IDENTIFICATION AND OPTIMIZATION OF LEMONGRASS ESSENTIAL OILS EXTRACTED BY HYDRODISTILLATION AND MICROWAVE ASSISTED HYDRODISTILLATION METHODS

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ABSTRACT

The use of plant extracts in different commercial sectors such as pharmaceutical, food and chemical industries has gained renewed attention in the last few decades. Existing conventional extraction methods are known for their economic impact due to high energy, toxic solvents, time consumption as well as causing environmental impact by releasing CO_2 and untreatable waste. Thus, microwave technique has been developed recently for extraction of natural products from plant materials due to the need of a rapid, safe and cost effective method in this field. Current study has been carried out to investigate the efficiency of microwave assisted hydrodistillation (MAHD) over conventional hydrodistillation (HD) for the extraction of essential oils from Lemongrass (*Cymbopogon citratus*). The oil extraction process was based on three major operating parameters namely the water to plant material ratio, microwave power level and extraction time and their interaction has been evaluated by using one-factor-at-a-time experiments (OFAT). In comparison of both extraction techniques, MAHD resulted in shorter extraction time to obtain optimum yield of 1.464% which was about 90 min compare to 150 min in HD. The results from OFAT experiments were being analyzed in optimization study of MAHD system using Response Surface Methodology (RSM) based on central composite design (CCD). All considered factors were statistically significant for extraction efficiency, while the most important factor was water to plant material ratio. Water to plant material ratio of 8:1, microwave power level of 250W and extraction time of 90 min were determined as the optimal parameter configuration with yield of 1.461%. This proved the experimental value was in good agreement with predicted model value. Besides, this optimum parameters condition was also being used for qualitative analysis to determine the chemical composition of Lemongrass oil by Gas chromatography-mass spectrometry (GC-MS) analysis in order to study the effects of extraction method and extraction time on chemical composition. As a result, more than 34 constituents were being identified. Five key compounds were present in all the extracts of both methods and they were neral, geranial, β-myrcene, geraniol and linalool. MAHD extract seems to contain higher amount of oxygenated compound. The oil obtained under optimized condition of both methods was selected for cytotoxicity study using brine shrimp lethality (BLT) assay. The results shows HD extracts, with higher β-myrcene content, was more toxic than the one obtained by MAHD with LC_{50} value of 13ppm and 18ppm respectively. To attain qualitative understanding of microwave extraction process, temperature analysis was being carried out to perform heat generation calculation. It shows the volume rate of heat generation decreasing within irradiation time due to the decreasing of dielectric properties. The evaluation on cost, energy and environmental impact by MAHD and HD shows the conventional method utilizes three time higher energy compared to the recent technique. Due to the substantial saving of time, cost and energy with no significant changes in its constituents, MAHD process found to be a good alternative green technology to conventional methods. Results obtained in this study have exposed the capability of MAHD techniques as another promising method for extraction of essential oils. Further works are nevertheless required to provide deeper understanding of the mechanism involved to facilitate the development of an optimum system applicable to the industry.

ABSTRAK

Penggunaan ekstrak tumbuhan dalam sektor perdagangan yang berbeza seperti industri farmaseutikal , makanan dan kimia telah mendapat perhatian baru dalam beberapa dekad yang lalu. Kaedah pengekstrakan konvensional sedia ada terkenal dengan kesan ekonomi mereka kerana tenaga yang tinggi, pelarut toksik, penggunaan masa dan juga menyebabkan kesan alam sekitar dengan melepaskan CO_2 dan bahan buangan yang tidak boleh dirawat . Oleh itu, teknik gelombang mikro telah dibangunkan baru-baru ini untuk pengekstrakan produk semula jadi daripada bahan tumbuhan kerana keperluan sebuah kaedah yang cepat, selamat dan kos efektif dalam bidang ini. Kajian semasa telah dijalankan untuk menyiasat kecekapan MAHD daripada HD dalam pengekstrakan minyak pati Serai (*Cymbopogon citratus*). Proses pengekstrakan minyak telah berdasarkan tiga parameter operasi utama iaitu nisbah air kepada bahan mentah, tahap kuasa gelombang mikro dan masa pengekstrakan dan interaksi mereka telah dinilai dengan menggunakan ujikaji satu-faktor -di- satu -masa (OFAT). Dalam perbandingan kedua-dua teknik pengekstrakan, MAHD mengambil masa pengekstrakan yang lebih pendek untuk mendapatkan hasil optimum 1.464 % iaitu kira-kira 90 min berbanding dengan 150 minit dalam HD. Keputusan daripada eksperimen OFAT telah dianalisis dalam kajian pengoptimuman sistem MAHD menggunakan Permukaan Sambutan Kaedah (RSM) berdasarkan reka bentuk komposit pusat (CCD) . Semua faktor yang dipertimbangkan adalah statistik yang signifikan untuk kecekapan pengekstrakan, manakala faktor yang paling penting ialah nisbah air kepada bahan mentah. Berdasarkan eksperimen, nisbah air kepada bahan mentah bernilai 8:1 , tahap kuasa gelombang mikro 250W dan masa pengekstrakan 90 min telah ditentukan sebagai konfigurasi parameter optimum dengan hasil sebanyak 1.461 %. Ini membuktikan nilai eksperimen adalah dalam perjanjian yang baik dengan nilai yang diramalkan oleh modal. Selain itu, ini keadaan parameter optimum telah juga digunakan untuk analisis kualitatif untuk menentukan komposisi kimia minyak Serai melalui Gas kromatografi - spektrometri jisim (GC- MS) dengan tujuan untuk mengkaji kesan kaedah pengekstrakan dan masa pengekstrakan keatas komposisi kimia . Hasilnya, lebih daripada 34 bahan sebatian telah dikenal pasti . Lima sebatian utama telah ditemui dalam kesemua ekstrak adalah neral, geranial, β - myrcene , geraniol dan linalool . Ekstrak MAHD menunjukkan kandungan sebatian oksigen yang lebih tinggi daripada HD. Minyak yang diperoleh di bawah keadaan optimum bagi kedua-dua kaedah telah dipilih untuk kajian cytotoxicity menggunakan kaedah ketoksikan terhadap udang air garam (BLT). Keputusan menunjukkan ekstrak HD adalah lebih toksik daripada yang diperolehi melalui MAHD dengan nilai LC_{50} sebanyak 13ppm dan 18ppm, masing-masing . Untuk mencapai pemahaman kualitatif proses pengekstrakan mikro , analisis suhu telah dijalankan untuk melaksanakan pengiraan penjanaan haba. Ia menunjukkan kadar jumlah penjanaan haba berkurangan dalam masa penyinaran disebabkan oleh penurunan sifat dielektrik . Penilaian dalam kos, tenaga dan kesan alam sekitar oleh MAHD dan HD menunjukkan kaedah konvensional menggunakan tenaga tiga kali lebih tinggi berbanding dengan teknik yang terkini ini . Oleh kerana penjimatan yang banyak dalam masa, kos dan tenaga tanpa sebarang perubahan penting dalam juzuk , proses MAHD didapati teknologi hijau alternatif yang baik. Keputusan yang diperolehi dalam kajian ini telah mendedahkan keupayaan teknik MAHD sebagai satu lagi kaedah yang menjanjikan untuk pengekstrakan minyak pati. Kerja-kerja selanjutnya namun diperlukan untuk memberi pemahaman yang lebih mendalam tentang mekanisme yang terlibat bagi memudahkan pembangunan sistem optimum yang sesuai dengan industri.

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P_T	Total Pressure
p_A	Vapor Pressure of A
p_W	Empirical Constant
Wt_A	Weight of A
Wt_w	Weight of Water
MW_A	Molecular Weight of A
MW_W	Molecular Weight of Water
y_A	mole fraction of A
y_W	mole fraction of water
K	Equilbrium Constant of Hydrolysis
q_{mw}	Volumetric rate of heat generation
ρ	Density
ρ_{mix}	Density of mixture
ρ_s	Density of samples
ρ_w	Density of water
C_p	Heat Capacity
$C_{P,mix}$	Heat capacity of mixture
$C_{P,s}$	Heat capacity of samples
$C_{P,w}$	Heat capacity of water
dT/dt	Rate of temperature increase
ϕ	Volume fraction
ε'	Dielectric constant
$\varepsilon'_{r',w}$	Dielectric constant of water

ε'_S	Dielectric constant of samples
ε''	Dielectric loss
$\varepsilon''_{r,w}$	Dielectric loss of water
ε''_S	Dielectric loss of samples
D_P	Penetration Depth
Mg	Moisture content
T	Temperature
λ_O	Wavelength of microwave energy
Л	Pi
Y	Response Variable

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BBD	Box-Behken Designs
BLT	Brine shrimp lethality
CAMD	Compressed air microwave distillation
CCD	Central Composite Design
CID	Controlled instantaneous decomposition
CO_2	Carbon Dioxide
DCM	Dichloromethane
DMSO	Dimethyl Sulfoxide
EAE	Enzyme-assisted extraction
FC	Foot Cell
GC-MS	Gas Chromatography-Mass Spectrometry
HC	Head Cell
HD	Hydrodistillation
$K_2Cr_2O_7$	Potassium Dichromate
MAD	Microwave-assisted distillation
MAHD	Microwave Assisted Hydrodistillation
MASE	Microwave- assisted solvent extraction
MHC	Mother Head Cell
MHG	Microwave hydrodiffusion and gravity
Na_2SO_4	Anhydrous Sodium Sulfate
OFAT	One factor at a time
PEF	Pulsed-electric field
PLE	Pressurized liquid extraction

R	Correlation coefficient
R^2	Coefficient of multiple determinations
RSM	Response Surface Methodology
SDE	Simultaneous distillation extraction
SFE	Supercritical fluid extraction
SFME	Solvent-free microwave hydrodistillation
SPME	Solid phase micro-extraction
SC	Stalk CCell
UAE	Ultrasonic assisted extraction
VMHD	Vacuum microwave hydrodistillation

CHAPTER 1

INTRODUCTION

1.1 RESEARCH BACKGROUND

Essential oils extracted from a wide variety of plants and herbs have been a source material for the manufacture of foodstuffs, cosmetics, cleaning products, fragrances, herbicides and insecticides; this industry dates back to centuries ago. Nowadays, plant essential oil has also gained considerable attention for aromatherapy, a branch of alternative medicine that claims that essential oils and other aromatic compounds have curative effects. Due to the growing concern of consumers over the ingredients from natural sources and the awareness about potentially harmful synthetic spices additives, the global demand for essential oils is increasing. As a consequence, an effective extraction method in terms of cost, quality and environmental friendliness are being investigated by a number of researchs to satisfy the needs of this quality-demanding product.

Cymbopogon (Lemongrass) is a genus of about 55 species of grasses, (of which the type species is *Cymbopogon citratus*) native to warm temperature and tropical regions of the Old World and Oceania (Edwin et al, 2012). Lemongrass (*Cymbopogon citratus*) is a tall perennial grass that contains 1 to 2% essential oil on a dry basis. Lemongrass (*Cymbopogon citratus*) essential oil is characterised by a high content of citral (composed of neral and geranial isomers). Citral is used as a raw material for the production of ionone, vitamin A and beta-carotene (Carlson et al, 2001). The chemical composition of Lemongrass (*Cymbopogon citratus*) essential oil varies widely on genetic diversity, habitat and agronomic treatment of the culture (Paviani et al, 2006). Lemongrass (*Cymbopogon citratus*) is widely used as an essential ingredient in Asian cuisines due to its sharp lemon flavour. The essential oil of lemongrass has also been used to treat a wide variety of health conditions such as acne, athlete's foot, excessive perspiration, flatulence, muscle aches, oily skin, and scabies (Brian, and Ikhlas, 2002). Moreover, a number of bioactivity studies have proved that various components of this essential oil contain antimicrobial, antifungal, antibacterial and mosquito-repellent properties. Because of these desirable qualities, Lemongrass (*Cymbopogon citratus*) oil is of great use and value in the agriculture sector, especially for the protection of stored agricultural products such as the staple food crop, maize (Masamba et al, 2003).

The techniques for the extraction of volatile oils have also been reported to cause variations in the volatile compounds of samples (Hong et al, 2010). Driven by the potential applications of essential oil extracted from Lemongrass (*Cymbopogon citratus*), studies were conducted based on conventional methods like solvent extraction, steam distillation, hydrodistillation (HD), as well as novel techniques like supercritical fluid extraction and extraction using dense carbon dioxide (Desai and Parikh, 2012). Amongst these methods, HD has been the most common approach in extracting essential oils from the medicinal herbs and plants. However, HD method suffers from some disadvantages including loss of volatile compounds, long extraction duration, and they are known to be energy-intensive. Furthermore, elevated temperatures can cause partial or full degradation of natural constituents, which are vulnerable to structural changes under steam distillation conditions. The conventional solvent extraction method is likely to involve loss of more volatile compounds during removal of the solvent (Mohsen 2012). In order to overcome this sort of drawbacks, an advance and improved method such as microwave-assisted extraction (Hong, 2010), ohmic-assisted hydrodistillation (Mohsen 2012), subcritical water extraction (Mohammad et al., 2007), and ultrasound-assisted extraction (Porto, 2009) have been applied to shorten extraction time, improve extraction yield and reduce operational costs. Microwave-assisted procedures for isolating essential oils have become attractive for use in laboratories and industries due to its effective heating, fast energy transfer, and its environmental friendliness.

Most of the studies on extraction of essential oil from Lemongrass (*Cymbopogon citratus*) were done using the Hydrodistillation process (Edwin et al., 2012; Bankole and Joda, 2004; Masamba et al., 2003). There is also a number of publications dealing with the extraction of Lemongrass (*Cymbopogon Citratus*) using advanced technologies such as Supercritical Carbon Dioxide Extraction by Huynh et al., 2008, Pressurised Liquid Extraction by Nur et al., 2013 and High Pressure Carbon Dioxide Extraction using Molecular Sieves by Pavani et al., 2006. However, to our best knowledge, no extraction study was carried out for Lemongrass using the Microwave-Assisted Hydrodistillation method and in identifying its optimum operation condition. Therefore, the objective of the present investigation is to study the effects of the main operating parameters, namely extraction time, irradiation power and water to plant material ratio, on the extraction yields of Lemongrass (*Cymbopogon Citratus*) essential oil. The chemical composition of the essential oil was analysed by GC–MS. In addition, the cytotoxicity properties of the essential oil extracted under optimised conditions were evaluated by means of lethality test using brine shrimp. Comparative evaluation between MAHD and conventional HD is aimed at highlighting the benefits of the advance method. Furthermore, Response Surface Methodology (RSM) has been used to optimise extraction of essential oil from lemongrass (*Cymbopogon citratus*) with regards to extraction time, microwave power, and water-to-raw material ratio.

1.2 PROBLEM STATEMENT

A study conducted over a decade ago in 1996-97 estimated the demand for essential oils for the year at 14,900 tons. The growth rates it estimated then was for domestic and export markets at 9 and 25% respectively. The demand and supply gap was then projected at 8,000 tons. It is difficult to guess the actual produce and supply. What is perceived is that there is a good demand for essential oils.

This increasing demand of essential oil, mainly for commonly used essential oils such as lemongrass oil, has opened up wide opportunities for global marketing, leading to the requirement of competitive product in market which comes with all the advantages in terms of cost, quality and production time. As mentioned earlier, essential oil is a volatile component. Therefore, it is vital to identify the best extraction technique, so that a higher yield of essential oil with good quality can be isolated.

HD is the most common approach to the extraction of essential oils from medicinal herbs and plants. However, these conventional methods present several drawbacks such as long extraction duration, potential loss of volatile constituents, and high energy use to name a few. Therefore, it is not suitable for the current market requirement hence developing an alternative extraction technique that is rapid, sensitive, safe, and energy-efficient is highly desirable. As a result, to improve this existing extraction process, a more active and efficient enhancement can be added; microwave is one of them.

MAHD method is a more recent technique used to recover volatile components such as essential oils. In this method, plant material placed in a Clevenger apparatus is heated inside a microwave oven for a short period of time to extract the essential oil whereby heat is produced by microwave energy. The sample reaches its boiling point very rapidly, leading to a very short extraction or distillation time. With the microwave distillation technique, it is possible to achieve distillation with the indigenous water of the fresh plant material (Kürkçüoğlu and Baser, 2010). This method could support the current demand of essential oil and is believed to be able to close the gap between the current demand and production rate. Besides, it also found to be energy and cost saving "green" extraction method. MAHD advantageous over conventional method makes it to be an alternative extraction method in this field.

1.3 SIGNIFICANT OF THE STUDY

The rationale of this proposed research project is to examine the performance of MAHD method in the extraction of essential oil from Lemongrass (*Cymbopogon citratus*), and to compare the result with conventional hydrodistillation based on their extracted yield. The results of this research would signify MAHD to be an advantageous technique over other extraction methods including for industrial applications.

There are various advantages of using MAHD method for this extraction purpose. Although the distillation was accomplished in a shorter time, an oil yield through this process is slightly higher compared to the conventional extraction method. This would go well to supply the ever increasing rate of demand for essential oil from lemongrass. This shorter period of consumption for extraction leads to lower power consumption and this reduces the operating cost as well. In addition, MAHD does not utilize any chemicals. Therefore, the essential oil extracted from this method is essentially pure and safe. These criteria are very important for essential oil such as lemongrass oil since they are mostly used for culinary and medicinal purposes.

There were a number of studies published comparing MAHD and HD in various plant materials to prove the advantages of MAHD over HD. However, most of those studies focused the comparison based on their yield and constituents. This recent study also focuses on the biological activity of the oil extracted through these methods through the conducting of cytotoxicity studies. In addition, RSM was conducted in order to determine the optimum parameter condition of the MAHD that affects the production of essential oil. The temperature analysis was carried out to qualitative understand the mechanism of MAHD system in extraction of natural products. Finally, a brief evaluation on cost, energy and environmental impact between MAHD and HD were performed to understand the real beneficial of MAHD in industrial level.

1.4 OBJECTIVES

There are three objectives of this research. These are:

1. To investigate the potential of MAHD for the extraction of essential oils from Lemongrass (*Cymbopogon citratus*),

2. To compare chemical composition of the extracts with those of HD

3. To optimise the extraction of essential oils by applying Response Surface Methodology (RSM), and

4. To investigate the components and biological activity of the essential oil from Lemongrass (*Cymbopogon citratus*) obtained by MAHD, as compared with HD.

1.5 SCOPES OF STUDY

There are some important tasks to be carried out in order to achieve the objectives of this study. Seven important scopes have been identified for this research in achieving the objectives:

1. To analyse the effects of MAHD parameters in relation to the condition on lemongrass oil yield namely extraction time, water-to-raw material ratio, and microwave power level,

2. To compare the lemongrass oil yield between conventional HD and MAHD methods based on the parameters' effects in extraction of Lemongrass (*Cymbopogon citratus)* oil,

3. To analyse the chemical composition of lemongrass oil by using Gas Chromotography-Mass Spectrometry (GC-MS),

4. To optimise the extraction parameters based on lemongrass oil yield by applying Central Composite Design (CCD) of RSM using Design Expert computer software,

5. To understand the cytotoxicity properties of Lemongrass (*Cymbopogon citratus*) oil extracted under optimised conditions from both conventional HD and MAHD methods which was evaluated by means of lethality test using brine shrimp,

6. To determine the effect of dielectric properties on MAHD extraction system by analyze the temperature profile, and

7. To study the effect of optimum operational condition on the operation cost, energy applied and environmental impact based on the yield of essential oil obtained from Lemongrass (Cymbopogon citratus).

1.6 Layouts of the Thesis

Chapter 1: Discuss the research background that covers the reasons that leads for current study being performed. Supported by the present industrial needs and the improvement that is required for enhance the essential oil production to fulfill its demand. The scope of study that covers the objectives of this research is indicated to support the requirement of each experimental process.

Chapter 2: The aim of this chapter is to review and analyze previous researches in relation to current studies which focused on their methodologies, findings and recommendations, so that appropriate improvement can be adapted and applied for advance studies. The main topics to be discussed in this chapter are essential oil, Lemongrass (Cymbopogon citratus), extraction methods, GC-MS, biological study and design of experiment.

Chapter 3: The aim of this chapter is to present the materials and methods involved in this study. It revolves around three major parts; materials and equipment used in the experiment, experimental methods and procedures, and finally, analysis and interpretation of data. These discussions may provide a detailed picture of the experimental flow used to achieve the objectives of this study.

Chapter 4: This chapter covers the results, illustrated in the form of graphs and tabulated data, as obtained using the method discussed in Chapter 3. In addition, the results of HD were compared to those of MAHD, focusing on factors that could influence the efficiency of both methods in extracting essential oil from Lemongrass (Cymbopogon citratus). Previous research studies in this field were also discussed in this chapter to compare their outcomes with the findings of the present study

Chapter 5: In this chapter, the outcomes of this research work were briefly explained. The significance of current study is analyzed according to the results obtained so that the objectives are achieved.

CHAPTER 2

LITRATURE REVIEW

2.1 INTRODUCTION

The aim of this chapter is to review and analyze previous researches in relation to current studies which focused on their methodologies, findings and recommendations, so that appropriate improvement can be adapted and applied for advance studies. The main topics to be discussed in this chapter are essential oil, Lemongrass (*Cymbopogon citratus*), extraction methods, GC-MS, biological study and design of experiment.

2.2 ESSENTIAL OIL

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the "oil of" the plant from which they were extracted, such as oil of clove. An oil is "essential" in the sense that it contains the characteristic fragrance of the plant that it is taken from.

2.2.1 Background

Essential oils were mankind's first medicine which has a long history. It is being used by the ancient civilizations of Egypt, Greece, India, and Rome since more than 5,000 years ago. The ancient civilizations of Mesopotamia utilized machines for obtaining essential oils from plants. According to Husnu and Gerhard, (2010) the first systematic studies of constituents from essential oil may be attributed to French chemist named M. J. Dumas (1800-1884) who carried out his research in hydrocarbon and oxygen as well as sulfur and nitrogen containing constituents and published them in 1833. The most important identifications have been carried out by O. Wallach who is an assistant of Kekule. Wallach found that some terpenes known under different names upon their botanical sources were often chemically identical. As the result, he decided to isolate the individual oil constituents and classify their basic properties. From 1884-1914, he wrote about 180 articles that are abridged in his book titled *Terpene Campher* (Wallach, 1914). This book consist of all the information on terpenes during that period of time and in 1887 he suggested that the terpenes should be constructed from isoprene units. To acknowledge this valuable finding, in 1910, Wallach was honored with the Noble Prize for Chemistry "in recognition of his outstanding research in organic chemistry and especially in the field of alicyclic compounds". The advancement in analytical methods in the course of the half century has brought exponential growth in this field which contributes knowledge on other constituents of essential oil.

Essential oils, also known as volatile oil and ethereal oil, are natural products consisting mixtures of volatile aroma compounds that can be obtained from a whole plant or plant part of known taxonomic origin. It is in form of concentrated, hydrophobic, typically lipophilic liquid having a distinctive scent, flavor, or essence of the plant. Essential oil may have two major components which are terpene hydrocarbon and oxygenated compounds. Terpene hydrocarbon can be divided into two group; monoterpenes and sesquiterpenes. While oxygenated compounds are phenols, monoterpenes, and sesquiterpenes alcohols, aldehydes, ketons, esters, lactones, coumarins, ethers, and oxides. Monoterpenes compounds are found in nearly all essential oil and have a structure of 10 carbon atoms and at least one double bond. The 10 carbon atoms are derived from two atoms isoprene units. Monoterpenes react readily to air and heat sources. These components have antiinflammatory, antiseptic, antiviral, and antibacterial therapeutic properties. Sesquiterpenes consist of 15 carbon atoms and have complex pharmacologicalactions. It has anti-inflammatory and anti-allergy properties. The biological activity of monoterpenes and sesquiterpenes has been the subject of a number of research studies (Domenico et al., 2005; Tamires et al., 2012; Rita et al., 2013; Anna, 1986; Zhang et al., 2013; Veeramuthu., 2012).