EXTRACTION OF ESSENTIAL OIL FROM MURRAYA KOENIGII LEAVES USING ULTRASONIC-ASSISTED SOLVENT EXTRACTION METHOD

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ABSTRACT

The objective of this research is to extract essential oils from *M. koenigii* leaves by using ultrasonic-assisted solvent extraction method. The major constituent of *M. koenigii* has been reported as caryophyllene and 3-carene which is responsible for the aroma and flavor. This research has focused on the influence of ultrasonic, various natures of solvents, sonication times and also drying method towards the extraction of *M.koenigii* essential oil. Two types of solvents are used in this research which is ethanol and hexane. In this research, the methods of drying, grinding, extraction, separation and analysis are used and the sample is separated from solvents by using a rotary evaporator to get the essential oil. The sample was analyzed by using a GC-MS to identify the component of *M. koenigii* essential oil. In this research, the most suitable solvent to produce higher percentage yield is by using ethanol (ultrasonic-assisted solvent extraction of fresh leaves for 30 minutes) and the percentage of oil yield also increased with increasing the time. The major component in *M. koenigii* leaves is caryophyllene and hexane on the other hand is the best solvent to be used to extract caryophyllene

ABSTRAK

Objektif penyelidikan ini adalah bagi mengekstrak minyak pati *M. koenigii* dengan menggunakan ultrasonik bagi membantu pengekstrakan dengan pelarut. Konstituen mejar *M koenigii* telah dilaporkan sebagai kariofilena dan 3-carene yang adalah bertanggungjawab untuk bau dan rasa minyak pati *M. koenigii*. Penyelidikan ini telah tertumpu pada pengaruh ultrasonik, sifat semulajadi pelbagai pelarut, masa pensonikan, dan juga kaedah pengeringan terhadap pengekstrakan minyak pati *M.koenigii*. Dua jenis pelarut adalah digunakan dalam penyelidikan ini yang merupakan etanol dan heksana. Dalam penyelidikan ini, kaedah-kaedah bagi pengeringan, pengisaran, pengekstrakan, pemisahan dan analisis adalah digunakan dan sampel dipisahkan dari pelarut dengan menggunakan satu penyejat berputar untuk mendapat minyak pati. Sampel itu telah dianalisis dengan menggunakan GC-MS untuk mengenal pasti komponen minyak pati *M.koenigii*. Dalam penyelidikan ini, pelarut paling sesuai untuk menghasilkan peratusan lebih tinggi hasil adalah dengan menggunakan etanol (ultrasonik dibantu pengekstrakan pelarut daun segar selama 30 minit) dan peratusan hasil minyak juga bertambah dengan bertambahnya masa. Komponen utama dalam daun *M. koenigii* merupakan kariofilena dan heksana sebaliknya adalah pelarut yang terbaik untuk mengekstrak kariofilena

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LIST OF ABBREVIATIONS

GC	-	Gas Chromatograph
MS	-	Mass Spectrometer
(L.)	-	Linn, Carl Linnaeus
kHz	-	Kilo Hertz
GHz	-	Giga Hertz
FKKSA	-	Fakulti Kejuruteraan Kimia & Sumber Asli
W	-	Watt
mN	-	Millinewton
cm	-	Centimeter
mmHg	-	Mlillimeter of Mercury
cP	-	Centipoise

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

M. koenigii (L.) Spreng (family: Rutaceae) or its common name curry leaf tree is a small strong smelling perennial shrub commonly found in forests as undergrowth, cultivated in India for its aromatic leaves and for ornament. Essential oil composition of the leaves has been studied by various workers. The major constituent responsible for the aroma and flavor has been reported as pinene, sabinene, caryophyllene, cadinol and cadinene [1-5]. Essential oils from *M. koenigii* serves as an important part in soap making ingredients, lotions, massage oils, diffusers, potpourri, scent, air fresheners, body fragrance, perfume oils, aromatherapy products, bath oils, towel scenting, spa's, incense, facial steams, hair treatments, and more [6]. There are several methods to extract essential oil from herb and spices like steam distillation, hydrodistillation, and solvent extraction but this study focus on a new, applicable method of essential oil extraction that is ultrasonic-assisted solvent extraction method. This extraction method is a combination of solvent extraction and ultrasonic extraction method. The steps required for the preparation of the material prior to extraction (including aspects concerning plant selection, collection, identification, drying, grinding and weighing) and analyzing method for the essential oil composition are detailed.

1.2 Problem Statement

The increasing importance of essential oils as pharmaceutical and aromatherapy assist besides their traditional role in cosmetics not only as a potent ingredient but also as a fragrance donor has opened up wide opportunities for global marketing. Essential oils, which are natural volatile extracts of plant material (flowers, leaves, stems, or roots, seeds, or seed peel, depending on the plant) generally distilled by steam, hold high export potential in future. Thus it is crucial that the correct or suitable extraction technique applied to preserve quality of the essential oil extracted.

Common extraction method like steam distillation has the advantage of no solvent residues but it can be a destructive process since heat is used and the oil does go through some chemical change [7]. Solvent extraction is the safest method for extracting high quality oil because some herbs and spices cannot be extracted from distillation method but it also has the disadvantage of having low yield and have remaining of solvents in the essential oils.

This study is conducted in order to introduce ultrasonic extraction as one of applicable method to extract essential oil. Ultrasonic-assisted solvent extraction is a combination of solvent and ultrasonic extraction can be considered an alternative to conventional extraction techniques. This combination can provide more yield compared if we just use only solvent extraction. Even though the effect of ultrasound have been studied in over hundreds of herbal and spice species, to the author’s knowledge, its effect to *M. koenigii* plant, has not been investigate yet. Further focus is given on the factors that can influence the selection of the method and suitable solvent for extraction.

1.3 Objective

The objective of this research is to extract essential oils from *M. koenigii* leaves by using ultrasonic-assisted solvent extraction method.

1.4 Scope of Research Work

This research focus on four main scopes:

(i) Investigate the effect of solvent nature on extraction in terms of percentage yield and extraction of major components in *M. koenigii* leaves.

(ii) Investigate the effect of ultrasonic-assisted solvent extraction.

(iii) Investigate the effect of sonication time on extraction.

(iv) Analyze the product composition from the extraction process to obtain the major component.

CHAPTER 2

LITERATURE REVIEW

2.1 Extraction/leaching – general principles

Figure 2.1 below shows the analytical procedure for extraction/leaching with various solvents in order to isolate the analytes from plant material, as a rule, in order of increasing polarity of the extracting agent [8-9]. By using of various solvents, extracts containing different analytes can be obtained (Extracts P, Q, R, and S). There are procedure should be carried out in several steps so that particular analytes are present in one extract only, while others are present in different extracts - A in Figure 2.1. Application of additional operations, for example extract purification, results in obtaining further fractions (Fractions I, II) - B in Fig. 1. Each of the fractions can then be chromatographically separated into individual components - C in Figure 1.

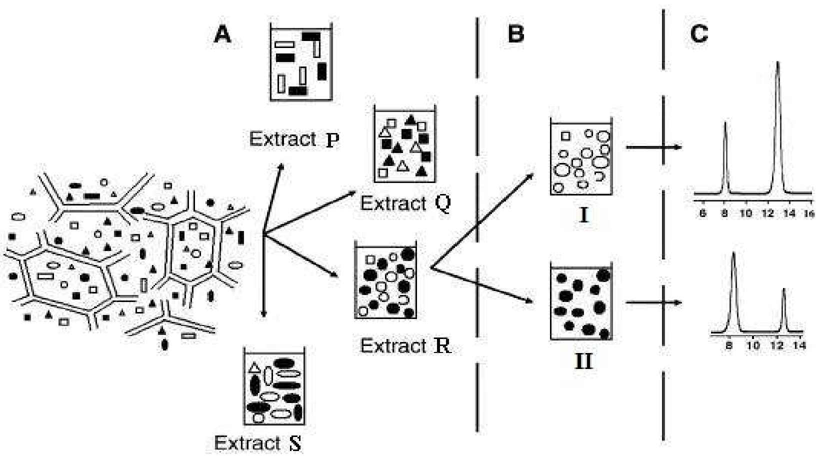

Figure 2.1: Separation steps used for isolation of plant metabolites [20].

2.2 Essential Oil

An essential oil is a liquid that is generally steam or hydro-distilled from flowers, leaves, bark and roots of plants and trees and are the compounds responsible for the aroma and flavor associated with herbs, spices, and perfumes. The formation and accumulation of essential oils in plants have been reviewed by Croteau (1986), Guenther (1972) and Runeckles and Mabry (1973) [10-11]. Chemically, the essential oils are primarily composed of mono- and sesquiterpenes and aromatic polypropanoids synthesized via the mevalonic acid pathway for terpenes and the shikimic acid pathway for aromatic polypropanoids.

The essential oils from aromatic plants are for the most part volatile and thus, lend themselves to several methods of extraction such as hydrodistillation, water and steam distillation, direct steam distillation, and solvent extraction [10, 12-14]. The specific extraction method employed is dependent upon the plant material to be distilled and the desired end-product. The essential oils which impart the distinctive aromas are complex mixtures of organic constituents, some of which being less stable, may undergo chemical alterations when subjected to high temperatures. In this case, organic solvent extraction is required to ensure no decomposition or changes have occurred which would alter the aroma and fragrance of the end-product. Mostly, essential oils are clear, however there are some exceptions. For example the essential oil of *M. koenigii* leaves having dark yellow color.

2.3 *Murraya Koenigii* (L.) Spreng

M. koenigii (L.) Spreng (Rutaceae) is one of the two species of Murraya found in Peninsular Malaysia. The plant usually cultivated for its aromatic leaves is normally used for natural flavoring in curries and sauces [15]. Originated in Tarai regions of Uttar Pradesh, India, it is now widely found in all parts of India and it adorns every house yard of Southern India and is also cultivated in Sri Lanka, China, Australia and the Pacific islands [16]. This plant is also distributed in Andaman

Islands and throughout Central and Southeast Asia [17]. The plant was spread to Malaysia, South Africa and Reunion Island by South Asian immigrants. Parts of the plant have been used as raw material for traditional medicine formulation in India [18]. The plant is used in Indian system of medicine to treat various ailments [19-21]. *M. koenigii* leaves and roots can be used to cure piles and allay heat of the body, thirst, inflammation and itching. The aromatic leaves, which retains their flavour and other qualities even after drying, are slightly bitter, acrid, cooling, weakly acidic in tastes and are considered as tonic, anthelminthic, analgesic, digestive, appetizing and are widely used in Indian cookery for flavouring food stuffs. Below are the figures of *M. koenigii* tree plant (Figure2. 2) and leaves (Figure 2.3):

Figure 2.2: *M. koenigii* tree plant.

Figure2. 3: *M. koenigii* leaves.

2.3.1 Chemical Structure of *M. koenigii* leaves

The leaves are reported to have rich source of carbohydrates, proteins, amino acids and alkaloids, and are rich in minerals, vitamins A and B [22-23]. They also a rich source of calcium, but due to the presence of oxalic acid in high concentration (total oxalates, 1.35%; soluble oxalates, 1.15%), its nutritional availability is affected. The leaves also contain a crystalline glucocide, koenigiin and a resin. By analysis of concentrated essence of *M. koenigii* leaf, Macleod and Pieris [4] obtained mainly terpenes. According to them the most important constituent of *M. koenigii* are β-caryophyllene, β-gurjunene, β-elemene, β-phellendrene and β-thujene.

The composition of the essential oil of *M. koenigii* may differ at different places. Earlier investigations on Indian curry leaf oil, hydrodistilled from fresh leaves, led to the identification of α-pinene, β-pinene, β-caryophyllene, isosafrole, lauric and palmitic acids [24]. Later, Sri Lankan oil was reported to contain monoterpenes (15.9%) and sesquiterpenes (80.2%) with β-phellandrene, β-caryophyllene, β-gurjunene, β-elemene, and α-selinene as the main constituents. However, Chinese oil was reported to contain α- and β-pinenes, β-caryophyllene and γ-elemene as main constituents, whereas curry leaf oil from Malaysia was shown to be rich in monoterpenes and oxygenated monoterpenes (ca. 85%) with α-pinene, limonene, β-phellandrene, terpinen-4-ol and β-caryophyllene as the main contents [25]. Chowdhury [26] reported that leaves on hydrodistillation gave 0.5% essential oil on fresh weight basis, having dark yellow color, spicy odor and pungent clove-like taste. It has following characteristics:

• Specific gravity (25^{o}C)	0.9748
• Refractive index (25^{o}C)	1.5021
• Optical rotation (25^{o}C)	+ 4.8 [6]
• Saponification value	5.2
• Saponification value after after acetylation	54.6
• Moisture	66.3%
• Protein	6.1%
• Fat (ether extract)	1.0%
• Carbohydrate	18.7%
• Fibre	6.4%
• Mineral matter	4.2%
• Calcium	810 mg/100 g of edible portion
• Phosphorus	600 mg/100 g of edible portion
• Iron	3.1 mg/100 g of edible portion
• Carotene (as vitamin A)	12 600 IU/100 g
• Nicotinic acid	2.3 mg/100 g
• Vitamin C	4 mg/100 g
• Thiamine and riboflavin	absent

The essential oil of Indian curry leaf collected from two different places in India has been investigated for its composition. The oils from the two places were found to contain mostly monoterpenes and oxygenated monoterpenes. The main items identified are α-pinene (19.0-19.7%), sabinene (31.8-44.85), b-pinene (4.2-4.7%), a-terpinene (1.3-4.3%), β-phellandrene (6.5-7.9%), γ-terpinene (3.9-7.1%) and terpinen-4-ol (5.2-9.9%). Although many of these compounds have already been reported in Malaysian curry leaf oil, there are marked differences between the oils, which suggests that the curry leaf plant exists in different chemical varieties. More recently, Jasim et al. (2008) [27] reported that gas chromatography mass spectroscopy (GC-MS) analysis of the chemical composition of the leaf of *M. koenigii* from Bangladesh were found to contained 39 compounds of which the major is 3-carene (54.2%) followed by caryophyllene (9.5%). The results were completely differ from those reported by previous workers, Raina et al. (2002) [28] and Walde et al. (2005) [29], where pinene, caryophyllenes and phellandrenes were the predominant compounds. Below are the figure of 3-carene (Figure 2.4) and caryophyllene (Figure 2.5):

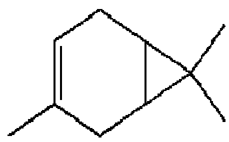

Figure 2.4: 3-carene.

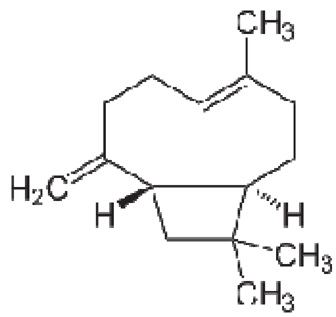

Figure 2.5: Caryophyllene.

2.3.2 Uses of *M. koenigii* leaves

M. koenigii leaves are used in traditional medicine, for example ayurvedic and unani medicine [30-32]. The plant is credited with tonic, stomachic and carminative properties. The green leaves are used to treat piles, inflammation, itching, fresh cuts, dysentery, vomiting, burses and dropsy. The green leaves are also eaten raw as a cure for diarrhea and dysentery; bruised and applied externally to cure eruptions; given as a decoction with bitters as a febrifuge; and in snake bite. Moreover its leaves have a potential role in the treatment of diabetes. Hypoglycemic action on carbohydrate metabolism was reported in rats fed with *M. koenigii* leaves [33].

M. koenigii leaf also found to exert antioxidant properties in rats fed a high fat diet [11]. There were lower levels of hydroperoxides, conjugatedienes and free fatty acids in the liver and heart of rats supplemented with *M. koenigii* leaves compared to rats fed on the high fat diet alone. Activities of superoxide dismutase, catalase, and glutathione transferase were increased in the heart and liver of rats supplemented with *M. koenigii* leaves. Activities of glutathione reductase, glutathione peroxidase and glucose-6-phosphate dehydrogenase were also increased in the liver and the concentration of gluthathione was decreased. Thus supplementing a high fat diet with 10% *M. koenigii* leaf can prevent the formation of free radicals and maintain the tissues at normal levels.

The undiluted essential oil exhibited strong antibacterial and antifungal activity when tested with microorganisms [34]. Even the crude leaf extracts of *M. koenigii* leaf plant are reported to possess antibacterial activity [35]. An essential oil, a glucoside and koeinigin are reported from the species. The essential oil is used in soaps and perfume industry.

2.4 Extraction Methods for *M. koenigii* leaves

Various methods have being employed these days for extracting essential oils from different spices and herbs. From the journal, hydrodistillation and solvent extraction are the common extraction methods being used to extract *M. koenigii* leaves.

2.4.1 Hydrodistillation

Hydrodistillation is one of the oldest methods of extraction used. The spice is fully immersed in hot water. The result is a soup, which carries aromatic molecules of the plant. The method is not much in use these days, because of the risk of overheating the plant and subsequent loss of the oil. The method is best suitable for spice in dry and powdered form of roots and barks.

2.4.2 Solvent Extraction

Many herbs and spices cannot be extracted from distillation method, so solvent extraction is the safest method for extracting high quality oil. In this process, the spices or herbs plants are immersed in the solvent and the 'separation' is performed chemically. These include pigments, volatile molecules and non-aromatic waxes. The herbs and spices are then subjected to low pressure distillation and the volatile oil is then separately collected. It should be noted that, even with the most advanced techniques, absolutes extracted in this manner do contain traces of solvent. In solvent extraction, solvent selection is important

2.4.2.1 Solvent

A solvent is a liquid or gas that dissolves a solid, liquid, or gaseous solute, resulting in a solution [36]. Solvents usually have a low boiling point and evaporate easily or can be removed by distillation, leaving the dissolved substance behind. Basically there are two categories of solvent that is organic and inorganic solvent. Organic solvents are commonly used in dry cleaning (e.g. tetrachloroethylene), as paint thinners (e.g. toluene, turpentine) and glue solvents (acetone, methyl acetate, ethyl acetate), in spot removers (e.g. hexane, petrol ether), in detergents (citrus terpenes), in perfumes (ethanol), and in chemical syntheses. The use of inorganic solvents (other than water) is typically limited to research chemistry and some technological processes. The selection of an appropriate solvent is guided by theory and experience. Generally a good solvent should meet the following criteria [37]:

a) It should be inert to the reaction conditions.
b) It should dissolve the reactants and reagents.
c) It should have an appropriate boiling point.
d) It should be easily removed at the end of the reaction.

As a rule of thumb we should know that non-polar reactants will dissolve in non-polar solvents whilst polar reactants will dissolve in polar solvents. There are three measures of the polarity of a solvent:

a) Dipole moment
b) Dielectric constant
c) Miscibility with water

Molecules with large dipole moments and high dielectric constants are considered polar. Those with low dipole moments and small dielectric constants are classified as non-polar. Generally solvents with a dielectric constant of less than 15 are considered non-polar [38]. On an operational basis, solvents that are miscible with water are polar, while those that are not are non-polar. Technically, the dielectric constant measures the solvent's ability to reduce the field strength of the electric field surrounding a charged particle immersed in it. This reduction is then compared to the field strength of the charged particle in a vacuum [39].

2.4.2.2 Solvents Classification

Solvents can be broadly classified into three categories according to their polarity namely polar protic, polar aprotic and non-polar.

Polar Protic Solvents

In this term, protic refers to a hydrogen atom attached to an electronegative atom (oxygen). This means, polar protic solvents are compounds that can be represented by the general formula ROH. The polarity of the polar protic solvents comes from the bond dipole of the O-H bond. The large difference in electronegativities of the oxygen and the hydrogen atom, combined with the small size of the hydrogen atom, warrant separating molecules that contain an OH group from those polar compounds that do not. Examples of polar protic solvents are water (HOH), methanol (CH_3OH), and acetic acid (CH_3CO_2H).