THE NOVEL GREEN EXTRACTION OF ESSENTIAL OIL FOR QUALITY PHARMACEUTICAL PRODUCTS

by

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LIST OF ABBREVIATIONS

SFVE	Solvent free vacuum extraction
HD	Hydro distillation
SFE	Supercritical fluid extraction
EO	Essential oil
SEM	Scanning electron microscope
GC	Gas chromatography
GCMS/MS	Gas chromatography mass spectrometry / mass spectrometry
FID	Flame ionization detector
MS	Mass spectrometer
DPPH	2, 2-diphenyl-1-picryhydrazyl
UV-VIS	Ultra violet visible spectrophotometer
AVG	Average
L	Latent heat
P	Power
W	Watts

Pengekstrakan Hijau Novel Minyak Pati Bagi Produk Farmaseutikal Berkualiti

ABSTRAK

Minyak pati ialah metabolit sekunder yang dihasilkan oleh tumbuh-tumbuhan aromatik yang mempunyai pelbagai aplikasi dalam industri. Kaedah konvensional untuk mengekstrak minyak pati daripada tumbuh-tumbuhan adalah seperti penyulingan wap, penyulingan hidro dan pengekstrakan pelarut yang mana mempunyai banyak kelemahan yang membawa kepada penyelidikan mengenai teknik baru untuk mengatasi kelemahan ini. Kajian ini dijalankan untuk memperkenalkan satu kaedah baru untuk mengekstrak minyak pati di mana dalam kaedah baru ini, proses pengekstrakan telah dilakukan dalam sistem vakum tertutup tanpa penambahan sebarang pelarut ataupun air. Kaedah baru ini adalah proses pengekstrakan dalam sistem tertutup di mana semua sebatian minyak pati akan terperangkap di dalam sistem untuk mengelakkan kehilangan komponen berharga. Kajian pengekstrakkan untuk kaedah bebas pelarut ini telah dijalankan untuk kedua-dua skala makmal dan skala perintis. Untuk skala makmal, proses pengekstrakan menggunakan kaedah bebas pelarut dan kaedah penyulingan hidro telah dijalankan untuk perbandingan. Kulit dari *Citrus sinensis* dan daun dari *Chromolaena odorata*, *Cymbopogon nardus* dan *Baeckea frutescens* telah diekstrak untuk minyak pati mereka. Hasil ekstrak dan ciri-ciri fizikal minyak pati yang diperolehi dari kedua-dua kaedah telah dinilai. Hasil minyak pati yang diperolehi untuk kesemua empat tumbuhan yang digunakan adalah lebih tinggi dengan menggunakan kaedah bebas pelarut berbanding dengan kaedah penyulingan hidro. Kaedah pelarut bebas telah meningkatkan hasil ekstrak sebanyak 100% untuk *Chromolaena odorata*, 76% untuk *Cymbopogon nardus*, 92% untuk *Baeckea frutescens* dan 174% untuk *Citrus sinensis*. Minyak pati daripada *Baeckea frutescens* telah dipilih untuk analisis selanjutnya. Gambaran morfologi kelenjar minyak pati *Baeckea frutescens* sebelum dan selepas pengekstrakan menggunakan kedua-dua kaedah tersebut ditentukan dengan menggunakan Mikroskop Pengimbas Elektron (SEM). Minyak pati kemudiannya dianalisis dengan menggunakan Kromatografi Gas (GC) dan Kromatografi Gas - Spektrometri Massa / Spektrometri Massa (GC-MS / MS) untuk sebatian kimia. Seperti yang dijangkakan daripada pecahan sebatian, kaedah bebas pelarut mempunyai lebih banyak sebatian berbanding kaedah penyulingan hidro. Satu sebatian baru iaitu 3-carene telah diperoleh dengan menggunakan kaedah bebas pelarut. Kewujudan 3-carene dalam minyak pati ini tidak pernah dilaporkan sebelum ini. Sebatian yang diperoleh daripada kaedah bebas pelarut didapati mengandungi jisim molekul yang lebih ringan berbanding dengan sebatian daripada kaedah penyulingan hidro yang mempunyai pecahan jisim molekul yang lebih berat. Ini adalah selaras dengan ramalan bahawa kaedah baru ini dapat mengekalkan sebatian bertakat didih lebih rendah yang juga mempunyai jisim molekul yang lebih ringan. Dengan hasil meyakinkan dari skala makmal, skala perintis untuk kaedah bebas pelarut telah direka dan dibangunkan. Skala perintis pengekstrak minyak pati mempunyai kapasiti maksima 30 liter untuk bahan tumbuhan. Oleh kerana kaedah pengekstrakan baru ini lebih mudah, kurang langkah pemprosesan, sifar sisa pelarut dan kurang penggunaan masa, maka, kaedah ini sesuai untuk aplikasi perindustrian seperti pengeluaran produk farmaseutikal sebagai komponen aktif dalam ubat-ubatan atau sebagai bahan-bahan wangian dalam aromaterapi dan minyak wangi.

The Novel Green Extraction of Essential Oil for Quality Pharmaceutical Products

ABSTRACT

Essential oils are secondary metabolites produced by aromatic plants which have various applications in the industries. The conventional method to extract essential oil from plants such as steam distillation, hydro distillation and solvent extraction have many drawbacks that lead to the research on the new technique to overcome the flaws. This research was conducted to introduce a new method to extract essential oils where in this new method, the extraction process has been done in a closed vacuum system without the addition of any solvents or water. The novelty of this method is the closed system extraction process where all the compounds of the essential oil were trapped inside the system to prevent the loss of valuable components. The extraction studies for this solvent free method was conducted for both laboratory scale and pilot scale. For the laboratory scale, extraction process using solvent free method and hydro distillation method was conducted for comparison. The peels from *Citrus sinensis* and the leaves from *Chromolaena odorata, Cymbopogon nardus* and *Baeckea frutescens* have been extracted for their essential oils. The essential oils collected from both methods were measured for their yield and physical characterizations. From the results obtained, it showed that the essential oil yield for all the four plants used was significantly higher by using solvent free method compared to hydro distillation method. The yield for solvent free method has been increased by 100% for *Chromolaena odorata*, 76% for *Cymbopogon nardus,* 92% for *Baeckea frutescens* and 174% for *Citrus sinensis*. *Baeckea frutescens* essential oil was selected for further analysis. The morphology image of *Baeckea frutescens* essential oil glands before and after extraction using both methods were determined using Scanning Electron Microscope (SEM). The essential oil was then being analyzed using Gas Chromatography (GC) and Gas Chromatography - Mass Spectrometry/Mass Spectrometry (GC-MS/MS) for their chemical constituents. As expected from the compound elucidations, solvent free method has more compounds compared to hydro distillation method. One new compound which is 3-carene has been obtained by using solvent free method. The existence of 3-carene in this essential oil was not been reported before. It was also discovered that the compounds obtained from solvent free method contained lighter molecular mass compared to the compounds from hydro distillation method which have mostly heavier molecular mass fractions. This is in alignment with the prediction that the new method can preserve lower boiling point compounds which would have lighter molecular mass. With the convincing results from the laboratory scale, the pilot scale for the solvent free method has been designed and developed. The pilot scale essential oil extractor has a maximum of 30-liter capacity for the plant material. As the new method of extraction were more convenient, less processing steps, zero solvent waste and less time consumptions, hence, it is suitable for industrial application such production of pharmaceutical products as active components in medicines or as fragrance ingredients in aromatherapy and perfumery.

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Plants produce a variety of compounds that can be divided into primary metabolites and secondary metabolites. Primary metabolites are essential for the survival of the plant which includes sugars, proteins, and amino acids. Secondary metabolites are not essential to plants survival but play significant roles in allowing the plant to adapt to its environment (Hunter, 2009). Essential oils are secondary metabolites produced by aromatic plants. Plants produce essential oils that give plants their distinctive smells, protect the plants and play a role in plants pollination (Charles, 2011). Essential oils are naturally occurring volatile aromatic compounds that are found in the seeds, bark, stems, roots, flowers, and other parts of plants, where they can be both beautifully and powerfully fragrant (Hunter, 2009). All plants that contain essential oil have the aromatic ring in their compounds structure where these aromatic compounds are highly volatile and easily sublime at room temperature. They are called volatile because they change state quickly. The physical and chemical properties of the volatile aromatic compounds that compose essential oils allow them to quickly diffuse through the air and directly interact with the sense in the nose.

Over 3,000 varieties of volatile aromatic compounds have been identified to date. The type of volatile aromatic compounds present in an essential oil determines both the oils aroma and the benefits it offers (Hunter, 2009). Essential oils have an extremely captivating characteristic which offers different qualities and different biological properties. The expression biological includes all activity that these blends of volatile mixes apply to people, animal, and plants. The nature of an essential oil varies from plant to plant, within botanical families, and from species to species (Baser & Buchbauer, 2010). The delicate ratio of aromatic constituents found in any given essential oil are what make it unique and give it specific benefits. Essential oils can be used in a wide range of physical wellness applications. They can be used as single essential oils or in complex essential oil blends depending on user experience and desired benefit (Hunter, 2009).

Essential oils have many applications in the industry. Such applications may be found in the cosmetic industry as ingredients in fragrances and cosmetics, in the food industry as aromas and flavourings, in the pharmaceutical industry as active components of medicines and as antibacterials/antimicrobials, and even in aromatherapy (Chamorro et al., 2012). Nowadays essential oils are especially used in pharmaceutical products. Pharmaceutical products are a primary component of both modern and traditional medicine. It is crucial that such products are safe, effective, good quality, and are prescribed and used rationally.

The composition of the compounds in essential oils can vary depending on the time of the day, season, geographic location, method and duration of distillation, year grown, and the weather, making every step of the production process a critical determinant of the overall quality (Hunter, 2009). Common methods used to extract essential oils include steam distillation, hydro distillation, solvent extraction, and cold pressing. Every method has their advantages and disadvantages and the extraction yield differs from method to method. The characterization step is important because even essential oils originating from the same botanical species may have differing chemical compositions. These variations might be due to the presence of different chemotypes that depend on the plants adaptation to the surrounding environment, as well as its state of development. It is the composition of the essential oils that result in their particular properties and grade value (Chamarro et al., 2012).

In practice, hydro distillation and solvent extraction are two of the most widely used methods to extract essential oils. Hydro distillation can be achieved by one of the two methods; Clevenger distillation where the plant material is boiled in water, or steam distillation where hot steam is passed through a bed of the plant material. The hot vapor containing the essential oil and water is then passed through a condenser to condense the vapor back into a liquid. Most essential oils do not mix with water well in the liquid phase so they can simply be separated by simple decantation. Care must be taken not to heat the samples for too long because it can lead to the hydrolysis of esters, polymerization of aldehydes, or decomposition of other components (Charles, 2011). The typical extraction period is from 15 to 30 minutes. The extraction period also influences not only the yield but also the extract composition.

Solvent extraction can be used to extract essential oils from plants that cannot withstand the high heat used in hydro distillation. Solvent extraction method utilizes organic solvents such as methanol, ethanol, acetone, or hexane, depending on the polarity of the compounds to be extracted. During solvent extraction, the plant material is put into a bath of solvents which extracts the essential oil from the plant material. This mixture is then filtered to remove the unwanted plant material and then the filtrate is distilled under reduced pressure to remove the organic solvent. Alcohol is then used to re-dissolve the essential oils. Oils obtained through this method are not suitable for use in cosmetics or food industries as trace amount of solvent may still remain. This residue may lead to skin irritations or other side effects. Although this method is fast and inexpensive, since it produces a non-pure oil, it is mostly used in oils that are put into perfumes and not on the skin.

Up to now, several new extraction processes have been reported for the extraction of essential oil. The aim behind the development of these new extraction technologies was the possible improvement of its yield and chemical constituents. Driven by the demand and interest on enhancing the quality and quantity of essential oil, the current technology of extracting essential oil will continue to improve and upgrade to extract the valuable essential oil. In the last few years, there has been an increasing demand for new green process technologies, starting from the idea that pollution and hazards have to be eliminated at the source, thus reducing environmental impact and costs. As a consequence, an increased interest exists for improvement, design, and development of new green processes for the extraction of essential oil.

Green chemistry is a general term that covers the invention, design, and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances. Whereas green extraction of the natural product defines as the design of extraction processes which will reduce energy consumption, allows the use of alternative solvents and renewable natural products, and ensure a safe and high-quality extract (Chemat et al., 2012). Currently, there has been growing interest in this green and sustainable extraction methods of natural products in the multidisciplinary area of applied chemistry, biology and technology. In relation to the principal of green extraction process, an innovative technology of using closed vacuum system for essential oil extraction was invented and tested to discover its potential. This extraction method did not use water or any chemical solvents and was done under a reduced pressure environment. It works on the principle that boiling point of a substance is lowered when the pressure is reduced. This technique applies to any plant that has volatile compounds. The extraction process was done in a vacuum closed system. The important key is the closed system where all the compounds from all range of boiling points were trapped inside the system, thus preventing the possibility of losing the valuable compounds. Vacuum condition is needed to extract and release the oil from the plant's oil cells by means of evaporation. The pressure in the system is reduced, making it possible to extract essential oil at a lower temperature. The essential oil produced from this technique will be purer as no solvent used for extraction as well as increasing the yield of extraction as the effect of closed system used. This technique was invented based on the facts that chemical compounds in essential oils have a wide range of boiling point range from low to high, hence by fractionating at high temperature might cause degradation of low boiling point compounds of the essential oil. Therefore, by using this technique, extraction of essential oil can be done at a lower temperature to preserve and recover the compounds from all range of boiling point.

1.2 Problem Statement

Essential oils are used in a wide variety of consumer goods such as detergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectioneries, soft drinks, distilled alcoholic beverages, and even insecticides. The world production and consumption of essential oils and perfumes therefore has a significant market. Production technology is an essential element to improve the overall yield and quality of essential oil. Essential oils are obtained from plant raw material by several extraction methods (Wang & Weller, 2006). In the process of modernization of traditional herbal medicine, the extraction and characterization of essential oil is an important task as many botanical drugs have the bioactive component presence in their essential oils (Suwaibah et al., 2012).

In ancient time, essential oil has been obtained from plants by the distillation technique. Currently, the conventional methods for extraction of essential oil that are widely used by the industry are the steam distillation, hydro distillation and solvent extraction. One of the disadvantages of conventional techniques is related to the thermolability of essential oils components which undergo chemical alterations such as hydrolyze, isomerization, and oxidation due to the high applied temperatures (Chamarro et al., 2012). The quality of extracted essential oils will therefore be reduced especially if the extraction time is long. The disadvantages related to these techniques have led to the searching for new alternative extraction processes. It is important that extraction methods could maintain essential oils chemical composition and natural proportion in its original state. Since economy, competitiveness, eco-friendly, sustainability, high efficiency and good quality become keywords of the modern industrial production, the development of essential oils extraction techniques has never been interrupted.

Conventional techniques are now not the only way to extract the essential oils. Supercritical Fluid Extraction (SFE) is one of the modern techniques used for the extraction of essential oil. In contrast to the conventional method, the extraction by supercritical fluid extraction is better because the essential oil produce is high quality and solvent free (Rezzoug et al., 2005). SFE is the process of separating one component from another matrix using supercritical fluids as the extracting solvent (Rozzi et al., 2002). Extraction is usually from a solid matrix but can also be from liquids. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extraction and metal cation extraction (Pourmortazavi et al., 2007). This extraction method produces a higher yield, higher diffusion coefficient, and lower viscosity. Many essential oils that cannot be extracted by hydro distillation can be obtainable with carbon dioxide extraction. Nevertheless, this technique is not cost effective due to the price of this equipment is very expensive and it is not easily handled (Chanthai et al., 2012).

Novel techniques acknowledge by green extraction concept and principles, have constantly emerged in recent years for obtaining natural extracts with a similar or better quality to that of conventional methods. New extraction techniques must also reduce extraction times, energy consumption, solvent use and CO_2 emissions. Hence, this study is being conducted with the aim to design and develop a new and green technique for the extraction of essential oil from various plant materials without any addition of any chemical solvents or water. The solvent free vacuum extraction process is done under reduced pressure, lowering the boiling points of the compounds. The goal is to heat and separate the components of essential oils at temperatures below their decomposition point while also preserved all valuable compounds in a closed system extraction process.

1.3 Objective

The main objective of this research is to introduce a new novel method to extract essential oil from various plant materials. This new solvent free method, which does not use any chemical solvents or water, should be able to produce a higher yield with a better quality of essential oil. This study also embarks on the following specific objectives:

1) To extract and compare essential oils extracted from both solvent free method and hydro distillation method in terms of yield, time of extraction and temperature for extraction.

2) To analyze the essential oil produced from solvent free method and hydro distillation method using Gas Chromatography (GC) and to elucidate the chemical constituents of the essential oil using Gas Chromatography-Mass Spectrometry-Mass Spectrometry (GC-MS/MS).

3) To establish a correlation between bioactivity of the extract through antioxidant activity test for essential oil extracted using solvent free method and hydro distillation method.

4) To propose a suitable design for the pilot scale reactor of solvent free method for possible commercialization and industrial applications.

1.4 Scope of Study

This study will focus on the introduction of a new method to extract essential oil. Various plant materials were used to test the effectiveness of the new solvent free method and only significant findings would be reported. The equipment used for this method was custom made by the glassblower using borosilicate glass material that can withstand high vacuum pressure and high temperature. A comparative study has been done for both solvent free method and hydro distillation method using several plant materials in order to evaluate and validate their efficiency. The results from this comparative study were collected and analyzed using Gas Chromatography (GC) and Gas Chromatography Mass Spectrometry/Mass Spectrometry (GC-MS/MS). The specific scope of this study aimed at comparing the yield, extraction time, physical appearance in terms of colour and odour of the essential oils, the chemical composition and **Error! Bookmark not defined.** activity of essential oil extracted by using hydro distillation and solvent free vacuum extraction method. Meanwhile, the design for pilot scale equipment was made to develop the prototype and compare the yield produced from the pilot scale prototype to the laboratory scale equipment.

CHAPTER 2

LITERATURE REVIEW

2.1 Essential Oil

Essential oils are plant-based volatile oils with strong aromatic components that are made up of different chemical compounds. For example, alcohols, hydrocarbons, phenols, aldehydes, esters, and ketones are some of the major components of essential oil (Katiyar et al., 2010). An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from the plant. They are also known as aromatic oils, fragrant oils, steam volatile oils, ethereal oils, or simply as the oil of the plant material from which they were extracted, such as oil of clove. Essential oil compositions are commonly a mixture of terpenes, mainly monoterpenes and sesquiterpenes, aliphatic compound and aromatic compound (Basappa et al., 2015). In general, the effective components of essential oils can be classified into two groups. The volatile fraction of the oil is the first group which mainly composed of monoterpenes, sesquiterpenes, aliphatic aldehydes, alcohols, and esters. Meanwhile, the nonvolatile fraction is the second group that contains hydrocarbons, sterols, fatty acids, carotenoids, coumarins, waxes, and flavonoids (Mehdi & Hadi, 2011). Therefore, the main fraction of the essential oil is the volatile compounds that composed the majority of the oil.

The advantages of essential oils are their flavor concentrations and their similarity to their corresponding sources. The majority of them are fairly stable and contain natural antioxidants and natural antimicrobial agent as on citrus fruits (Basappa et al., 2015). Essential oils are usually colorless, particularly when fresh. Nevertheless, with age, essential oil may oxidize which resulting the color becomes darker. Therefore, essential oil needs to be stored in a cool, dry place tightly stoppered and preferably full in amber glass containers.

The nature of an essential oil varies from plant to plant, within botanical families, and from species to species. The delicate ratio of aromatic constituents found in any given essential oil are what make it unique and give it specific benefits. Even with pure essential oils the composition of the oil can vary depending on the time of day, season, geographic location, method and duration of distillation, year grown, and the weather, making every step of the production process a critical determinant of the overall quality of the essential oil product. Essential oils can be used for a wide range of emotional and physical wellness applications. They can be used as single essential oils or in complex essential oil blends depending on user experience and desired benefit (Hunter, 2009).

There are about 10 % of the essential oil that is commercially important out of approximately 3000 essential oils that have been identified up to now. These valuable natural products are used in various industries such as cosmetics, perfumes, foods, pharmaceuticals and many more. The research on the essential oils and their biological activities are still growing which involves researchers around the world. This is due to the facts that the composition of essential oils may vary considerably between plant species and varieties. Such varieties include seasonal and geographic conditions, climates, and harvest periods. In addition, the composition of essential oils from different parts of the same plant can also vary widely (Hunter, 2009).

Essential oils are generally a pale to clear or slightly yellowish liquids, mostly insoluble in water, with specific gravities between 0.80 to 1.20. Some essential oil