be seen on the gland (Figure 4.13c). This suggests that HD technique is accompanied by some degree of aggressiveness leading to high mechanical strain of the oil glands. The effect of this would be an explosive rupture of the glands in order to retrieve the oil. This could be the reason for the explosive disruption of the gland during HD extraction as revealed by the SEM observation

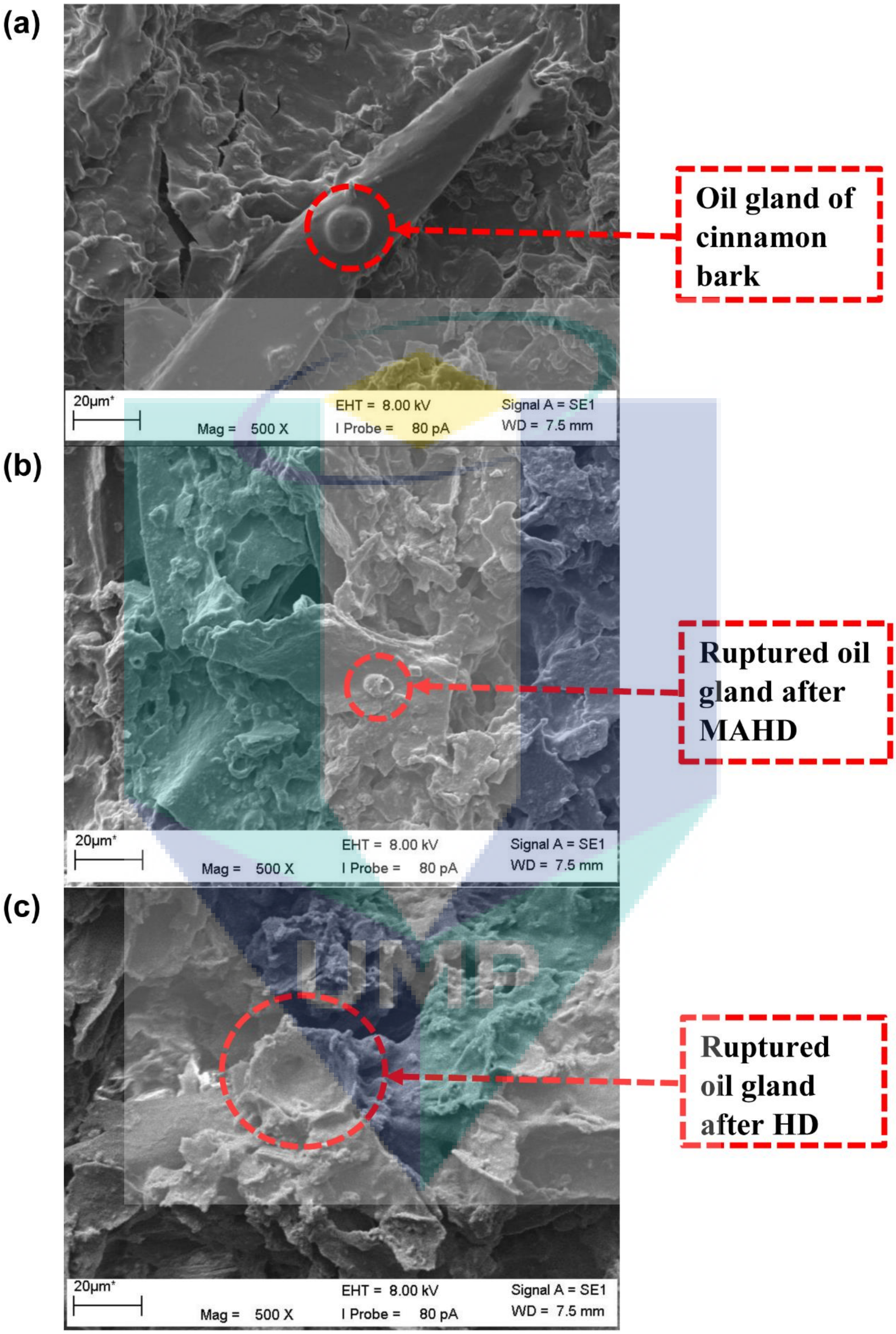


Figure 4.13: Scanning electron micrographs of oil cell glands of cinnamon bark (a) after pre-treatment (soaking for 30 min), (b) after MAHD extraction (75 min) and (c) after HD extraction (150 min).

## 4.5 Spectra Analysis of Essential Oil

The FTIR spectra for cinnamon bark oils obtained through HD and MAHD are illustrated in Figure 4.14. As can be seen from these spectra, there is an overlap of the absorption spectrum among each of the different oil components. This is due to the mixture complexity of volatile oils. The region from 1800-600 $cm^{-1}$ is highly informative as the peaks for the characteristic fingerprint of cinnamon bark oil lie within this range. Some of the observable peaks include 1668 $cm^{-1}$ and 1623 $cm^{-1}$ which represents the vibration stretching of the carbonyl C=O groups of an aldehyde. These peaks indicate that a high level of aldehydes and cinnamaldehydes are present in the oil extracted from the cinnamon bark (Adinew, 2014 and Singh and Ahmad, 2015). The peak at 1574 $cm^{-1}$ corresponds to C=C skeletal vibration from the aromatic rings of the aromatic components while the peak at 1449 $cm^{-1}$ is a representation of the vibrational bending absorption of C─OH from the alcohol moieties. The peak at 1294 $cm^{-1}$ is a characteristic of $─CH_2$ swing in alkanes and =C─H in-plane bending absorption of the aromatic rings, whereas the peak at 1250 $cm^{-1}$ is attributed to symmetric expansion of C─O─C from aromatic acid ester and vibrational stretching of C─OH groups from phenolic components. This peak is often associated with absorptions of eugenol and esters present in volatile oils (Li et al., 2013b). The peak at 1119 $cm^{-1}$ and 1072 $cm^{-1}$ corresponds to C─O stretching vibration and deformational vibration of C─OH groups. The peak at 970 $cm^{-1}$ is an attribute of vibrational bending absorption of C─H groups while the peak at 748 $cm^{-1}$ corresponds to =CH vibrational absorption of benzene rings. The peak at 686 $cm^{-1}$ is a representation of alkenes vibration absorption. Summary of the important peaks and their representations are included in Table 4.6. Generally, it can be seen that there is much resemblance in the spectra of oils obtained from these two methods. Due to the complexity of these oils and overlapping fingerprint of their chemical composition, it becomes seemingly difficult to state any major differences in their components. This is an indication that MAHD is suitable for extracting volatile oils from cinnamon barks without necessarily causing any adverse change to the chemical composition of the oil.

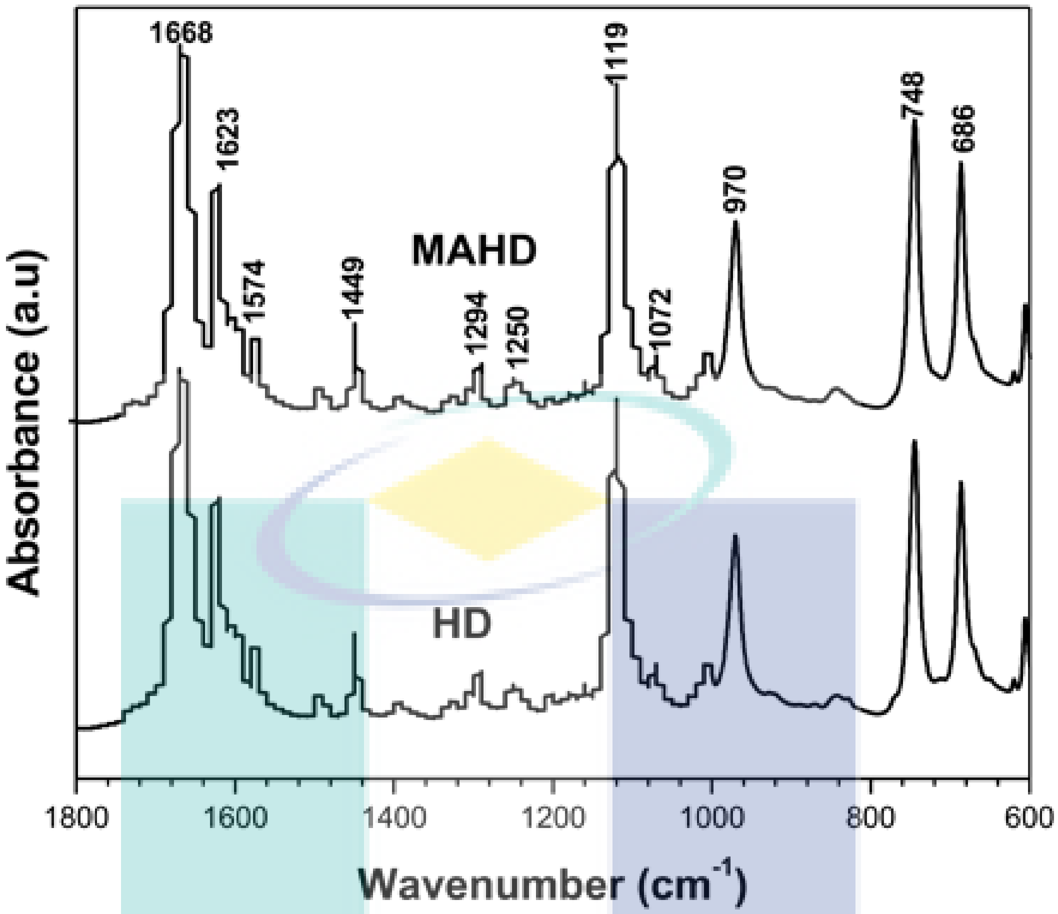


Figure 4.14: FTIR spectra of cinnamon bark oil obtained through MAHD and HD.

Table 4.6
Summary of functional groups present in cinnamon bark oil obtained through HD and MAHD

| **Functional group representation** | **Position of bands (cm$^{-1}$)** | |
|---|---|---|
| | **HD** | **MAHD** |
| C=O stretching vibration | 1668, 1623 | 1668, 1624 |
| C=C skeletal vibration of aromatic ring | 1574 | 1574 |
| C─OH absorption bending vibration | 1449 | 1449 |
| $CH_2$ swing and aromatic =C─H in-plane bending absorption | 1294 | 1294 |
| C─O─C symmetric expansion and phenolic C─OH stretching vibration | 1250 | 1250 |
| C─O stretching vibrations and C─OH deformation vibration | 1119, 1072 | 1119, 1072 |
| C─H bending vibration | 970 | 970 |
| C=H vibration of benzene ring | 748 | 745 |
| C=C vibration of benzene ring | 686 | 687 |

## 4.6 Cinnamon Bark Oil

The quality of cinnamon bark oil extracted through MAHD method was compared with those of conventional HD method in terms of chemical classes and chemical constituents. According to Karakaya et al. (2014), the standard reference for quantifying an essential oil is based on the HD extraction method. Therefore, the chemical compounds present in cinnamon bark oil extracted through MAHD method was compared with HD method to check the similarity of the chemical composition present between the two methods. In this section, the chemical compositions of the crude extract of cinnamon bark oil was analysed at various extraction time on the optimum conditions at microwave power level of 225 W and constant operating power supply of 350 W for MAHD and HD methods respectively at fixed water: cinnamon powder ratio of 8:1.

### 4.6.1 Identification of Cinnamon Bark Oil Crude Extract

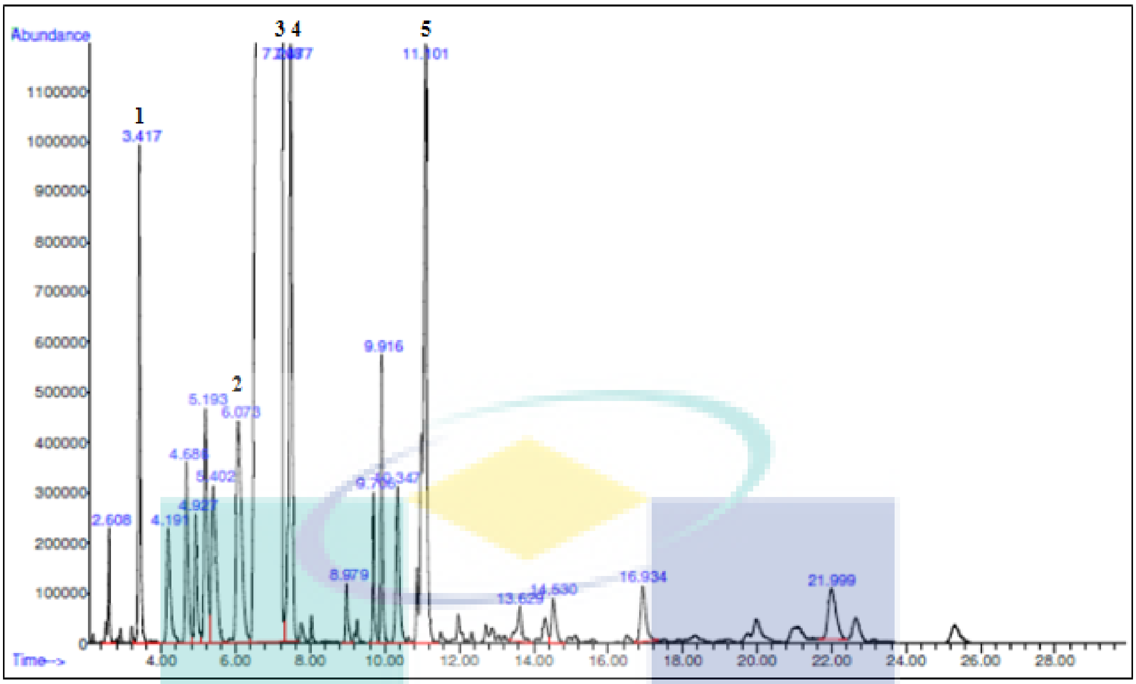
Cinnamon oil crude extract obtained through MAHD and HD methods were subjected to GC-MS analysis. Figures 4.15 and 4.16 illustrate the GC-MS chromatogram of bark oil crude extract during 30 min extraction period by MAHD (225 W) and HD (350 W) at fixed water: cinnamon powder ratio of 8:1 respectively. The rest of GC-MS chromatograms of MAHD and HD for various extraction time are attached in APPENDIX A. The nine chemical components identified in all extraction period from both MAHD and HD methods are eucalyptol, linalool, benzenepropanal, borneol, benzaldehyde, trans-cinnamaldehyde, coumarin, alpha-amorphene, (+)-delta-cadinene and caryophyllene oxide.

Total of 19 and 22 components were detected in GC-MS chromatogram for cinnamon bark oil at 30 min extraction period by MAHD and HD respectively. Trans-cinnamaldehyde is the marker and significant component in all extraction time for both extraction methods. For 30 min extraction period, the retention time of trans-cinnamaldehyde was 7.210 min and 7.345 min for MAHD and HD respectively.

As can be seen from the Figure 4.15, there were mainly five compounds which occupied high percentage peak area compared to other compounds present in the crude extract of cinnamon oil. These compounds are linalool (3.417 min), benzaldehyde (6.073

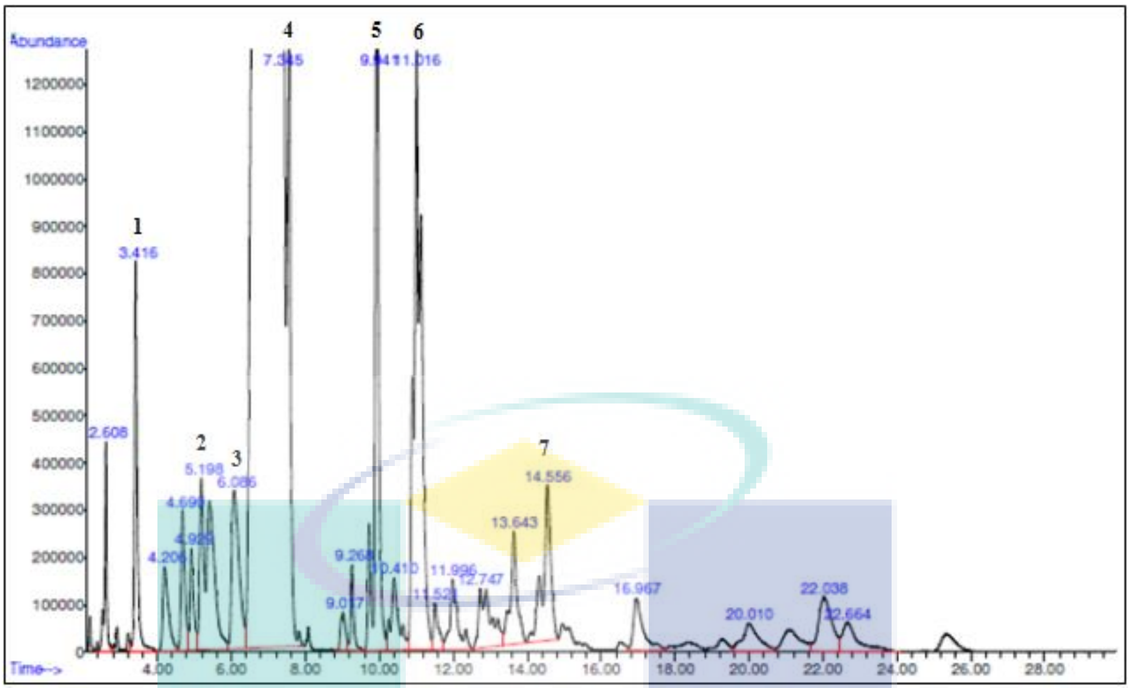
min), trans-cinnamaldehyde (7.210 min), anethole (7.475 min) and cinnamyl acetate (11.101 min). When compared to other extraction time of GC-MS chromatogram like 60 min, 90 min and 120 min by MAHD, four compounds were present in all the extraction period which are benzaldehyde, trans-cinnamaldehyde, anethole and cinnamyl acetate. As for 60 min and 120 min extraction period by MAHD, cis-cinnamaldehyde (5.404 min and 5.421 min respectively) was present in high percentage peak area. At 90 min and 120 min extraction period, coumarin was present at retention time of 10.422 min and 10.442 min respectively and caryophyllene exhibited retention time of 11.010 min for 90 min extraction time by MAHD.

Figure 4.16 illustrates the seven major compounds identified through their high peak area from essential oil obtained via HD as suggested by MS library. The seven compounds identified after 30 min extraction period by HD are linalool (3.416 min), α-terpineol (5.198 min), benzaldehyde (6.086 min), trans-cinnamaldehyde (7.345 min), copaene (9.939 min), caryophyllene (11.016 min) and (+)-delta-cadinene (14.557 min). α-Terpineol was a significant compound of 30 min (5.198 min) , 120 min (5.128 min) and 150 min (5.063 min). Besides that, cis-cinnamaldehyde was found to be highly present in extraction period of 90 min (5.263 min) and 180 min (5.269 min) by HD. Trans-cinnamaldehyde or also known as Cinnamaldehyde, (E) was the most significant and abundant component exhibit in all the extraction time of HD with retention time of 7.345 min (30 min), 7.210 min (60 min), 7.039 min (90 min), 7.157 min (120 min), 7.192 min (150 min) and 7.193 min (180 min). Bornyl acetate was present in 90 min extraction period at retention time of 7.345 min while anethole was present at 60 min and 120 min extraction time at retention time of 7.475 min and 7.445 min. Moreover, alpha-cubebene was rich component at extraction period of 90 min (9.880 min) and 150 min (9.792 min) whereas copaene was identified at 30 min (9.939 min), 60 min (9.880 min), 120 min (9.875 min) and 180 min (9.798 min). Coumarin component is often regarded as a toxic compound in cinnamon that may cause hepatic toxicity. It has a high influence in liver damage for rats and may as well be harmful for human beings. U.S Food and Drug Administration has therefore placed a ban on high percentage of coumarin content from food flavouring agent when discovered that it has the potential for hepatotoxic effects and it was also perceived to be a carcinogenic compound (FDA, 2015 and Lake, 1999). Then Lake (1999), did further research on the carcinogenicity effects of coumarin compound to ascertain its carcinogenicity tendencies (Lake, 1999). Interestingly, he found that coumarin is a non-genotoxic compound meaning it showed negative response towards carcinogenic tests. Therefore, European Food Safety Authority (EFSA), (EFSA, 2007), set 0.1 mg/kg of coumarin content as a tolerable daily intake (TDI) dosage (EFSA, 2007). In essence, it would be better if the content of courmarin compound is less in the extracted oil. It was found that in 60 min (10.304 min), 120 min (10.292 min), 150 min (10.175 min) and 180 min (10.181 min) extraction period in HD method, coumarin compound recorded a relative peak area range of about 1.24 % to 1.41 %. On the other hand, comparing the coumarin component obtained in MAHD extraction method, coumarin component was evident in only two extraction periods. It was identified in 90 min and 120 min extraction periods but with low relative peak area of 1.12 % and 1.15 % respectively. Based on the observation, advance MAHD extraction technique shows produced lower concentration of coumarin component compared to conventional HD. Besides that, caryophylenne was contained in all extraction period from 30 min to 180 min with the retention time of 11.016 min, 10.963 min, 10.804 min, 10.957 min, 10.839 min and 10.839 min with peak area percentage from 3.26 % till 4.27 % in HD extraction method. At last, delta-cadinene was found at 30 min and 180 min with respective retention time of 14.557 min and 14.222 min through HD technique.



| | | | |
|---|---|---|---|
| 1 | Linalool (3.417 min) | 4 | Anethole (7.475 min) |
| 2 | Benzaldehyde (6.073 min) | 5 | Cinnamyl acetate (11.101 min) |
| 3 | Trans-cinnamaldehyde (7.210 min) | | |

Figure 4.15: GC-MS chromatogram of MAHD at 30 min extraction time at fixed water: cinnamon powder ratio of 8:1 and microwave power of 225 W.



| | | | |
|---|---|---|---|
| 1 | Linalool (3.416 min) | 5 | Copaene (9.939 min) |
| 2 | α-Terpineol (5.198 min) | 6 | Caryophyllene (11.016 min) |
| 3 | Benzaldehyde (6.086 min) | 7 | (+)-delta-Cadinene (14.557 min) |
| 4 | Cinnamaldehyde, (E)- (7.345 min) | | |

Figure 4.16: GC-MS chromatogram of HD at 30 min extraction time at fixed water: cinnamon powder ratio of 8:1 and constant operating power supply of 350 W.

### 4.6.2 Classification of Cinnamon Bark Oil Crude Extract

The chemical components of cinnamon oil crude extract obtained through MAHD and HD were compared based on their chemical classes detected from various extraction periods. The composition of bark oil crude extract was analysed by GC-MS and almost a total of 36 chemical compounds were identified. The compounds with concentration more than 0.1 % are tabulated in Table 4.7. Chemical component with relative content that less than 0.1 % is regarded as negligible otherwise referred to as trace component present in an essential oil (Geng et al., 2011). The chemical components of cinnamon oil crude extract obtained by both HD and MAHD revealed similar compositional properties. This indicates that the important components obtained from oils extracted through HD were also present in MAHD. There were nine chemical compounds detected to be present at each extraction time through both extraction methods. However, only one of the compounds is often regarded as the active and marker compound of *Cinnamomum cassia* oil which is trans-cinnamaldehyde otherwise known as Cinnamaldehyde, (E)- (Geng et al., 2011; Li et al., 2013b; Ooi et al., 2006 and Shan et al., 2007). Results from this study conforms to this rule as can be seen from the table that bark oil crude extract obtained via both MAHD and HD methods are abundantly rich in trans-cinnamaldehyde compound. The percentage composition of this compound is 84.43 % and 79.55 % for MAHD and HD respectively at the optimum extraction conditions. Table 4.7 illustrates the molecular formulae of cinnamon bark oil crude extract, relative content in percentage for each extraction period and classification into chemical classes. Figures 4.17 and 4.18 illustrate the distribution of cinnamon oil crude extract functional group acquired through MAHD and HD extraction methods at various extraction periods. Table 4.8 comprises the mean chemical composition that was present at optimum conditions: 225 W of microwave power, 75 min of extraction time and 8:1 of water: cinnamon powder ratio for MAHD and 350 W of operating power, 150 min of extraction period and 8:1 of water: cinnamon powder ratio for HD.

In this research, chemical compounds were classified into four groups: oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and other oxygenated compounds. Generally, essential oils contain large abundance of monoterperne ($C_{10}$) and sesquiterpene ($C_{15}$) compounds. In cases whereby oxygen molecule is attached to either the monoterpene or sesquiterpene compound, it is referred to as oxygenated monoterpene or oxygenated sesquiterpene respectively. Based on these chemical compounds classes, the chemical components of cinnamon oil crude extract were classified. Initially, the relative content (%) of chemical components present in the bark oil crude extract were compared with the time allocated for the extraction through each extraction technique. As can be seen in the Table 4.7, extraction of cinnamon bark oil crude extract through MAHD produced more oxygenated fraction including marker component (trans-cinnamaldehye) and lower amount of sesquiterpene hydrocarbon compared to HD. For instance, MAHD recorded oxygenated component in the range of 95.13 %–98.30 % and sesquiterpene hydrocarbons in the range of 1.18 %-3.33 % while HD recorded about 85.35 %–88.52 % for oxygenated fraction and 9.19 %-11.74 % for sesquiterpene hydrocarbons. MAHD could therefore be proposed as a suitable technique for obtaining higher percentage of oxygenated compounds from cinnamon bark. Although there is no much difference in the overall percentage of essential oil obtained through MAHD and HD as the values can be seen to be close. On the overall, higher fraction of oxygenated compounds and low amount of sesquiterpene hydrocarbon was produced through MAHD, whereas for HD, reverse is the case. Oxygenated compound obtained through MAHD is about 8.58 % higher than what was obtained through HD. The longer extraction time associated with HD might lead to decomposition or degradation of some active compounds via hydrolysis or transesterification making them to transform into hydrocarbon (sesquiterpene) compounds (Asbahani et al., 2014 and Gavahian et al., 2015). These compounds however contribute less to the aroma properties of essential oils making them less important. Aromatherapy properties present in essential oils have been reported to possess great medicinal and curative properties (Fornari et al., 2012).

As can be seen from the Figure 4.17, the oxygenated monoterpene compounds decrease as the time increase from 30 min to 120 min. The reason could be associated with prolong heating of the cinnamon matrix, the components in the cinnamon oil crude extract might be thermally degraded or vaporise due to low molecular weight of oxygenated monoterpene compounds in the range of 148 to 164. Besides that, another obvious observation was that as the time increases, the sesquiterpene hydrocarbon chemical class compounds also increases. The reason is because the sesquiterpene hydrocarbons have higher molecular weight than oxygenated monoterpene compounds (about 204). Therefore, the components may not be easily degraded even after longer extraction period. As for oxygenated sesquiterpenes, when the extraction period increases, the oxygenated sesquiterpenes compounds also increase. This is because the molecular weight of oxygenated sesquiterpenes is also quite high, in the range of 220 - 222, literally higher than oxygenated monoterpenes. This might enhance their stability, making them less susceptible to degradation even after longer period of extraction at 225 W of irradiation power.

Figure 4.18 illustrates the distribution of chemical classes of bark oil crude extract by HD. From the figure, it can be seen that cinnamon bark oil crude extract obtained through HD contains more sesquiterpene hydrocarbon compared to oxygenated monoterpenes and oxygenated sesquiterpene compounds. However for MAHD, fraction of the oxygenated compounds is higher than hydrocarbons. This further depicts the advantages of MAHD whereby the extraction method requires shorter extraction period and smaller amount of solvent to extract. Since this method requires shorter extraction period, there is high possibility to protect the volatile compounds from evaporation.

Besides that, sufficient amount of solvent (water) in the cinnamon matrix during extraction process ensures that the plant matrix is not thermally degraded and that the volatile compounds are not lost (Asbahani et al., 2014). Besides that, the solvent (water) used in this studies has high dielectric properties which means that water has high capability to absorb more microwave energy, convert the energy into heat energy and dissipate the energy to the cinnamon matrix. Therefore, it would be reasonable to accept that more polar compound (water) easily absorb the microwave irradiation and form a better interaction between the electromagnetic waves and cinnamon matrix. Hence more oxygenated aromatic components may be obtained through MAHD method. This is in agreement with observations from similar studies on chemical compositions of different plant materials obtained through MAHD and HD extraction methods. From these researches, it was reported that the more oxygenated compounds were obtained through MAHD method (Djouahri et al., 2013; Golmakani and Rezaei, 2008; Liu et al., 2012 and Ranitha et al., 2014).

Table 4.8 tabulates the summary of mean composition of chemical components in crude extract (%) according to chemical classes and oxygenated fraction of extracted cinnamon bark oil through MAHD and HD. Triplicate experiments were done to validate the optimum conditions to compare and evaluate the chemical components and quality of cinnamon bark oil crude extract obtained from MAHD and HD extraction methods. It is noteworthy however, that large quantity of essential oil from a particular extraction method does not necessarily guarantee good quality of the oil. Based on Table 4.8, the percentage of chemical compound in crude extract (%) at optimum conditions was primarily compared with the marker component which is trans-cinnamaldehyde. It can be observed that at the optimum conditions, the mean value of trans-cinnammaldehyde present in MAHD and HD were 84.43±0.70 and 79.55±0.45 respectively. From previous findings, it was stated that the maximum content of trans-cinnammaldehyde which was extracted from C*innamomum cassia* through conventional method, HD was only around 33.95–76.40 % (Geng et al., 2011). It was however, stated that the content of cinnamon bark oil may be influenced by the age and segment of the tree (Geng et al., 2011). Results from this studies demonstrated that high content of trans-cinnammaldehyde was present in both methods and the highest cinnamon bark oil was obtained through MAHD which is about 84.43 %. Thus the compounds obtained through MAHD are of immense benefit to the pharmaceutical and other related industries that utilise materials with high aromatherapy properties and high toxicity to insect and pest (Petrakis et al., 2014). In our opinion, the higher oxygen compounds obtained from MAHD can be associated with minimal degree of damage on the cinnamon powder and its composition during extraction (as illustrated by the SEM image in Figure 4.13b), owing to the efficient heat distribution. It can also be associated with the shorter extraction time which prevented the constituents from possible partial degradation which is common with HD (Gavahian et al., 2015). This indicates that essential oil obtained from cinnamon bark through MAHD has high potential to be used as alternative medicine.

Table 4.7
Relative content (%) of chemical components present in crude extract of cinnamon bark oil extracted through MAHD and HD for various extraction time

| **No.** | **Components** | **Molecular Formula** | **Relative content (%) for each extraction time (min)** | | | | | | | | | | **References** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **MAHD** | | | | **HD** | | | | | | |
| | | | **30** | **60** | **90** | **120** | **30** | **60** | **90** | **120** | **150** | **180** | |
| 1 | Eucalyptol | $C_{10}H_{18}O$ | 0.26 | 0.17 | 0.14 | 0.14 | 0.46 | 0.48 | 0.37 | 0.52 | 0.50 | 0.54 | Singh et al., 2007 |
| 2 | Guaiacol | $C_7H_8O_2$ | - | - | - | - | - | 0.22 | - | 0.22 | 0.32 | 0.34 | Ravindran et al., 2003 |
| 3 | Linalool | $C_{10}H_{18}O$ | 1.27 | 0.93 | 0.81 | 0.86 | 1.07 | 0.72 | 0.76 | 0.73 | 0.70 | 0.72 | Li et al., 2013b |
| 4 | Benzenepropanal | $C_9H_{10}O$ | 0.56 | 0.69 | 0.59 | 0.86 | 0.48 | 0.54 | 0.62 | 0.55 | 0.64 | 0.65 | Li et al., 2013b; Geng et al., 2011 and Deng et al., 2014 |
| 5 | Borneol | $C_{10}H_{18}O$ | 0.65 | 0.50 | 0.43 | 0.46 | 0.51 | 0.71 | 0.41 | 0.73 | 0.43 | 0.74 | Li et al., 2013b; Geng et al., 2011 and Deng et al., 2014 |
| 6 | cis-α-Terpineol | $C_{10}H_{18}O$ | 0.45 | 0.34 | 0.29 | 0.30 | 0.39 | - | - | - | 0.30 | - | Li et al., 2013b |
| 7 | α-Terpineol | $C_{10}H_{18}O$ | 0.91 | 0.71 | 0.62 | 0.63 | 1.83 | 0.56 | - | 1.48 | 1.48 | 0.54 | Li et al., 2013b and Deng et al., 2014 |
| 8 | cis-Cinnamaldehyde | $C_9H_8O$ | 0.98 | 1.01 | 0.97 | 1.05 | - | 0.95 | 1.74 | - | - | 1.06 | Li et al., 2013b and Deng et al., 2014 |
| 9 | Benzaldehyde | $C_{10}H_{12}O$ | 1.51 | 1.17 | 1.05 | 1.00 | 1.17 | 0.88 | 0.88 | 0.90 | 0.74 | 0.75 | Li et al., 2013b and Deng et al., 2014 |
| 10 | trans-Cinnamaldehyde | $C_9H_8O$ | 84.18 | 85.47 | 84.12 | 83.96 | 79.94 | 79.00 | 79.94 | 79.05 | 79.91 | 79.47 | Li et al., 2013b; Geng et al., 2011 and Deng et al., 2014 |
| 11 | Bornyl acetate | $C_{12}H_{20}O_2$ | - | - | - | - | - | - | 1.59 | - | - | - | Ravindran et.al., 2003 |
| 12 | Anethole | $C_{10}H_{12}O$ | 2.86 | 2.22 | 1.97 | 1.80 | - | 1.85 | - | 1.60 | - | - | Deng et al., 2014 |
| 13 | Acetophenone | $C_8H_8O$ | - | - | - | - | - | - | 0.18 | 0.30 | 0.45 | 0.49 | Li et al., 2013b |
| 14 | Cinnamyl alcohol | $C_9H_{10}O$ | - | - | tr | 0.10 | - | - | - | - | - | - | Geng et al., 2011 |
| 15 | Eugenol | $C_{10}H_{12}O_2$ | 0.18 | 0.18 | 0.19 | 0.20 | 0.18 | 0.30 | - | - | - | 0.33 | Li et al., 2013b |

Table 4.7 continued.

| No. | Components | Molecular Formula | Relative content (%) for each extraction time (min) |  |  |  |  |  |  |  |  |  | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  |  |  | MAHD |  |  |  | HD |  |  |  |  |  |  |
|  |  |  | 30 | 60 | 90 | 120 | 30 | 60 | 90 | 120 | 150 | 180 |  |
| 16 | α –Cubebene | $C_{15}H_{24}$ | 0.72 | - | tr | tr | 0.25 | 0.23 | 2.60 | 0.53 | 2.15 | 0.29 | Deng et al., 2014 |
| 17 | Geranyl acetate | $C_{12}H_{20}O_2$ | 0.37 | 0.31 | 0.31 | 0.29 | - | - | - | 0.29 | 0.30 | - | Li et al., 2013b |
| 18 | Copaene | $C_{15}H_{24}$ | - | 0.79 | 0.89 | 0.87 | 3.29 | 2.60 | 0.40 | 2.28 | - | 2.60 | Singh et.al., 2007 |
| 19 | Coumarin | $C_9H_8O_2$ | 0.71 | 0.90 | 1.12 | 1.15 | 0.55 | 1.24 | 0.83 | 1.31 | 1.41 | 1.38 | Geng et al., 2011 |
| 20 | Caryophyllene | $C_{15}H_{24}$ | - | - | 1.01 | 0.98 | 4.27 | 3.36 | 3.69 | 3.39 | 3.36 | 3.26 | Li et al., 2013b |
| 21 | Cinnamyl acetate | $C_9H_{10}O$ | 3.04 | 2.99 | 2.14 | 1.85 | - | - | - | - | - | - | Geng et al., 2011 |
| 22 | trans- α –Bergamotene | $C_{15}H_{24}$ | - | - | - | - | 0.23 | 0.10 | 0.18 | tr | 0.22 | 0.24 | Deng et al., 2014 |
| 23 | α-Humulene | $C_{15}H_{24}$ | - | 0.16 | 0.18 | 0.17 | 0.53 | 0.47 | 0.45 | 0.40 | 0.50 | 0.61 | Jiang and Song, 2010 |
| 24 | α-Muurolene | $C_{15}H_{24}$ | - | - | - | tr | 0.74 | 0.70 | 0.64 | 0.76 | 0.68 | 0.70 | Li et al., 2013b |
| 25 | 2-Methoxy cinnamaldehyde | $C_{10}H_{10}O_2$ | - | - | - | - | - | - | - | - | 0.26 | 0.26 | Geng et al., 2011 |
| 26 | α –Amorphene | $C_{15}H_{24}$ | 0.22 | 0.27 | 0.34 | 0.32 | 0.76 | 0.75 | 1.70 | 0.79 | 0.53 | 0.55 | Singh et al., 2007 |
| 27 | γ –Muurolene | $C_{15}H_{24}$ | - | - | tr | - | - | - | - | - | tr | - | Deng et al., 2014 |
| 28 | 1s-cis-Calamenene | $C_{15}H_{22}$ | - | - | 0.24 | 0.23 | - | 0.49 | - | 0.47 | 0.39 | - | Geng et al., 2011 |
| 29 | (+)-δ-Cadinene | C15H24 | 0.24 | 0.28 | 0.35 | 0.35 | 1.24 | 0.74 | 0.22 | 0.70 | 0.71 | 1.22 | Golmohammad et al., 2012 |
| 30 | Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- | $C_{15}H_{24}$ | - | - | - | 0.41 | 0.43 | 0.23 | - | 0.22 | 0.23 | 0.23 | Li et al., 2013b |
| 31 | Caryophyllene oxide | $C_{15}H_{24}O$ | 0.37 | 0.35 | 0.38 | 0.34 | 0.51 | 0.39 | 0.39 | 0.36 | 0.45 | 0.35 | Deng et al., 2014 |

Table 4.7 continued.

| No. | Components | Molecular Formula | Relative content (%) for each extraction time (min) | | | | | | | | | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | MAHD | | | | HD | | | | | | |
| | | | **30** | **60** | **90** | **120** | **30** | **60** | **90** | **120** | **150** | **180** | |
| 32 | Tetradecanal | $C_{24}H_{28}O$ | - | - | - | - | - | 0.18 | 0.17 | 0.16 | 0.23 | 0.22 | Geng et al., 2011 |
| 33 | Calarene | $C_{15}H_{24}$ | - | - | - | - | - | - | - | - | 0.11 | - | Thantsin , 2008 |
| 34 | Patchoulene | $C_{15}H_{24}$ | - | - | - | - | - | 0.30 | - | - | - | 0.14 | Deng et al., 2014 |
| 35 | Cedrene | $C_{15}H_{24}$ | - | - | - | - | - | - | - | 0.28 | 0.31 | - | Li et al., 2013b |
| 36 | β –Cadinene | $C_{15}H_{24}$ | - | - | - | - | - | - | 0.30 | - | - | 0.34 | Jiang and Song, 2010 |
| 37 | τ –Muurolol | $C_{15}H_{26}O$ | - | - | - | 0.27 | - | 0.33 | 0.33 | 0.32 | 0.33 | 0.33 | Geng et al., 2011 |
| 38 | Cadelene | $C_{15}H_{18}$ | - | - | - | - | - | - | - | - | tr | tr | Tung et al., 2008 |
| **Chemical classes** | | | | | | | | | | | | | |
| Oxygenated monoterpenes | | | 8.09 | 6.22 | 5.50 | 5.39 | 5.61 | 5.50 | 2.42 | 5.96 | 4.41 | 3.88 | |
| Sesquiterpene hydrocarbons | | | 1.18 | 1.50 | 3.01 | 3.33 | 11.74 | 9.97 | 10.58 | 9.82 | 9.19 | 10.18 | |
| Oxygenated sesquiterpenes | | | 0.37 | 0.35 | 0.38 | 0.61 | 0.51 | 0.72 | 0.72 | 0.68 | 0.78 | 0.68 | |
| Other oxygenated compounds | | | 89.84 | 91.37 | 89.25 | 89.26 | 80.97 | 79.13 | 85.07 | 81.88 | 83.26 | 83.61 | |
| **Total** | | | **99.48** | **99.44** | **98.14** | **98.59** | **98.83** | **95.32** | **98.79** | **98.34** | **97.64** | **98.35** | |
| Oxygenated fraction | | | 98.3 | 97.94 | 95.13 | 95.26 | 87.09 | 85.35 | 88.21 | 88.52 | 88.45 | 88.17 | |

tr: trace compound (<0.10 %) -: not detected
"-": no compounds detected

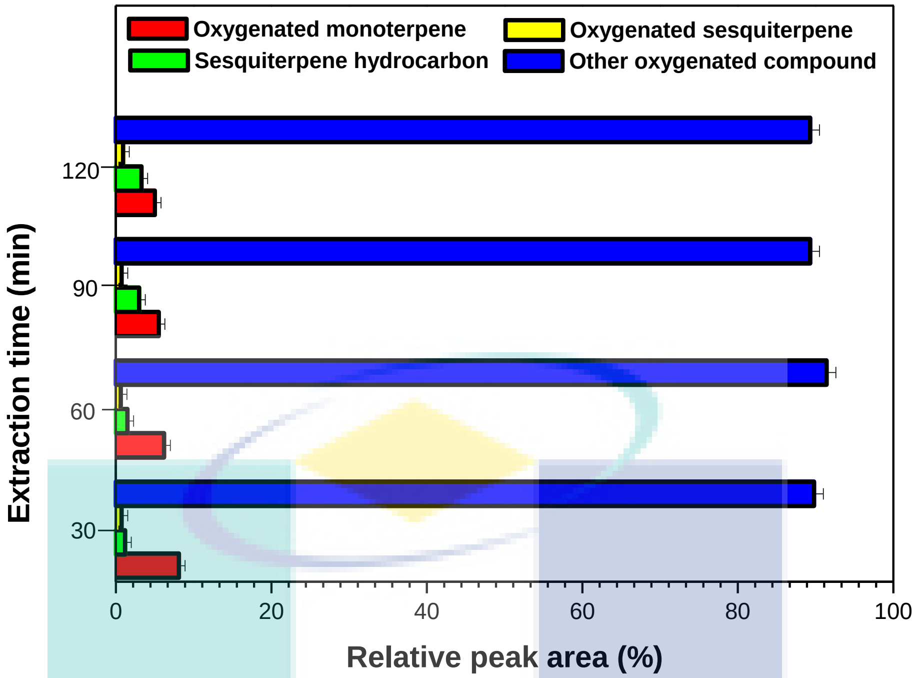


Figure 4.17: Composition of chemical classes at various extraction time obtained through MAHD.

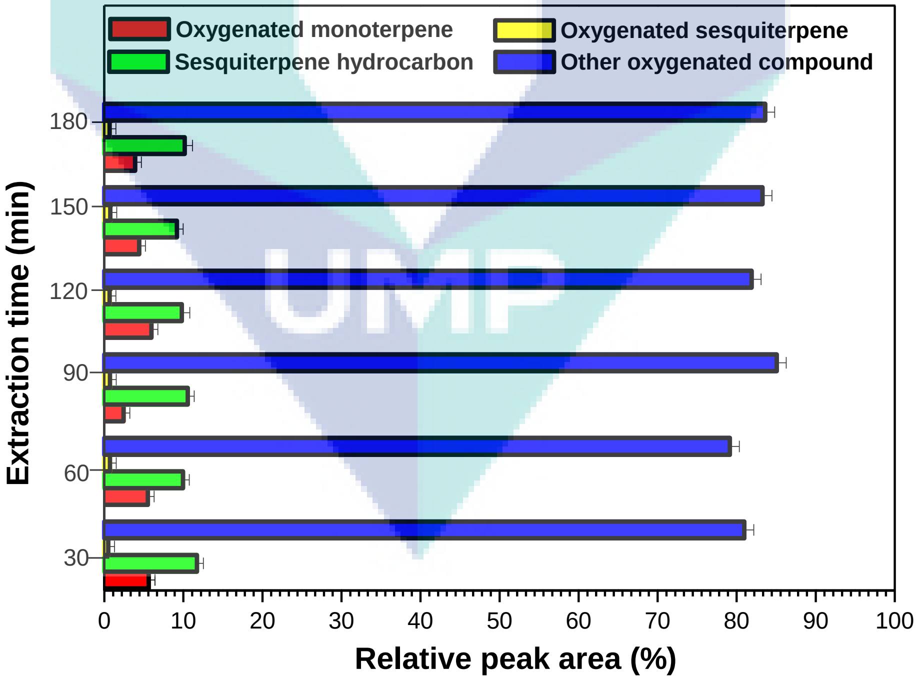


Figure 4.18: Composition of chemical classes at various extraction time obtained through HD.

Table 4.8
Mean chemical compositions (%) of the crude extract of cinnamon bark oil extracted through MAHD and HD at optimum conditions

| No | Components | Molecular Formula | Percentage of chemical components in crude extract[a] (%) | |
|---|---|---|---|---|
| | | | **MAHD** | **HD** |
| | **Oxygenated monoterpenes** | | **6.31** | **4.56** |
| 1 | Eucalyptol | $C_{10}H_{18}O$ | 0.18 ± 0.06 | 0.48 ± 0.06 |
| 2 | Linalool | $C_{10}H_{18}O$ | 0.97 ± 0.21 | 0.78 ± 0.14 |
| 3 | Borneol | $C_{10}H_{18}O$ | 0.51 ± 0.10 | 0.59 ± 0.16 |
| 4 | L-α-Terpineol | $C_{10}H_{18}O$ | 0.35 ± 0.07 | 0.12 ± 0.18 |
| 5 | α-Terpineol | $C_{10}H_{18}O$ | 0.72 ± 0.13 | 0.98 ± 0.71 |
| 6 | Benzaldehyde | $C_{10}H_{12}O$ | 1.18 ± 0.23 | 0.89 ± 0.16 |
| 7 | Anethole | $C_{10}H_{12}O$ | 2.21 ± 0.46 | 0.58 ± 0.89 |
| 8 | Eugenol | $C_{10}H_{12}O_2$ | 0.19 ± 0.01 | 0.14 ± 0.16 |
| | **Sesquiterpene hydrocarbons** | | **2.09** | **10.29** |
| 9 | alpha.-Cubebene | $C_{15}H_{24}$ | tr | 1.01 ± 1.07 |
| 10 | Copaene | $C_{15}H_{24}$ | 0.64 ± 0.43 | 1.86 ± 1.33 |
| 11 | Caryophyllene | $C_{15}H_{24}$ | 0.50 ± 0.57 | 3.56 ± 0.38 |
| 12 | α-Muurolene | $C_{15}H_{24}$ | tr | 0.70 ± 0.04 |
| 13 | trans-alpha-Bergamotene | $C_{15}H_{24}$ | - | 0.17 ± 0.07 |
| 14 | α-Humulene | $C_{15}H_{24}$ | 0.13 ± 0.09 | 0.49 ± 0.07 |
| 15 | alpha-Amorphene | $C_{15}H_{24}$ | 0.29 ± 0.05 | 0.85 ± 0.43 |
| 16 | 1s-cis-Calamenene | $C_{15}H_{22}$ | 0.12 ± 0.14 | 0.23 ± 0.25 |
| 17 | (+)-delta-Cadinene | $C_{15}H_{24}$ | 0.31 ± 0.05 | 0.81 ± 0.38 |
| 18 | Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)- | $C_{15}H_{24}$ | 0.10 ± 0.21 | 0.22 ± 0.14 |
| 19 | Calarene | $C_{15}H_{24}$ | - | 0.18 ± 0.04 |
| 20 | Cedrene | $C_{15}H_{24}$ | - | 0.10 ± 0.15 |
| 21 | beta-Cadinene | $C_{15}H_{24}$ | - | 0.11 ± 0.17 |
| | **Oxygenated sesquiterpenes** | | **0.43** | **0.68** |
| 22 | Caryophyllene oxide | $C_{15}H_{24}O$ | 0.36 ± 0.02 | 0.41 ± 0.06 |
| 23 | τ- Muurolol | $C_{15}H_{26}O$ | 0.07 ± 0.14 | 0.27 ± 0.13 |
| | **Other oxygenated monoterpenes** | | **6.31** | **4.56** |
| 24 | Guaiacol | $C_7H_8O_2$ | - | 0.18 ± 0.15 |
| 25 | Benzenepropanal | $C_9H_{10}O$ | 0.68 ± 0.14 | 0.58 ± 0.07 |
| 26 | cis-Cinnamaldehyde | $C_9H_8O$ | 1.00 ± 0.03 | 0.63 ± 0.74 |
| 27 | trans-Cinnamaldehyde | $C_9H_8O$ | 84.43 ± 0.70 | 79.55 ± 0.45 |
| 28 | Bornyl acetate | $C_{12}H_{20}O_2$ | - | 0.27 ± 0.65 |

Table 4.8 continued.

| No | Components | Molecular Formula | Percentage of chemical components in crude extract$^a$ (%) | |
|---|---|---|---|---|
| | | | MAHD | HD |
| 29 | Acetophenone | $C_8H_8O$ | - | 0.24 ± 0.21 |
| 30 | Geranyl acetate | $C_{12}H_{20}O_2$ | 0.32 ± 0.03 | 0.10 ± 0.15 |
| 31 | Coumarin / cinnamic acid | $C_9H_8O_2$ | 0.97 ± 0.21 | 1.12 ± 0.35 |
| 32 | Cinnamyl acetate | $C_9H_{10}O$ | 2.51 ± 0.60 | - |
| 33 | Tetradecanal | $C_{24}H_{28}O$ | - | 0.16 ± 0.08 |
| | **Total** | | **98.74** | **98.36** |
| | Yield (%) | | 2.75 | 2.02 |
| | Oxygenated fraction | | 96.65 | 88.07 |

tr: trace compound (<0.10 %). -: not detected
$^a$ Mean ± Standard Deviation
'-': no compounds detected

On the other hand, numerous reports have been reported on the variation values of the chemical component in cinnamon bark oil. A total of 38 chemical compounds were acquired in the cinnamon bark oil crude extract that was extracted in this research, and 36 of them has concentration of more than 0.1 %. Comparatively, more chemical components were obtained by (Thantsin et al., 2008) (74 compounds), whereas only 10 compounds were identified by (Shan et al., 2007). By comparing the chemical components of the cinnamon oil crude extract from Malaysia and different studies from other regions of the world, the reports demonstrated that there were significant quantitative similarities between the chemical profiles of the major chemical compound of cinnamon bark oil. Some of the researchers confirmed trans-cinnamaldehye as the marker component in the cinnamon bark oil crude extract (Geng et al., 2011; Golmohammad et al., 2012; Li et al., 2013b; Shan et al., 2007; Singh et al., 2007 and Thantsin et al., 2008). They were reported that the relative percentage obtained in all the crude extracts were more than 50 %. Thus, the findings are in agreement with this current study which also demonstrates that trans-cinnamaldehyde is the marker compound of extracted cinnamon oil crude extract through MAHD and HD methods. However, the chemical composition of some other major components in cinnamon crude extract may vary based on the age, organ development and also segment of the tree (Cheng et al., 2009; Geng et al., 2011 and Wannes, et al., 2009). This statement was supported by the research work of (Geng et al., 2011), who stated that the *Cinnamomum cassia* shows huge variance at different growing stages in the yield and chemical composition of cinnamon bark oil. The findings also reported that the contents of cinnamon oil yield in the stem bark differed in the range of 0.41 – 2.61 % based on the age and segment of the tree. The highest oil was obtained from the stem bark age of 12 years meanwhile the highest trans-cinnamaldehyde component was acquired from the six years growth stem bark. On the other hand, there might be other factors responsible for the relative content of the main components of the essential oil, for instance seasonal changes might influence the yield and relative composition of an essential oil (Campeol et al., 2003; Gazim et al., 2010 and Hussain et al., 2008).

## 4.7 Heat Generation System and Dielectric Properties of MAHD Extraction

### 4.7.1 Introduction

The advantages of microwave assisted hydrodistillation (MAHD) extraction technique have been explored in many fields, especially in researches involving extraction of medicinal plants and herbs. This is often exploited either to obtain similar or most often better quality of essential oil yield compared to those obtained through conventional methods, at less energy consumption as well as rapid extraction time. In order to maximize these advantages of microwave extraction, study on the influence of dielectric properties of microwave extraction is important. The capability of a specific material or solvent to absorb microwave energy and dissipate heat energy depends on the dielectric constant (ε') and dielectric loss (ε'') of the material. Dielectric constant is the ability of a plant sample matrix to absorb irradiation energy whereas dielectric loss is conversion of irradiation energy into thermal energy (Mandal et al., 2007). Dielectric properties possessed by solvents are essential in microwave heating performance. Solvents which exhibit high dielectric properties are capable of absorbing more microwave energy and convert it into thermal energy which invariably could enhance the heating up of the plant matrix. On the other hand, solvents with low dielectric properties may not be able to support the effective heating of the plant matrix (Kormin et al., 2013). Based on this, in MAHD process, the solvent which has high dielectric properties would absorb more microwave energy, leading to the production of heat inside the plant material which is thereafter transferred outward. This process is known as volumetric heating. On the contrary, conventional methods heat surfaces first before the inner part of the plant material gets heated.

Generally, MAHD technique is accompanied by a very unique heating mechanism compared to other extraction techniques. MAHD uses the combination of both electric and magnetic fields to generate heat. This generated heat travels through molecules by ionic conduction and dipole rotation (Eskilsson and Bjorklund, 2000). Therefore, the plant material releases the essential oil effectively and rapidly through good heat distribution of microwave heating (Desai et al., 2010). The major focus of microwave extraction research is to improve the techniques of extraction towards more feasible, efficient and high productivity. Therefore, temperature analysis study is required to be carried out in order to understand the principle of heating using microwave. In a particular research, studies on the microwave heating mechanism was carried out on dielectric properties of cinnamon matrix (water + cinnamon powder) by examining its responses to the applied electromagnetic waves (Navarrete et al., 2011). In this present study, the efficiency of microwave was investigated on the parameters of volume rate of heat generation and penetration depth of cinnamon bark matrix. Besides that, the efficiency of water as a solvent in the extraction of cinnamon bark oil through MAHD was investigated by analysing the dielectric properties. The irradiation time was recorded from zero minute until the test sample reached its boiling point that is the induction time. The induction time is very important for plotting and analysing the temperature profile of cinnamon matrix. Based on the generation of temperature profile, the volumetric rate of heat generation can be calculated accordingly.

### 4.7.2 Temperature Analysis

Figure 4.19 illustrates the temperature profile of a mixture of water and cinnamon powder at the ratio of 8:1 and microwave power level of 225 W in relation to the increasing exposure time. From this graph, an exponential trend can be seen from 0 sec to 540 sec. The sharp exponential trend obtained herein can be attributed to properties of the solvent (water) which was used in the extraction process. Water is a polar solvent with great capability to absorb large microwave energy. Reason for this is that water has high dielectric constant (Kormin et al., 2013; and Shaharuddin et al., 2014) which means water has the high capability to store electrical energy in an electric field (microwave cavity). This also indicates water has high dipolar moment meaning the polarity of water bond (H-O-H) is strong (Mirza et al., 2003). As a consequence of that, water absorb more microwave energy and dissipate the microwave energy into heat energy. This heat energy may increase the temperature of the cinnamon matrix inside the microwave cavity. In addition, the temperature of the cinnamon mixture further increase with respect to the increasing irradiation time in particular when the irradiation time increases from 540 sec to 840 sec. However, upon increasing the irradiation time of the microwave beyond 840 sec, the temperature seemed to reach steady state. This is because at this time, the cinnamon mixture had reached its boiling point (100 $^0$C).

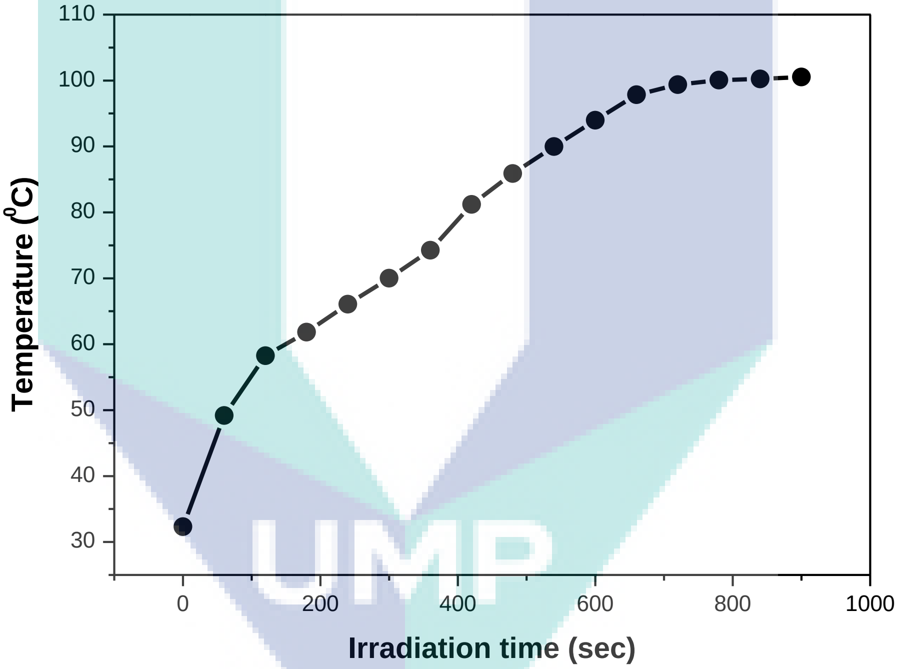


Figure 4.19: Temperature profile of cinnamon matrix extracted through MAHD at water: cinnamon powder ratio of 8:1 and microwave power of 225 W.

The average rate of increasing temperature was calculated at three segments from the graph including at the beginning of the irradiation time from 0 sec to 540 sec, followed by 300 sec and lastly 120 sec. The values gotten are 0.9735 $^0$C/s, 0.1784 $^0$C/s and 0.0074 $^0$C/s respectively. Figure 4.20 illustrates the temperature increase with respect to increasing irradiation time at a fixed microwave power of 225 W and water to cinnamon powder ratio of 8:1. The rate of temperature increase was calculated by dividing the temperature increase with irradiation period. As can be seen from the graph, the temperature increase becomes gradually reducing as the irradiation power increases; the only exception being the initial increase after just about 60 sec. This initial increase can be attributed to the quick heating system of the microwave due to the high polarity of water which might have caused it absorb microwave energy at a faster rate. However, the rate of temperature increase start to decline after 60 sec, due to the decreasing of dielectric properties of water (Fang and Lai, 1995 and Shaharuddin et al., 2014) which will be discussed extensively in the next section under volumetric rate of heat generation. Besides that, there might be other possible factors which may influence the decrease in the rates of temperature increase including physical properties of water, such as the boiling point, density and heat capacity (Kormin et al., 2013). For instance, when the boiling point of the cinnamon matrix increases, the mass of the cinnamon matrix began to decrease as some portion of the water start to evaporate from the cinnamon matrix during extraction process. Hence, the density and heat capacity of the cinnamon matrix also may decrease as the mass decreases (Navarrete et al., 2011). The level off values of these parameters might negatively influence the rate of temperature increase in relation with extended irradiation time.

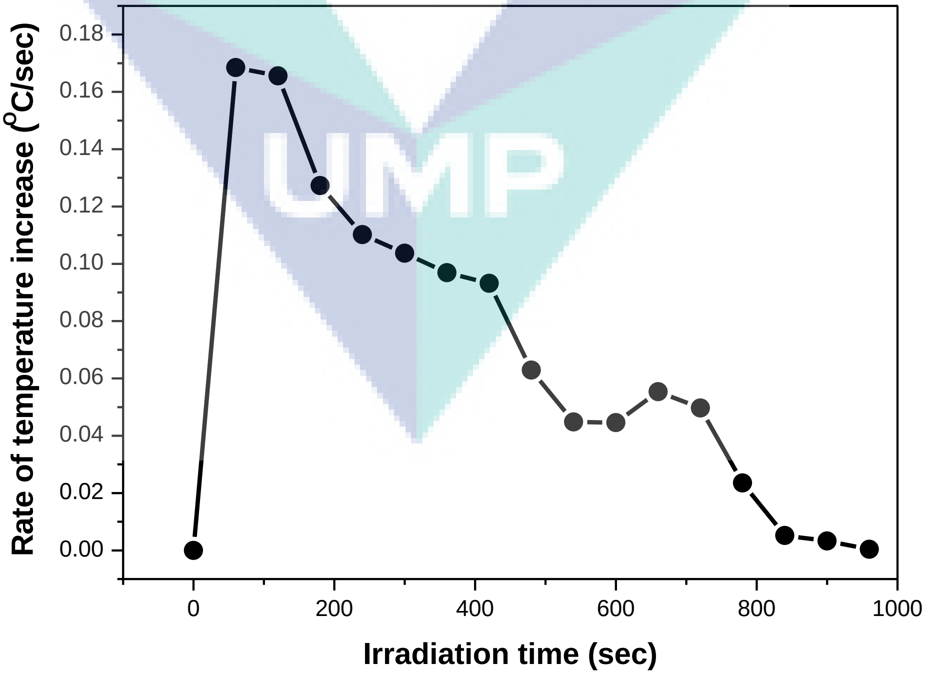


Figure 4.20: Rate of temperature increase profile of cinnamon matrix extracted through MAHD at water: cinnamon powder ratio of 8:1 and microwave power level of 225 W.

In another vein, various microwave power levels could also influence the ability of the water to absorb microwave energy. The ability of the materials to absorb microwave energy is closely associated to the pulse cycle. For instance, at higher microwave power level (250 W), the system reveals longer pulse time which resulted into larger rate of temperature increase (0.2807 $^0$C/sec) at initial irradiation time of 60 sec as depicted in APPENDIX B. However, at lower microwave power level (200 W), the pulse time is short and low rate of temperature increase (0.1050 $^0$C/sec) compared to 250 W power level was recorded at the same initial irradiation time of 60 sec. Hence it takes longer time to raise the temperature for the mixture to reach its boiling point due to low percentage of microwave absorbance by water at 200 W, as reported elsewhere (Kormin et al., 2013). Similar observation was also reported by Zhu et al. (2003), that longer pulse time at higher microwave power level strongly influenced the rise in rate of temperature and microwave power absorbance (Zhu et al., 2003). Likewise, this current research conforms to the observation reported previously. The reports gathered shows that at higher microwave power level longer pulse time and higher rate of temperature suitable to absorb more microwave energy was obtained (Kormin et al., 2013; Shaharuddin et al., 2014 and Zhu et al., 2003). However, in this study 225 W has been chosen to be the optimum microwave power level. This was selected after a careful consideration of the energy consumption, effect of microwave irradiation to human health, quality of essential oil extracted and yield of essential oil obtained. Furthermore, the critical importance of the optimization results obtained from section 4.3 explains clearly the reasons for selecting 225 W as the optimum microwave power level. The other irradiation power data for 200 W and 250 W of rate of temperature increase profile for MAHD can be obtained from APPENDIX B.

### 4.7.3 Volumetric Rate of Heat Generation

Figure 4.21 illustrates the volume rate of heat generation of cinnamon matrix as a function of irradiation time at 225 W of microwave power level and 8:1 water-to-cinnamon powder ratio. This graph shows that the volume rate of heat generation is inversely proportional to exposure time after 60 sec. At the initial 60 sec of irradiation period, the volume rate of heat generation reached highest point (0.0200 kcal/ cm$^3$.s) rapidly. However, as the irradiation time was prolonged, the volume rate of heat generation began to decline and attained plateau at 0.0006 kcal/ $cm^3$.s. Similar observation has been reported by Kormin et al. (2013) and Shaharuddin et al. (2014), that at higher microwave levels, the volume rate of heat generation decrease in relation with increasing microwave radiation time after 60 sec. Reason for that could be because water contain non-symmetric molecules that could induce temporary dipole movement inside the electromagnetic field and increase the volume rate of heat generation rapidly. However, the friction between a dipole molecule and surrounding molecules produce heat with the aid of external electric field. Therefore, when the temperature increase as a result of prolonged irradiation time, the dipole situated within the electric field rotates to align itself in the changing microwave field and thus the dielectric properties of water decline. As a result, the capability of water to absorb microwave energy also reduces. According to Rahmat (2002), the dielectric property of a polar solvent is fully dependent on the movement of dipole molecules within the structure (Rahmat, 2002). On the other hand, heating temperature determines the ability of a solvent to absorb sufficient microwave energy and dissipate the microwave energy into heat energy which may influence the dielectric properties of the solvent (Chan and Chen, 2002; Eskilsson and Bjorklund, 2000 and Saoud, 2004). The calculations of volume rate of heat generation for 225 W of microwave power level and 8:1 of water-to-raw material ratio are displayed in APPENDIX B.

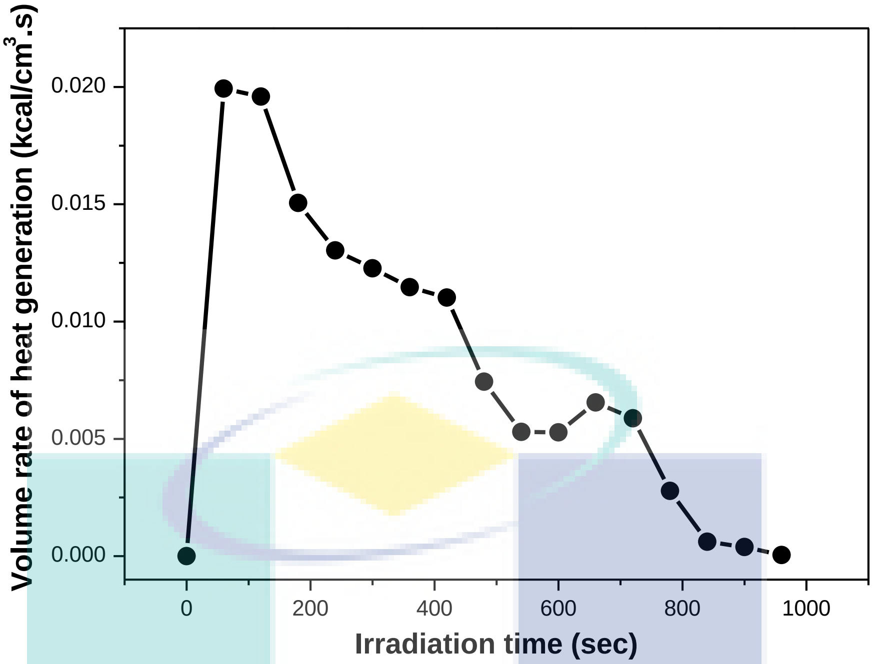


Figure 4.21: Volume rate of heat generation of cinnamon matrix extracted through MAHD at water: cinnamon powder ratio of 8:1 and microwave power level of 225 W for various microwave irradiation time.

### 4.7.4 Dielectric Properties

Water has high dipolar moment; it can therefore absorb microwave energy at a fast rate and easily convert it into thermal energy. This may help to heat the test sample more efficiently. The ability of a solvent to absorb the microwave energy and turn it into heat energy at a given temperature and frequency is known as loss tangent (tan δ). Loss tangent is expressed by dividing dielectric loss with dielectric constant (ε''/ ε'). Dielectric constant (ε') describes the capability of material molecules to be polarized by the applied electric field (Herrero et al., 2010). On the other hand, dielectric loss or also known as loss factor (ε'') is the magnitude value of the absorbed microwave irradiation energy converted into thermal energy (Desai et al., 2010). Figure 4.22 and 4.23 depicts the dielectric constant and dielectric loss of cinnamon matrix as a function of increasing temperature at 225 W of microwave power and 8:1 of water-to-raw material ratio respectively. Similar trend can be observed from both graphs as dielectric properties including dielectric constant and dielectric loss decrease with respect to temperature rise. Similar observation has been reported by (Navarrete et al., 2011), in which case as the temperature increase the dielectric properties of water and rosemary essential oil declined. The dielectric properties of cinnamon matrix are strongly influenced by plant moisture content and density. According to a particular research, Navarrete eand co-workers (2011), it was reported that the radiated microwave energy is strongly absorbed at points with higher moisture content due to the high percentage of water present which has high dielectric properties (Navarrete et al., 2011). As for current studies, the moisture content of the cinnamon bark is approximately 9.2 %. Therefore, the dielectric properties which are dielectric constant and dielectric loss were high at the initial stage of extraction. However, as the temperature rose, the water contained inside the plant material start to evaporate and led to water loss. Hence, the mass of the cinnamon matrix solution decrease, reducing the moisture content inside the plant material. This therefore made the density also decreases and invariably, the dielectric properties of cinnamon matrix decrease as the temperature increase. Calculations of dielectric constant and dielectric loss of cinnamon matrix for 225 W of microwave power level and 8:1 of water-to-raw material ratio are displayed in APPENDIX B.

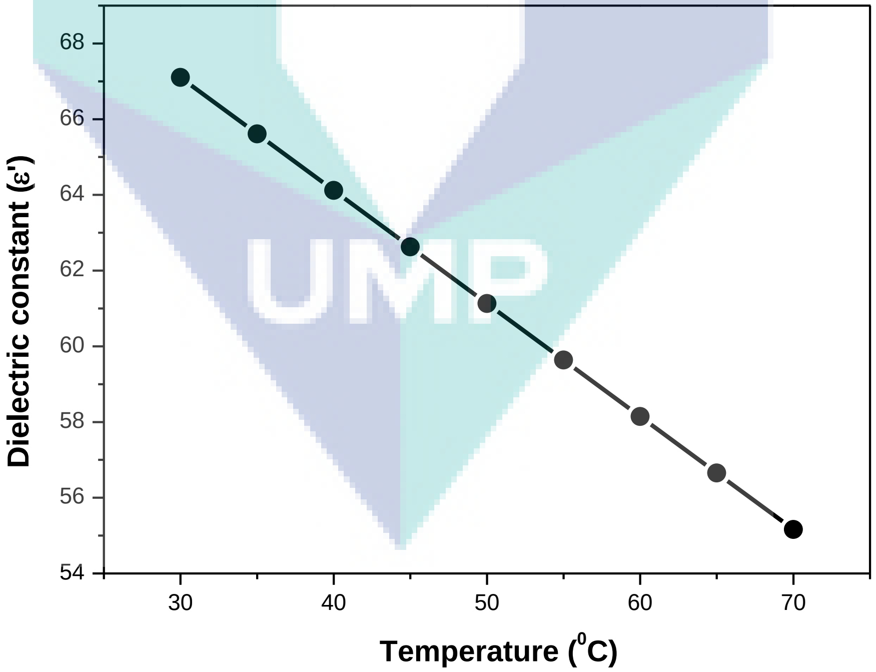


Figure 4.22: Dielectric constant (ε') at various temperature of cinnamon bark matrix extracted through MAHD at 225 W of irradiation power and 8:1 ratio of water to cinnamon bark powder.

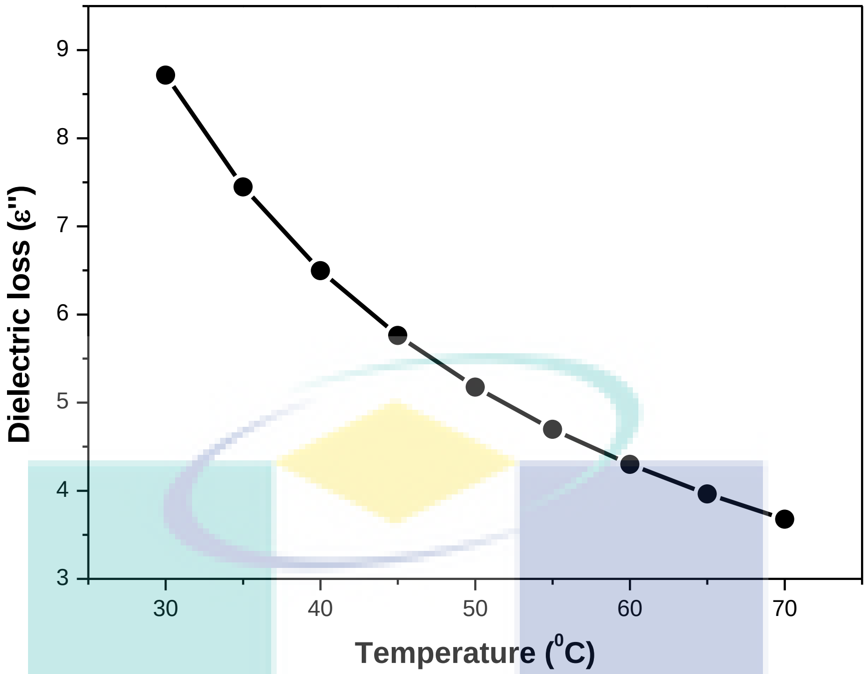


Figure 4.23: Dielectric loss (ε'') at different temperature of cinnamon bark matrix extracted through MAHD at 225 W of irradiation power and 8:1 ratio of water to cinnamon bark powder.

### 4.7.5 Penetration Depth

Depth of penetration, ($D_P$) is the depth that has reduced about 37 % of the initial value of power flux at its surface. When microwave radiation energy passes through or penetrates a material such as cinnamon matrix solution, the energy absorbed by the cinnamon matrix at a rate is associated with the loss tangent. Penetration is unlimited to the materials which are transparent to radiation energy whereas there is no possibility of penetration occurrence for materials which are opaque or reflective like metals. Generally, the penetration factor of irradiation energy is inversely proportional to the loss tangent of a material at a given frequency. Thus, penetration depth of irradiation energy is dependent on the dielectric properties of plant matrix and the frequency. Figure 4.24 shows the effects increasing temperature on penetration depth. This experiment was conducted at the optimum parameters of microwave extraction which are 225 W of microwave power and 8:1 ratio of water-to-cinnamon powder. The depth of penetration values was calculated to support the results on dielectric properties. This is because results of penetration depth gives the most convenient and efficient way to explain the dielectric constant and dielectric loss of a plant material and properties of the microwave heating. In fact, the penetration depth data has also been used to compare the ability of the plant sample to absorb radiation energy. As can be seen from the graph, a linear relationship between penetration depth and irradiation time was obtained. It was observed that the results obtained for penetration depth is contrary to what was obtained from the previous section for dielectric properties. This is in agreement with the report stating that penetration depth is inversely proportional to the dielectric loss (Kormin et al., 2013). A large value of penetration depth indicates that the plant material absorb very low percentage of microwave energy whereas short penetration depth indicates that surface heating is predominant (Saoud, 2004). Calculation for the penetration depth at the optimum parameters of microwave are include in the APPENDIX B.

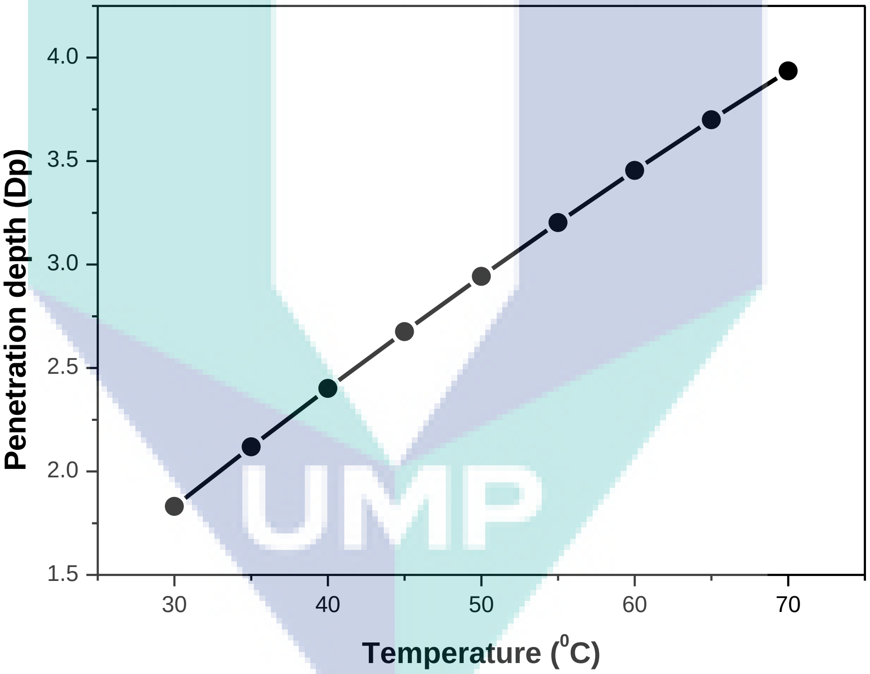


Figure 4.24: Penetration depth, Dp, at various temperature of cinnamon bark matrix extracted through MAHD at 225 W of irradiation power and 8:1 ratio of water to cinnamon bark powder.

## 4.8 Cytotoxicity Bioassay

Cytotoxicity test is used to measure the degree to which a substance could be toxic on certain cells. The brine shrimp *A. salina* lethality bioassay was employed here to measure and evaluate toxicity of essential oil in order to predict its suitability for pharmaceutical applications. Generally, this brine shrimp cytotoxicity bioassay test may be used as preliminary test to investigate anticancer, antitumor and pesticide activities. According to Elhardallou (2011), brine shrimp *A. salina* species correspond similarly to functionalities of mammalian cell (Elhardallou, 2011). Therefore, this cytotoxicity bioassay test contributes a significant impact on this research.

### 4.8.1 Cytotoxicity of Cinnamon Bark Oil

In this research, the cytotoxicity properties exhibited by *Cinnamomum cassia* (cinnamon) oil extracted through MAHD and HD were evaluated against brine shrimp nauplii. Table 9 comprises the cytotoxicity effects of various concentrations of crude extracts of cinnamon bark oil on *A. salina*. The finding shows that the degree of lethality is directly proportional to the concentration of the cinnamon oil crude extract. In other words, the rate of mortality increases with the increasing sample concentration. The percentage death of brine shrimp *A. salina* at different concentrations after 24 h was calculated according to probit by using Finney's method (Finney, 1952).

Table 4.9
$LC_{50}$ values of cinnamon bark oil crude extracts against *A. salina*

| **Crude Extract** | **Percentage of death at different concentration after 24 hours/ Dose** | | | | | **$LC_{50}$ (ppm)** | ***Toxicity Level** |
|---|---|---|---|---|---|---|---|
| | **1000 ppm** | **500 ppm** | **100 ppm** | **50 ppm** | **10 ppm** | | |
| MAHD | 100.00 | 96.67 | 76.67 | 43.33 | 13.33 | 51.21 | Very highly toxic |
| HD | 100.00 | 80.00 | 63.33 | 46.67 | 20.00 | 68.88 | Very highly toxic |
| DMSO (Negative Control) | | | 3.33 | | | *NA | *NA |
| $K_2Cr_2O_7$ (Positive Control) | NA | 80.00 | 60.00 | 20.00 | *NA | 130.17 | Highly toxic |

* refer to classification of the toxicity of essential oil of spices from (Bajracharya and Tuladhar, 2012)
* NA represents Not Applicable

The probit graph was plotted against log of concentration, ppm to obtain the intercept and slope from the linear regression in order to find out the 50 % lethal concentration ($LC_{50}$) value of brine shrimp mortality (Throne et al., 1995). Usually, the crude extracts that records lower value of $LC_{50}$ are classified as highly toxic. From Table 4.9, it can be seen that the $LC_{50}$ value obtained from MAHD crude extract is 51.21 ppm which value lies between the toxicity region (1–100 ppm: very highly toxic) as illustrated in Figure 4.25.

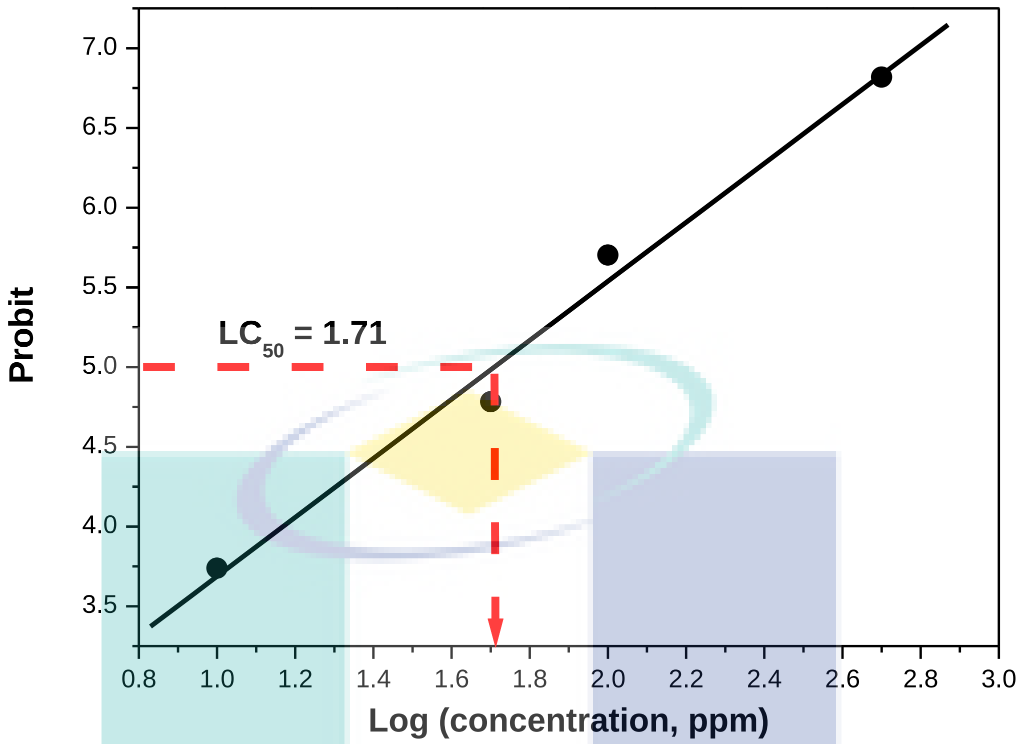


Figure 4.25: Probit graph for MAHD.

The crude extracts of cinnamon oil that extracted through HD recorded $LC_{50}$ value of 68.88 ppm. This result shows that the $LC_{50}$ value of HD crude extract also falls in the toxicity level (1 – 100 ppm: very highly toxic) as depicted in Figure 4.26. Both MAHD and HD crude extracts falls within similar categories which are very highly toxic. However, MAHD crude extract shows lower $LC_{50}$ value than HD which means that MAHD crude extract exhibits higher toxicity.

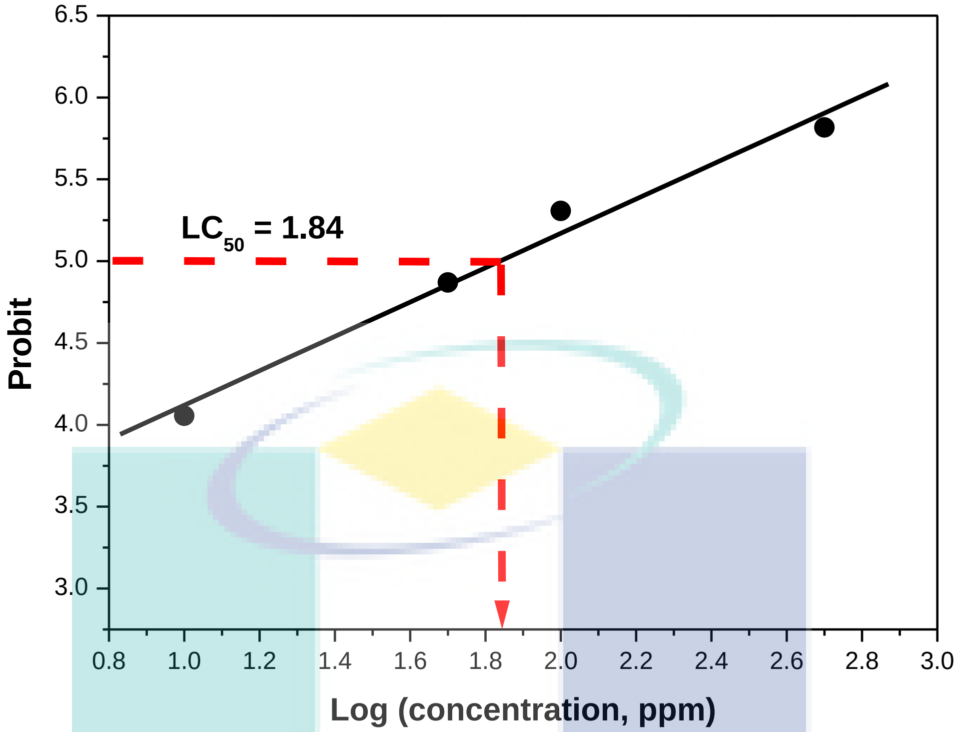


Figure 4.26: Probit graph for HD.

On the other hand, $LC_{50}$ value for positive control (Potassium dichromate, $K_2$-$Cr_2O_7$) was 130.17 ppm which represents the highly toxic level for the brine shrimp lethality of *A. salina* as shown in Figure 4.27. On the other hand most of the brine shrimp *A. salina* was active, in other words only one death was observed in the negative control (Dimethyl sulfoxide, DMSO). This indicates that the cinnamon bark oil crude extract is responsible for the observed deaths.

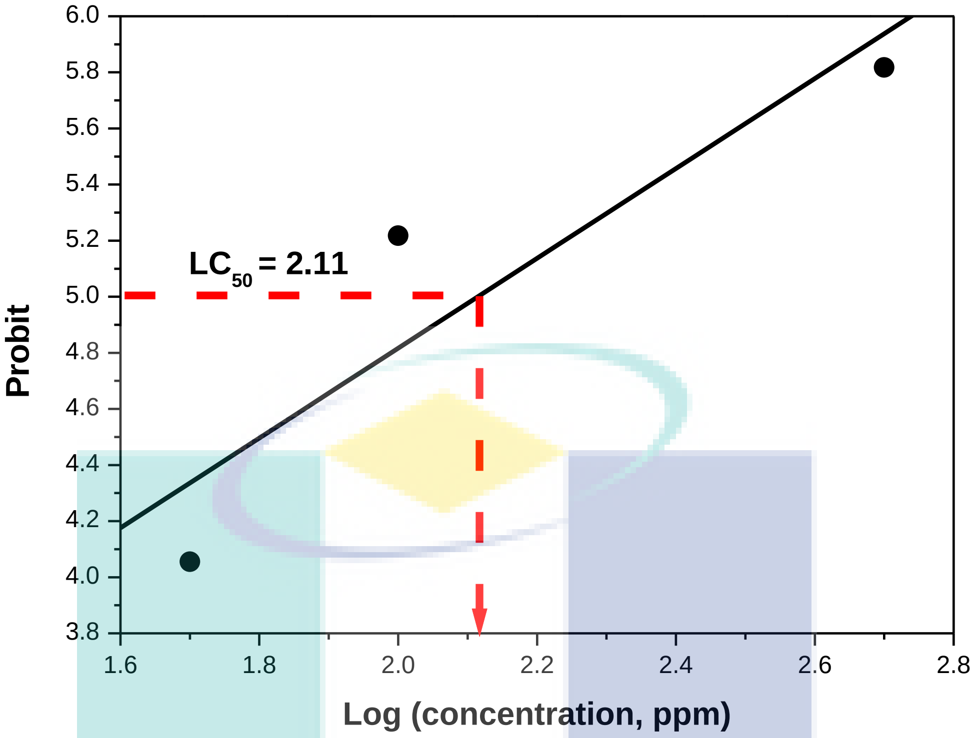


Figure 4.27: Probit graph for $K_2Cr_7O_2$.

From previous researches, it had been reported that, the cinnamon crude extracts extracted through HD showed $LC_{50}$ value of 72.1 ppm (Cheng et al., 2006) and 46.41 ppm (Bajracharya and Tuladhar, 2012) against brine shrimp *A. salina*. Hence, the value obtained for this present research are reasonable. This is because the $LC_{50}$ values obtained in this studies falls within the range of the reported values. However, so far to the best of our knowledge no bioassay studies have been carried out for the cinnamon bark oil crude extract obtained through MAHD. From our experiment, the toxicity trend of cinnamon oil crude extract is $K_2Cr_2O_7$ < HD < MAHD. Therefore, crude extract of bark oil obtained through MAHD is more toxic compared to HD and positive control. In essence, crude extracts obtained through both MAHD and HD techniques show high toxic $LC_{50}$ value which is less than 70 ppm. According to Meyer and co-workers (1982), any plant extract which exhibits $LC_{50}$ value less than 1000 ppm is considered as pharmacologically active and is toxic (Meyer et al., 1982). Interestingly, the cinnamon crude extract obtained through both methods here showed highly desirable toxicity against brine shrimp *A. salina* which highlights their potential as suitable pharmaceutical raw materials. The most prominent toxicity value being revealed by MAHD crude extract and therefore possessing better pharmaceutical (such as anticancer, pesticidal and antitumor) properties. The reason could be associated with the possession of higher amount of key compound which is trans-cinnamaldehye. According to Ooi et al. (2006) and Geng et al. (2011), most of the positive bioactive activities of cinnamon oil are related to cinnamaldehye content. Analysis on chemical constituents of cinnamon bark oil crude extract, confirmed that MAHD produced higher cinnamaldehyde content (85.43 %) than HD (80.18 %). Different researches have confirmed the wide range bioactive properties of cinnamon bark extract obtained through various methods (Bajracharya and Tuladhar, 2012; Cheng et al., 2006; Geng et al., 2011; Maridass, 2008; Mustaffa et al., 2013 and Sharififar et al., 2009). Thus this research is a step further through the reports presented on cinnamon bark extract through MAHD.

## 4.9 Energy, Economy and Environmental Impact

Based on the results acquired herein, it has been observed that cinnamon bark oil extracted through MAHD method exhibits higher yield and better quality compared to the conventional HD technique. Further effort is therefore being taken to critically draw a comparison between these two techniques through evaluation of their energy, economy and environmental implications. This is necessary to predict the potential of MAHD extraction method at the industrial level.

As discussed in section 4.2.1, extraction through HD took about 23 min to reach the boiling point of plant matrix by heating 200 mL of distilled water containing 25 g of cinnamon powder. The cinnamon oil was fully recovered at 150 min for HD method. Interestingly, MAHD method only required 9 min to reach its induction time at 225 W after which it began to produce cinnamon oil and within 75 min the cinnamon bark oil was fully recovered. Table 4.10 illustrates the summary of comparison on energy consumption and carbon dioxide release from both extraction methods. Based on the calculated result of energy consumption, the energy required to carry out extraction was 0.32 kWh for MAHD and 1.00 kWh for HD. The power consumption value has been determined from the microwave wattmeter and the electrical heater power supply. As can been seen from the table, HD requires more energy to produce essential oil, with over 3 times more energy requirement compared to MAHD. Noteworthy is the fact that the yield produced through MAHD at over 3 times less energy consumption is in no way inferior to the one produced through HD.

Concerning the environmental impact, the amount of carbon dioxide released to the environment is higher in the case of HD (807.02 g $CO_2$/g of cinnamon bark oil) than MAHD (252.00 g $CO_2$/ g of cinnamon bark oil). According to Ferhat and co-workers (2006), 1 kWh of energy consumption from coal or fuel releases almost 800 g of carbon dioxide to the environment during combustion process (Ferhat et al., 2006). The calculation of energy consumption and emission of carbon dioxide were present can be found in APPENDIX C.

In essence, based on the results obtained in this research we can conclude that MAHD method is an environmentally friendly extraction method that supports the idea and development of green technology. MAHD method is a chemical free method which uses minimal amount of water and optimum power to produce good quality of essential oil. In addition, MAHD method can be used to produce large amount of essential oil by using currently existing big scale microwave extraction reactors. Maximum of about 100 kg of fresh plant per time may be feed into the microwave reactor and the reactor can be modified to be used for MAHD extraction.

Table 4.10
Summary of energy consumption and $CO_2$ emission of MAHD and HD methods

| | **MAHD** | **HD** |
|---|---|---|
| Total operating time, min | 84 | 173 |
| Electricity consumption, kWh | 0.32 | 1.00 |
| $CO_2$ released, g | 252.00 | 807.20 |

# CHAPTER 5

# CONCLUSION AND RECOMMENDATIONS

## 5.1 Conclusion

In this study, eco-friendly essential oil was produced from *C. cassia* bark through advanced green extraction technique MAHD and conventional HD method. The effects of factors affecting the MAHD and HD were investigated through OFAT and design expert software. In the OFAT experiment, the optimum parameters obtained in the extraction of *C. cassia* bark oil through MAHD was water to powdered cinnamon bark ratio of 8:1, extraction time of 90 min and microwave power of 225W. Whilst, for the case of HD, it is water to plant material ratio of 8:1 and extraction period of 150 min at constant operating power of 350 W. In another vein, the optimum conditions obtained by MAHD extraction technique via design expert method were water to raw material ratio of 8:1, extraction time of 75 min and irradiation power of 225W. Among these two methods, results obtained through design expert method were concluded to be the optimum conditions for MAHD in this research.

At the optimum conditions, the full recovery of cassia bark oil yield was recorded to be 2.75 % and 2.02 % respectively for MAHD and HD. Besides that, MAHD technique only required half of the extraction time to fully recover the essential oil than HD method. MAHD technique was found not only to reduce extraction time but also reduces the energy consumption, minimizes release of carbon dioxide to environment and ensures the use of less. On the other hand, the crude extract of cassia bark was analyse through GC-MS to examine the classes of chemical compounds present in both extraction techniques. In comparison between the extraction methods, MAHD exhibits 9 % higher oxygenated compounds than HD. The oxygenated compounds commonly contribute large proportion to therapeutics effects. Also, the active compound in cassia bark oil, trans-cinnamaldehyde, was more in MAHD crude extract about 84.43 % than HD only 79.55 %. Furthermore, FTIR analysis carried out on cassia bark crude extract obtained from both extraction techniques reveals that the spectra of oils obtained from these two methods are almost similar. This indicates that all the major chemical compounds present in HD method is also present in MAHD methods. Therefore, MAHD method is a suitable technique for extracting volatile oils from *C. cassia* barks without necessarily causing any structural change to the chemical composition of the oil.

Preliminary test on cytotoxicity of the extracted crude extracts was also conducted. The cytotoxicity properties exhibited by cassia crude extract obtained through MAHD and HD were evaluated against *Artemia salina*, brine shrine nauplii. Interestingly, the crude extract obtained through MAHD shows lower $LC_{50}$ value of 51.21 ppm compared to HD which recorded 68.88 ppm. These results explained indicates that MAHD crude extracts exhibits higher toxicity than HD which revealed that MAHD crude extract possess better pharmaceuticals properties. In essence, MAHD technique is an environmentally friendly, economically feasible, safe, technically compatible, rapid extraction, and chemical free method which requires minimal volume of water as well as uses optimum power to produce excellent quality of essential oil. This emerging and innovative green technology extraction method can suitably replace the current existing conventional HD method.

## 5.2 Recommendations

Based on the observations reported in this research, the author would like to give the following recommendations for future work:

This research focused mainly on the potential of MAHD for the extraction of essential oil from *C. cassia* bark. MAHD method has been observed to be suitable for obtaining good quality essential oils from *C. cassia* bark. In order to further ascertain the suitability of this technique, it is recommended that subsequent research should focus on quantification of oils obtained through this technique.

Similarly, reports from this study which states that trans-cinnamaldehyde is the main active component in *C. cassia* bark oil is based on literature review and few analyses. The cytotoxicity test carried out and the observation reported is therefore presented for the crude extract with the assumption that trans-cinnamaldehyde is the active component. Unfortunately, there has not been any report on isolation of trans-cinnamaldehyde from *C. cassia* bark oil obtained via MAHD. It is therefore suggested that subsequent studies on cassia bark oil obtained via MAHD should extend to isolation of the active compound. Cytotoxicity of the isolated compounds can then by investigated based on the isolated compounds.

In another vein, it is highly recommended that scale up of the lab scale domestic microwave oven to pilot plant should be considered for future research work. With this, MAHD technique can be fully incorporated into industrial production processes for essential oil production.

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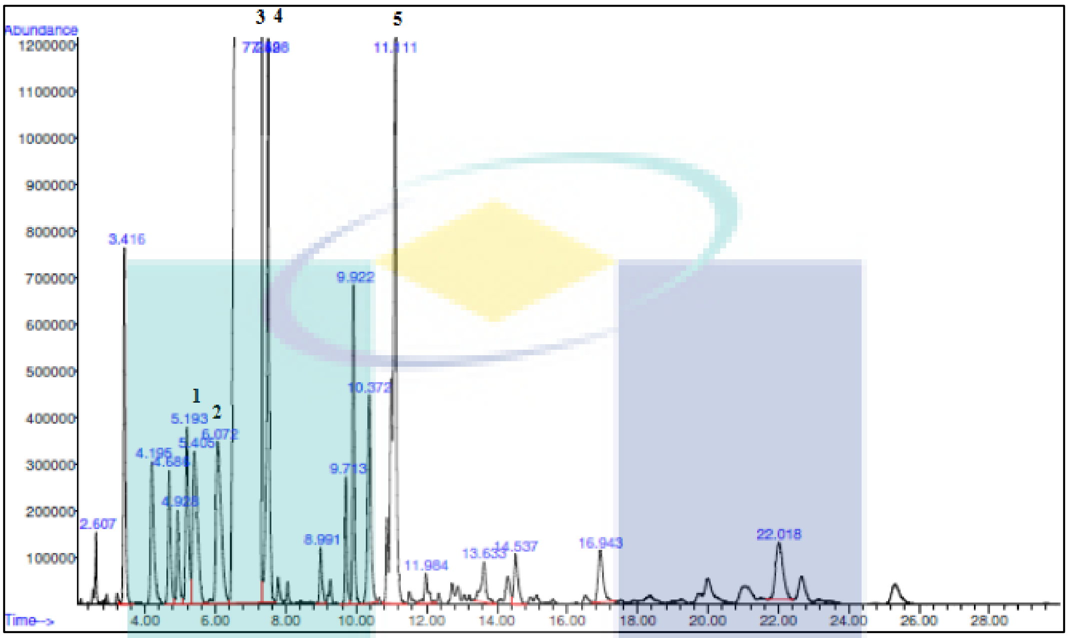
# APPENDIX A1

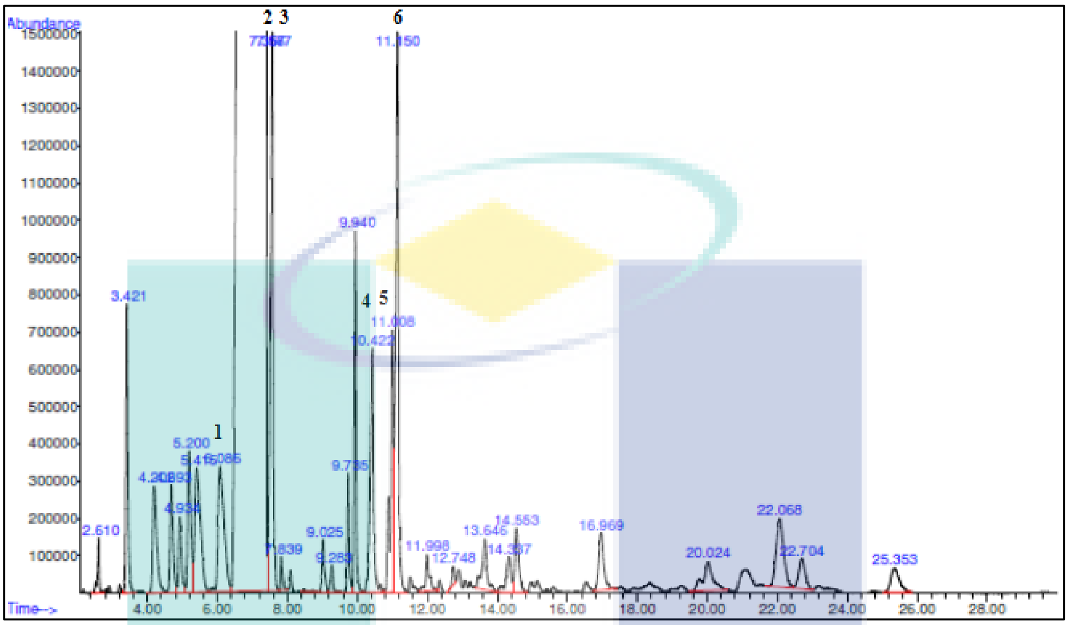
## GC-MS chromatogram of MAHD at 60 min extraction time at fixed water: cinnamon powder ratio of 8:1 and microwave power level of 225 W



| | | | |
|---|---|---|---|
| 1 | Cis-cinnamaldehyde (5.404 min) | 4 | Anethole (7.498 min) |
| 2 | Benzaldehyde (6.075 min) | 5 | Cinnamyl acetate (11.111 min) |
| 3 | Cinnamaldehyde, (E)- (7.251 min) | | |

# APPENDIX A2

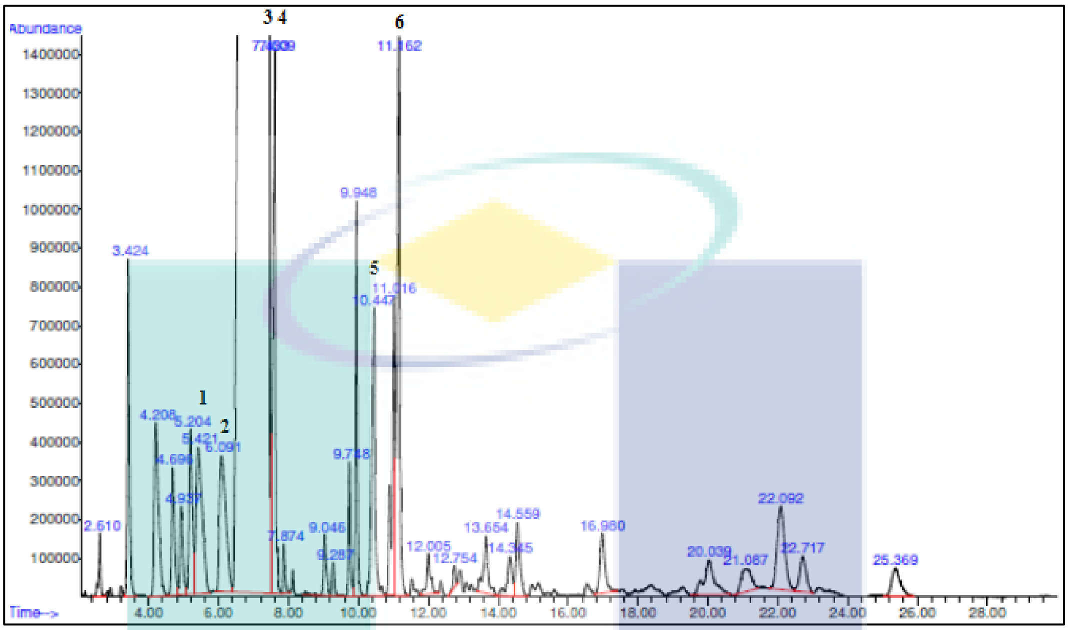
## GC-MS chromatogram of MAHD at 90 min extraction time at fixed water: cinnamon powder ratio of 8:1 and microwave power level of 225 W



| | | | |
|---|---|---|---|
| 1 | Benzaldehyde (6.086 min) | 4 | Coumarin (10.422 min) |
| 2 | Cinnamaldehyde, (E)- (7.369 min) | 5 | Beta- Caryophyllene (11.010 min) |
| 3 | Anethole (7.569 min) | 6 | Cinnamyl acetate (11.150 min) |

# APPENDIX A3

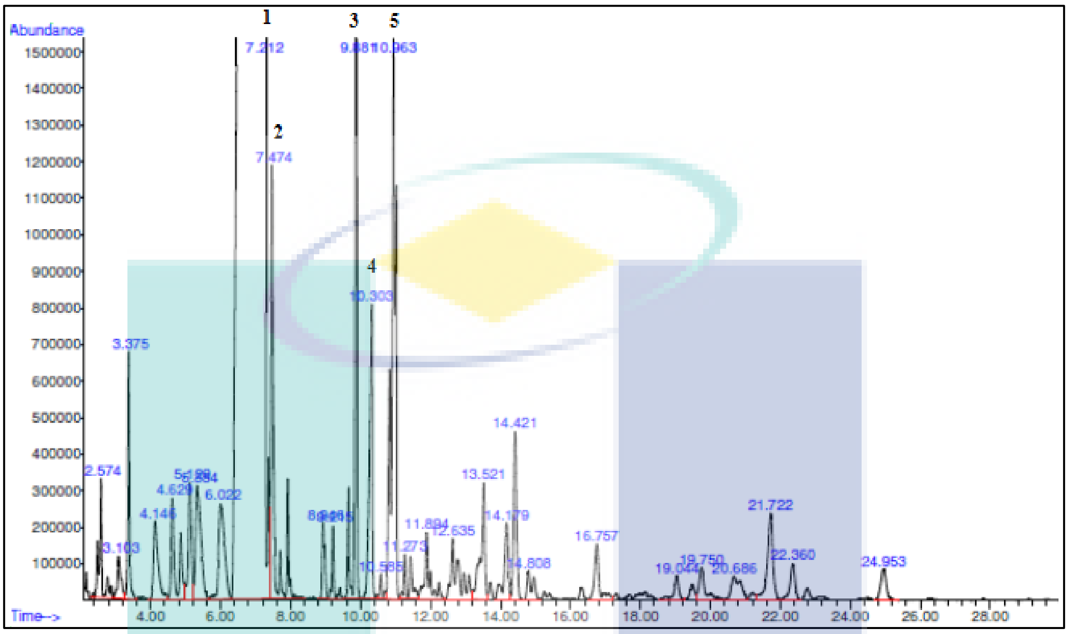
## GC-MS chromatogram of MAHD at 120 min extraction time at fixed water: cinnamon powder ratio of 8:1 and microwave power level of 225 W



| | | | |
|---|---|---|---|
| 1 | Cis-cinnamaldehyde (5.421 min) | 4 | Anethole (7.610 min) |
| 2 | Benzaaldehyde (6.091 min) | 5 | Coumarin ( 10.442 min) |
| 3 | Cinnamaldehyde, (E)- (7.434 min) | 6 | Cinnamyl acetate (11.162 min) |

# APPENDIX A4

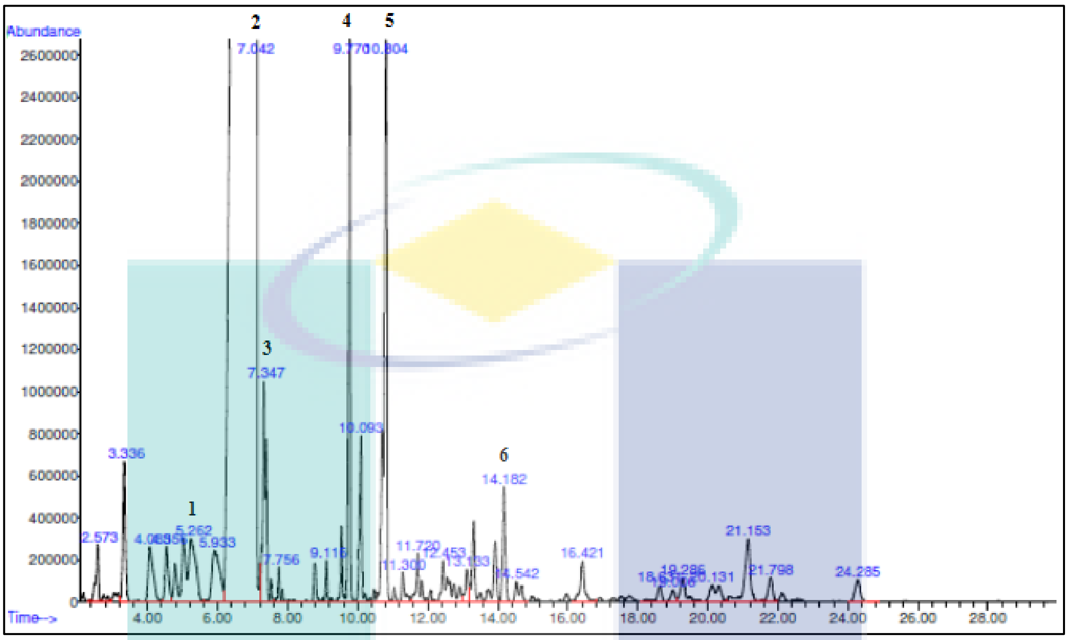
## GC-MS chromatogram of HD at 60 min extraction time at fixed water: cinnamon powder ratio of 8:1 and constant power supply of 350 W



| | | | |
|---|---|---|---|
| 1 | Cinnamaldehyde, (E)- (7.210 min) | 4 | Coumarin (10.304 min) |
| 2 | Anethole (7.475 min) | 5 | Beta-caryophyllene ( 10.963min) |
| 3 | Copaene (9.880 min) | | |

# APPENDIX A5

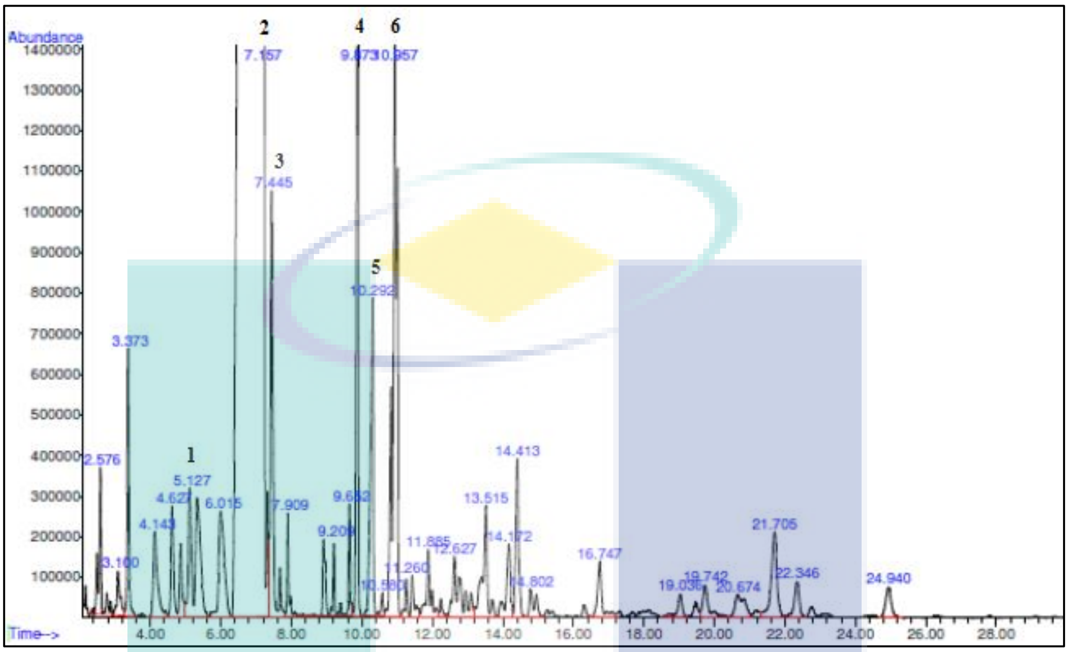
## GC-MS chromatogram of HD at 90 min extraction time at fixed water: cinnamon powder ratio of 8:1 and constant power supply of 350 W



| 1 | Cis-cinnamaldehyde (5.263 min) | 4 | alpha-Cubebene (9.769 min) |
|---|---|---|---|
| 2 | Cinnamaldehyde, (E)- (7.039 min) | 5 | Beta-caryophyllene ( 10.804 min) |
| 3 | Bornyl acetate (7.345 min) | 6 | (+)-delta-Cadinene (14.182 min) |

# APPENDIX A6

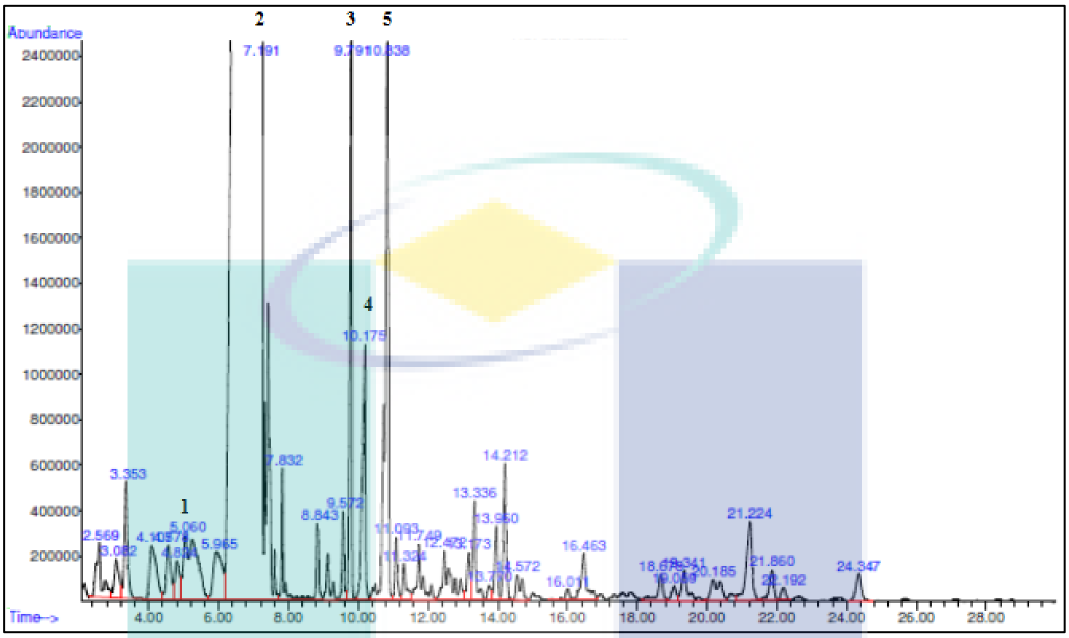
## GC-MS chromatogram of HD at 120 min extraction time at fixed water: cinnamon powder ratio of 8:1 and constant power supply of 350 W



| | | | |
|---|---|---|---|
| 1 | α-Terpineol (5.128 min) | 4 | Copaene (9.875 min) |
| 2 | Cinnamaldehyde, (E)- (7.157 min) | 5 | Coumarin ( 10.292 min) |
| 3 | Anethole (7.445 min) | 6 | Cinnamyl alcohol (10.957 min) |

# APPENDIX A7

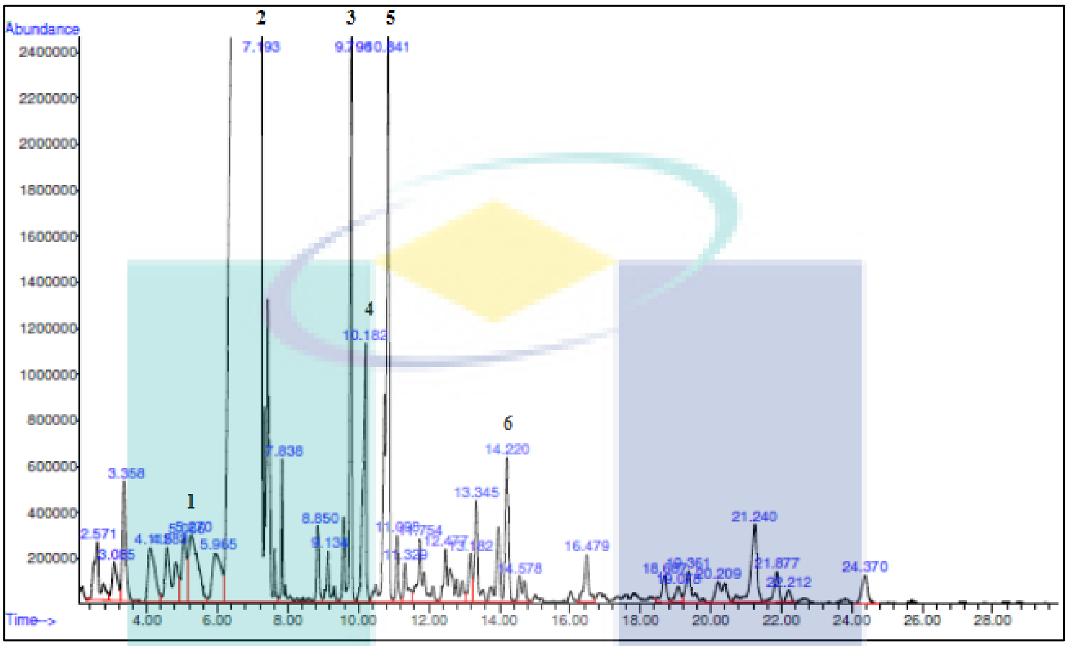
## GC-MS chromatogram of HD at 150 min extraction time at fixed water: cinnamon powder ratio of 8:1 and constant power supply of 350 W



| | | | |
|---|---|---|---|
| 1 | L-α-Terpineol (5.063 min) | 4 | Coumarin (10.175 min) |
| 2 | Cinnamaldehyde, (E)- (7.192 min) | 5 | Beta-caryophyllene ( 10.839 min) |
| 3 | alpha-Cubebene (9.792 min) | | |

# APPENDIX A8

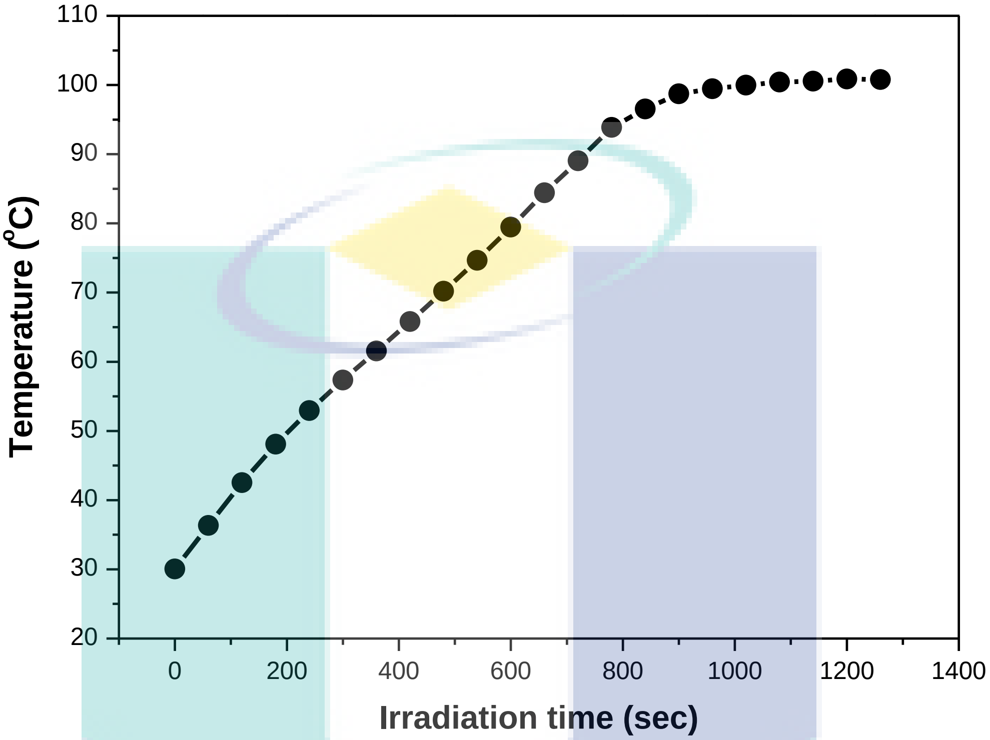
## GC-MS chromatogram of HD at 180 min extraction time at fixed water: cinnamon powder ratio of 8:1 and constant power supply of 350 W



| | | | |
|---|---|---|---|
| 1 | Cis-cinnamaldehyde (5.269 min) | 4 | Coumarin (10.181 min) |
| 2 | Cinnamaldehyde, (E)- (7.193 min) | 5 | Beta-caryophyllene ( 10.839 min) |
| 3 | alpha-Cubebene (9.798 min) | 6 | (+)-delta-Cadinene (14.222 min) |

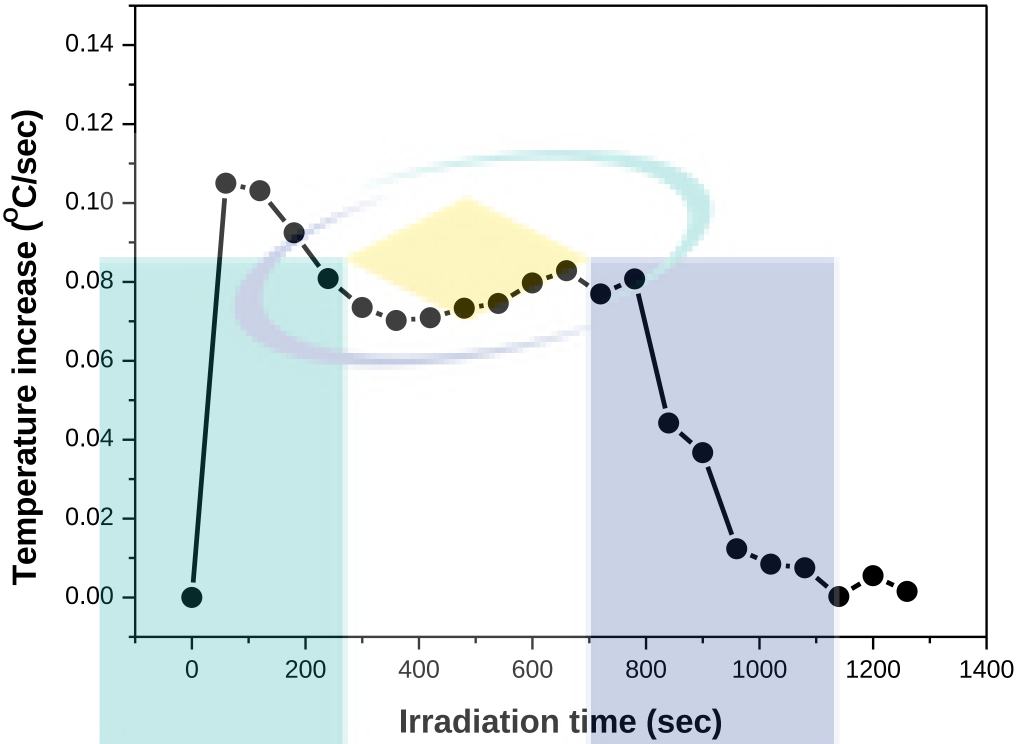
# APPENDIX B1

## Temperature profile of cinnamon matrix extracted through MAHD at water: cinnamon powder ratio of 8:1 and microwave power of 200 W.



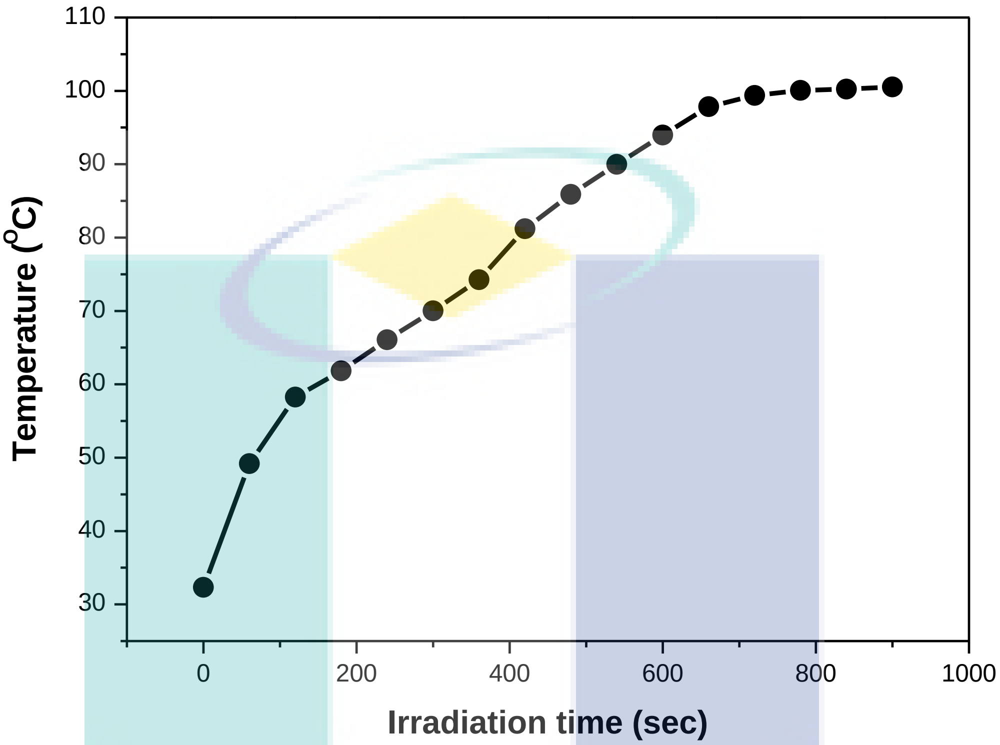
# APPENDIX B2

## Rate of temperature increase profile of cinnamon matrix extracted through MAHD at water: cinnamon powder ratio of 8:1 and microwave power of 200 W.



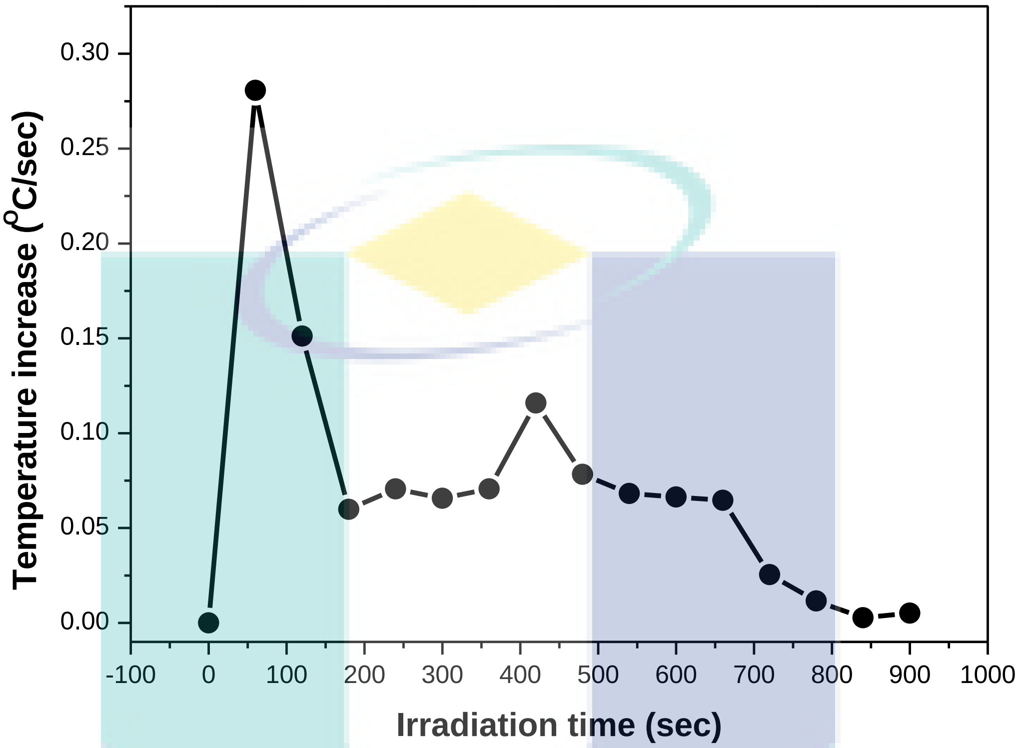
## APPENDIX B3

### Temperature profile of cinnamon matrix extracted through MAHD at water: cinnamon powder ratio of 8:1 and microwave power of 250 W.



## APPENDIX B4

### Rate of temperature increase profile of cinnamon matrix extracted through MAHD at water: cinnamon powder ratio of 8:1 and microwave power of 250 W.



# APPENDIX B5

## Calculations of volume rate of heat generation, Q at 225 W of microwave power and 8:1 of water-to-cinnamon powder ratio

$$Q_{mw} = \rho_{mix}\, x\, C_{p\,mix}\, x\, \frac{dT}{dt}$$

However, $\rho_{mix}$ and $C_{p\,mix}$ were calculated by simple mixing rule as follows:

$$\rho_{mix} = \rho_W \emptyset + \rho_s\,(1-\emptyset)$$
$$C_{p\,mix} = C_{p,W} \emptyset + C_{p,s}\,(1-\emptyset)$$

Where,

Φ : Volume fraction

Therefore, $\rho_{mix}$ calculated as follows:

$\rho_W$ = density of water at room temperature measured by using pycnometer equipment and obtained 0.9695 g/cm$^3$

$\rho_S$ = density of cinnamon bark powder measured by using pycnometer equipment and obtained 1.5915 g/cm$^3$

By inserting the mentioned values in the following equation for 8:1 of water-to-cinnamon powder ratio:

$$\rho_{mix} = \rho_W \emptyset + \rho_s\,(1-\emptyset)$$

$$\rho_{mix} = \left(\left(0.9695\, x\, \frac{8}{9}\right) + \left(1.5915\,\left(1-\frac{8}{9}\right)\right)\right)$$
= 0.8618 + 0.1768
= 1.0386 g/cm$^3$

$C_{Pmix}$ was calculated as follows:
$C_{P,W}$ = heat capacity of water at room temperature is 0.0009993 kcal/ g. $^0$C.
$C_{P,W}$ = heat capacity of cinnamon bark powder is 1.017 kcal/ g. $^0$C.

By inserting the mentioned values in the following equation for 8:1 of water-to-cinnamon powder ratio:

$$C_{p\,mix} = C_{p,W} \emptyset + C_{p,s}\,(1-\emptyset)$$

$$C_{Pmix} = (\left(0.0009993\ x\ \frac{8}{9}\right) + (1.017\ (1 - \frac{8}{9})))$$
$$= 0.0008883 + 0.113$$
$$= 0.1139\ \text{kcal/ g.}\ {}^{0}\text{C}$$

As a result, insert the calculated values in the following equation in order to obtain the volume of heat generation at different rate of temperature:

$$Q_{mw} = \rho_{mix}\ x\ C_{p\ mix}\ x\ \frac{dT}{dt}$$
$$Q_{mw} = 1.0386\ x\ 0.1139\ x\ \frac{dT}{dt}$$

Following is the table of summary of the volume of heat generation for 225 w of microwave irradiation power and 8:1 of water: cinnamon powder ratio:

| **Radiation time, t (sec)** | **Temperature, T ($^{0}$C)** | **Temperature increase, ΔT ($^{0}$C)** | **Rate of temperature increase, dT/dt ($^{0}$C/s)** | **Volume rate of heat generation, $q_{mw}$ (cal/s.m$^{3)}$** |
|---|---|---|---|---|
| 0 | 31.1233 | 0 | 0 | 0 |
| 60 | 41.2333 | 10.1100 | 0.1685 | 0.01993 |
| 120 | 51.1900 | 9.9567 | 0.1656 | 0.01959 |
| 180 | 58.8267 | 7.6367 | 0.1273 | 0.01506 |
| 240 | 65.4400 | 6.6133 | 0.1102 | 0.01304 |
| 300 | 71.6600 | 6.2200 | 0.1037 | 0.01227 |
| 360 | 77.4733 | 5.8133 | 0.0969 | 0.01146 |
| 420 | 83.0667 | 5.5934 | 0.0932 | 0.01103 |
| 480 | 86.8433 | 3.7766 | 0.0629 | 0.00744 |
| 540 | 89.5333 | 2.6900 | 0.0448 | 0.00530 |
| 600 | 92.2100 | 2.6767 | 0.0446 | 0.00528 |
| 660 | 95.5333 | 3.3233 | 0.0554 | 0.00655 |
| 720 | 98.5167 | 2.9834 | 0.0497 | 0.00588 |
| 780 | 99.9267 | 1.4070 | 0.0235 | 0.00278 |
| 840 | 100.2367 | 0.3100 | 0.0052 | 0.00062 |
| 900 | 100.4433 | 0.2066 | 0.0033 | 0.00039 |
| 960 | 100.6800 | 0.2367 | 0.0004 | 0.00005 |

# APPENDIX B6

## Calculations of dielectric constant and dielectric loss of mixture, at 225 W of microwave power and 8:1 of water-to-cinnamon powder ratio

$$\varepsilon'_W = 85.215 - 0.33583T$$
$$\varepsilon''_W = 320.685T^{-1.0268}$$

However, $\varepsilon'_{mix}$ and $\varepsilon''_{mix}$ were calculated by simple mixing rule as follows:

$$\varepsilon'_{mix} = \varepsilon'_W\emptyset + \varepsilon'_S(1-\emptyset)$$
$$\varepsilon''_{mix} = \varepsilon''_W\emptyset + \varepsilon''_S(1-\emptyset)$$

Where,
$\Phi$ : Volume fraction

Therefore, $\varepsilon'_{mix}$ was obtained as follows:

$\varepsilon'_S$ = dielectric constant of cinnamon bark powder was reported as 2.8 at 2450 MHz (Khan and Chandel, 2011)

$\varepsilon'_S(1-\emptyset) = 2.8 \times (1 - \frac{8}{9}) = 0.3111$

$\varepsilon'_W = 85.215 - 0.33583T$

Following is the table of summary of dielectric constants of mixture at 225 W of microwave power and 8:1 of water-to-cinnamon powder ratio:

| **T** | $\varepsilon'_W$ | $\varepsilon'_W\emptyset = \varepsilon'_W(\frac{8}{9})$ | $\varepsilon'_{mix}$ |
|---|---|---|---|
| 30 | 75.1401 | 66.7912 | 67.1023 |
| 35 | 73.4610 | 65.2986 | 65.6097 |
| 40 | 71.7818 | 63.8060 | 64.1172 |
| 45 | 70.1027 | 62.3135 | 62.6246 |
| 50 | 68.4235 | 60.8209 | 61.1320 |
| 55 | 66.74435 | 59.3283 | 59.6394 |
| 60 | 65.0652 | 57.8357 | 58.1468 |
| 65 | 63.38605 | 56.3432 | 56.6542 |
| 70 | 61.7069 | 54.8506 | 55.1619 |

Therefore, $\varepsilon''_{mix}$ was obtained as follows:

$\varepsilon''_S$ = dielectric loss of cinnamon bark powder was reported as 0.38 at 2450 MHz (Khan and Chandel, 2011)

$\varepsilon''_S\ (1 - \emptyset)$ = 0.38 x (1 - $\frac{8}{9}$) = 0.0422

$\varepsilon''_W = 320.685T^{-1.0268}$

Following is the table of summary dielectric loss of mixture at 225 W of microwave power and 8:1 of water-to-cinnamon powder ratio:

| **T** | $\varepsilon''_W$ | $\varepsilon''_W\emptyset = \boldsymbol{\varepsilon''_W(\frac{8}{9})}$ | $\boldsymbol{\varepsilon''_{mix}}$ |
|---|---|---|---|
| 30 | 9.7582 | 8.6740 | 8.7162 |
| 35 | 8.3297 | 7.4042 | 7.4464 |
| 40 | 7.2625 | 6.4555 | 6.4977 |
| 45 | 6.4352 | 5.7202 | 5.7624 |
| 50 | 5.7753 | 5.1336 | 5.1758 |
| 55 | 5.2369 | 4.6550 | 4.6972 |
| 60 | 4.7893 | 4.2572 | 4.2994 |
| 65 | 4.4114 | 3.9212 | 3.9635 |
| 70 | 4.0882 | 3.6340 | 3.6762 |

# APPENDIX B7

## Calculations of penetration depth at 225 W of microwave power and 8:1 of water-to-cinnamon powder ratio

$$D_p = \frac{\lambda_\circ \, x \, \sqrt{\varepsilon'}}{2\pi\varepsilon''}$$

Where,

$\lambda_\circ$ is the wavelength of microwave energy at 12.24cm for 2450 MHz.

### Penetration depth for various temperatures

| **T** | $\boldsymbol{\varepsilon'_W}$ | $\boldsymbol{\varepsilon''_W}$ | $\boldsymbol{D_p}$ |
|---|---|---|---|
| 30 | 67.1023 | 8.7162 | 1.8309 |
| 35 | 65.6097 | 7.4464 | 2.1192 |
| 40 | 64.1172 | 6.4977 | 2.401 |
| 45 | 62.6246 | 5.7624 | 2.6753 |
| 50 | 61.1320 | 5.1758 | 2.9428 |
| 55 | 59.6394 | 4.6972 | 3.2028 |
| 60 | 58.1468 | 4.2994 | 3.4551 |
| 65 | 56.6542 | 3.9635 | 3.6995 |
| 70 | 55.1619 | 3.6762 | 3.9357 |

# APPENDIX C1

## Calculation on Energy Consumption and Emission of Carbon Dioxide for MAHD

### Optimum process conditions for MAHD:

| | |
|---|---|
| Microwave power level, w | 225 |
| Water: cinnamon power ratio | 8: 1 |
| Extraction time, min | 75 |
| Induction time, min | 9 |
| Total operation time, min | 84 |

Energy consumed = $\frac{Watts\ x\ Hours\ operated}{1000}$

*800 g $CO_2$ is released to the environment to obtain 1kWh

Therefore,

Energy released by MAHD for full recovery of cassia oil:

= 225 W x 84 min x $\frac{1\ h}{60\ min}$ x $\frac{1\ kW}{1000\ W}$

**= 0.315 kWh**

Emission of $CO_2$ to the atmosphere by MAHD extraction:

= 0.315 x 800 g

**= 252 g $CO_2$ / g of cassia oil**

# APPENDIX C2

## Calculation on Energy Consumption and Emission of Carbon Dioxide for HD

### Optimum process conditions for HD:

| | |
|---|---|
| Operating power, w | 350 |
| Water: cinnamon power ratio | 8: 1 |
| Extraction time, min | 150 |
| Induction time, min | 23 |
| Total operation time, min | 173 |

Energy consumed = $\frac{Watts\ x\ Hours\ operated}{1000}$

*800 g $CO_2$ is released to the environment to obtain 1kWh

Therefore,

Energy released by HD for full recovery of cassia oil:

= 350 W x 173 min x $\frac{1\ h}{60\ min}$ x $\frac{1\ kW}{1000\ W}$

**<u>= 1.009 kWh</u>**

Emission of $CO_2$ to the atmosphere by HD extraction:

= 1.009 x 800 g

**<u>= 807.2 g $CO_2$ / g of cassia oil</u>**

## LIST OF POTENTIAL PUBLICATIONS

Nitthiyah Jeyaratnam, Abdurahman H. Nour, Ramesh Kanthasamy, Azhari Hamid Nour, A.R. Yuvaraj and John O. Akindoyo. Essential Oil from *Cinnamomum cassia* Bark through Hydrodistillation and Advanced Microwave Assisted Hydrodistillation. *Industrial Crops and Products*, *92*, 57-66.

Nitthiyah Jeyaratnam, Abdurahman Hamid Nour and John Olabode Akindoyo. Comparative Study between Hydrodistillation and Microwave-Assisted Hydrodistillation for Extraction of *Cinnamomum cassia* Oil. *ARPN Journal of Engineering and Applied Sciences*, *11*(4), 2647-2652.

Nitthiyah Jeyaratnam, Abdurahman H. Nour and John O. Akindoyo. The Potential of Microwave Assisted Hydrodistillation in Extraction of Essential Oil from *Cinnamomum cassia* (Cinnamon). *ARPN Journal of Engineering and Applied Sciences, 11*(4), 2179-2183.

Nitthiyah Jeyaratnam, Abdurahman H. Nour, Ramesh Kanthasamy and John O. Akindoyo. Screening of Microwave Assisted Hydrodistillation Parameters through Two Level Factorial Design for *Cinnamomum cassia* Bark Oil Extraction. *Submitted to RCS Advances.*

## CONFERENCES & PAPERS PRESENTED

**1.** Malaysian Technical Universities Conference on Engineering and Technology 2015 (Mucet 2015). Qualitative and Quantitative Analysis of Hydrodistillation and Microwave-Assisted Hydrodistillation Extracted *Cinnamomum cassia* Oil-A Comparative Study. ***11th-13th October 2015. KSL Resort Hotel, Johor Bahru, Johor, Malaysia.***

**2.** International Conference on Fluids and Chemical Engineering 2015 (FluidsChE 2015*)* The Potential of Microwave Assisted Hydrodistillation (MAHD) in Extraction and Characterization of Essential Oil from *Cinnamomum cassia* (cinnamon). ***25th-27th November 2015, Adya Hotel, Langkawi, Malaysia.***

**3.** National Conference for Postgraduate Research 2016 (NCON-PGR 2016). Mechanism of Microwave Assisted Hydrodistillation Studied through Heat Analysis. ***24th–25th September 2016. Universiti Malaysia Pahang, Pekan.***

**4.** International Conference of Chemical Engineering and Industrial Biotechnology 2016 (ICCEIB 2016). Microwave Assisted Hydrodistillation – An Advance and Green Extraction Technology. ***28th–30th November 2016. Melaka, Malaysia.*** **ACCEPTED.**

# EXHIBITION AND AWARDS

1. Asia Pacific Bioeconomy Innovation Awards (BIOMALAYSIA 2016). High through put production of *Cinnamomum cassia* (Cinnamon) Essential Oil by Microwave Hydro-Distillation. ***31st May–2nd June 2016, Kuala Lumpur Convection Center, Malaysia.***

2. International Festival Innovation on Green Technology (i-FINOG 2016). Prospects of Cinnamon Cassia (Cinnamon) Essential Oil Extracted via MAHD. ***15th–17th April 2016, Universiti Malaysia Pahang***. **Gold Prize.**

3. Creation, Innovation, Technology & Research Exposition (CITREX 2016). Prospects of Cinnamon Cassia (Cinnamon) Essential Oil Extracted via MAHD. ***7th–8th March 2016, Universiti Malaysia Pahang***. **Silver Award.**