EXTRACTION OF PANDANUS AMARYLLIFOLIUS ESSENTIAL OIL FROM PANDAN LEAVES

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ABSTRACT

The extraction process is the key operation for obtaining oils. The oils are extracted by chemical method where strong acids are used. The process using solvent extraction has been used for extraction of pandan leaves. In this experiment, there are four types of solvent will be used which is ethyl acetate, hexane,chloroform, and, methanol. The pandan leaves was sliced into small size before added before being put in a beaker containing two solvents and then the solution will be unstable for 2 4 and 6 hours. The effectiveness of different ratio of solvent process will be studied.The composition of the essential oils extracted by solvent from leaves parts of pandan (Pandanus amaryllifolius) were analyzed by using gas chromatography (GC-MS). The major component in Pandanus amaryllifolius was 2-acetyl-1-pyrroline but in present study only the minor component was obtained which were 3-Hexanol and 3-Hexanone. Significantly the result shows that the solvent ratio chloroform:ethyl acetate get a large amount of oils .

ABSTRAK

Proses pengekstrakkan adalah kunci utama untuk mendapatkan minyak. Minyak yang dihasilkan dengan kaedah kimia di mana asid kuat yang digunakan. Proses ekstrakkan menggunakan pelarut telah digunakan untuk ekstrakkan daun pandan. Dalam kajian ini, ada empat jenis pelarut yang akan digunakan adalah etil asetat, heksana, kloroform, dan metanol. Daun pandan itu dihiris dalam saiz kecil sebelum ditambah sebelum dimasukkan ke dalam sebuah gelas kimia mengandungi dua pelarut dan kemudian penyelesaian akan menjadi tidak stabil selama 2, 4 dan 6 jam. Keberkesanan nisbah yang berbeza daripada proses pelarut akan komposisi di kaji. Dari minyak oleh pelarut dari bahagian daun pandan (Pandanus amaryllifolius) dianalisis dengan menggunakan kromatografi gas (GC-MS). Komponen utama dalam amaryllifolius Pandanus adalah 2-asetil-1-pyrroline tetapi dalam kajian ini hanya bahagian-bahagian kecil yang diperolehi 3-Hexanol dan 3-Hexanone. Secara menyeluruh hasilnya menunjukkan bahawa nisbah pelarut kloroform: etil asetat mendapatkan jumlah besar minyak

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ISO	-	International Standards Organization
2-AP	-	2-Acetyl-1-Pyrroline
GCMS	-	Gas Chromatography Mass Spectrometry
FID	-	Flame ionization detector
RPM	-	Rotate per minute
PPM	-	Part per million

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Pandanus amaryllifolius is a tropical plant in the screwpine genus which is known commonly as '*pandan*' and used widely in Southeast Asian cooking. In Pandan odoratissimus, the flowers are the scented part of the plant, while, in Pandan amaryllifolius, the leaves are scented (Rahman *et al.*, 2001).It is an upright green plant with fan-shaped sprays of long, narrow, bladelike leaves and woody aerial roots. The plant is sterile, flowers only very rarely, and is propagated by cuttings. The plant is rare in the wild but cultivated widely for use as a flavoring in cooking. The leaves are used fresh or wilted, and are commercially available in frozen form in Asian grocery stores in nations where the plant does not grow. They have a nutty, botanical fragrance which enhances the flavor of Indonesian, Filipino, Malaysian, Thai, Vietnamese and Burmese foods, especially rice dishes and cakes. The leaves are sometimes steeped in coconut milk, which is then added to the dish. They may be tied in a bunch and cooked with the food. The leaves of the plant also have a repellent effect on cockroaches.

The key aroma compound in pandan is 2-acetyl-1-pyrroline (2-AP) (Laksanalamai and Ilangantilek, 2006) which is described as "pandan" by Asian and "popcorn and roasty" by Westerners (Harold et al., 2009). 2-AP is also a key odorant in Jasmine rice and Basmati rice. Yet another study found 3-methyl-2-(5H)-furanone as main volatile compound in pandanus leaves, besides 3-hexanol, 4-methylpentanol, 3-hexanone and 2-hexanone.

An aroma compound, also known as odorant, aroma, fragrance or flavor, is a chemical compound that has a smell or odor. A chemical compound has a smell or odor when two conditions are met. The compound needs to be volatile, so it can be transported to the olfactory system in the upper part of the nose, and it needs to be in a sufficiently high concentration to be able to interact with one or more of the olfactory receptors.

Aroma compounds can be found in food, wine, spices, perfumes, fragrance oils, and essential oils. As well many of the aroma compounds plays a significant role in the production of flavorants, which are used in the food service industry to flavor, improve and increase the appeal of their products.

2-Acetyl-1-pyrroline, abbreviated 2AP, with the IUPAC name 1-(3,4-dihydro-2H-pyrrol-5-yl) ethanone, is an aroma compound and flavor that gives white bread, jasmine rice and basmati rice, the spice pandan (Pandanus amaryllifolius) their typical smell. 2-Acetyl-1-pyrroline and its structural homolog, 6-acetyl-2, 3, 4, 5-tetrahydropyridine of similar smell, can be formed by Maillard reactions during heating of food such as in baked bread.

An essential oil is a concentrated, hydrophobic liquid containing volatile aroma compounds from plants. Essential oils are also known as volatile, ethereal oils, 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 carries a distinctive scent, or essence, of the plant.

In the present work, an attempt has been made to extract the flavour compound, 2-AP, from the scented leaves of Pandan amaryllifolius Roxb., either by conventional solvent extraction, or Likens–Nickerson simultaneous solvent-extraction-steam-distillation method, or by supercritical carbon dioxide.

Liquid-liquid extraction, also known as solvent extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. Liquid-liquid extraction is a basic technique in chemical laboratories, where it is performed using a separatory funnel. In other words, this is the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. By this process, a soluble compound is usually separated from an insoluble compound. Solvent extraction is used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes, and other industries.

In solvent extraction, a distribution ratio is often quoted as a measure of how well-extracted a species is. The distribution ratio is equal to the concentration of a solute in the organic phase divided by its concentration in the aqueous phase. Depending on the system, the distribution ratio can be a function of temperature, the concentration of chemical species in the system, and a large number of other parameters. In solvent extraction, two immiscible liquids are shaken together. The more polar solutes dissolve preferentially in the more polar solvent, and the less polar solutes in the less polar solvent. In this experiment, the nonpolar halogens preferentially dissolve in the nonpolar mineral oil.

1.2 Problem Statement

Pandanus Amaryllifolius or pandanus odorus (Pandan) widely used as cooking material in Malaysia. The climate of Malaysia is suitable for them to grow. They can be easily grown and need not much care for their growth. This project is conducted due to these facts:

1) It is a fact that many of this plant exist in Malaysia and actually only a small amount of this plant is need in cooking. So, there are enormous amount of pandan to be used as the raw material.
2) The fact that mainly the essential oils purely used in industrial is very commercial. Pure essential oil from pandan leaves is hard to get it and it will commercial cultivation and income in business such as shampoo, makes up, perfumes, cooking and so on.

Due to these facts, the initiative to invent a solution to get very optimum essential oils from natural material, in this case Pandanus Amaryllifolius is conducted. This research it is environmental friendly product which will not harm person, especially children from toxicity.

1.3 Objective

The goals of this research are to:

1) Extract essential oils of pandanus amaryllifolius from pandan leaves using solvent extraction method with hexane, chloroform, ethyl acetate, and methanol as the solvents.

2) Investigate the effect of time of extraction, and distribution ratio of the solvents with raw material to the yields of pandanus essential oil extraction.
3) The effect of time of extraction and distribution ratio of the solvents to the volatility components (3-Hexanol) of the pandanus essential oil extracts.

1.4 Scope

1) To study the effect of using different solvent for pandan leaves oil extraction.
2) To determine the optimum mixing of time for the extraction.

1.5 Rationale & Significance

The essential oils produces through this research will be used as oils and flavor for industrial that are widely used in cooking, perfumes, makes up and :

1) This research gives an advantage as it can provide an environmental friendly condition for more inexpensive and ecologically safe solutions.
2) Pandanus amaryllifolius plant is a type of plant that can be easily grown in Malaysia's weather and condition. It also does not require any special and burdensome treatment for their growth.
3) Pandan leaves are commonly focused on their traditional uses e.g. in cooking and for some medicinal purpose.
4) The solvent used are hexane, chloroform, methanol, and ethyl acetate, which is easy to obtain and cheap.
5) The operating system, solvent extraction method is simple.

CHAPTER 2

LITERATURE REVIEW

2.1 2-Acetyl-1-Pyrroline (2-AP).

The genus Pandanus from the family Pandanaceae comprises approximately 600 species that are widely distributed in tropical and subtropical regions (Tsugita *et al.* 2006). Nineteen species of Pandanus are recorded in India (Mohsin et al. 2002), out of which Pandanus tectorious Parkinson, J. Voy. S. Seas. and P. amaryllifolius Roxb. are exploited commercially by flavour industry. In Pandanus tectorious, the flowers are scented, while in P. amaryllifolius, the leaves are scented (William 2007). Throughout Southeast Asia the long, thin green leaves P. amaryllifolius are used in cooking to impart flavour and colour to rice, sweets, jellies and in many others food products (Laksanalamai et al., 2006)

Leaves are used in perfume industry and also medicinally important as diuretic, cardio-tonic, snit-diabetic and for skin disease (Lin et al., 2008). The Geneva based International Standards Organization (ISO) has included P. amaryllifolius in the document 676 that list109 herb and spice plant species useful as ingredients in food (Harold 2009). The distinct flavour aroma component of the leaves was characterized by (Grimm *et al.*, 2006) as 2-Acetyl-1-Pyrroline (2-AP) in amounts of pandan. This compound is the same that found in scented rices including famous Jasmine and Basmati rice. Hence, it is widely used to flavor ordinary rice as a substitute for the

expensive aromatic rice varieties (Buttery *at el.* 2003). We have found out lower epidermal papillae as the site of storage of 2-Acetyl-1-Pyrroline through histochemical test (Bergman *et al.* 2000).

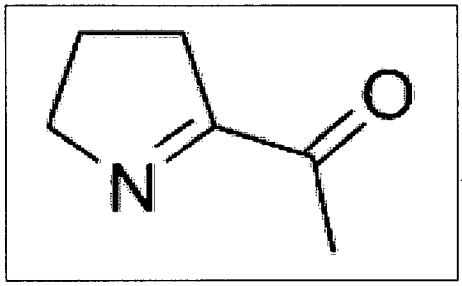

Figure 1.1 The chemical structure of 2-acetyl-1-pyrroline

The unique feature in our research plan involved one Pandanus species, Pandanus amaryllifolius (pandan wangi). P. amaryllifolius is a tropical plant in the screwpine genus which is known commonly as pandan and used widely in Southeast Asian cooking. It is characterized with upright green plant with fan-shaped sprays of long, narrow, bladelike leaves and woody aerial roots. The plant is sterile, flowers only very rarely, and is propagated by cuttings. Living sample of P. amaryllifolius can be observed at village in Kuantan, Pahang.

However, this occurrence is in contrast with other Pandanus species, e.g. P. tectorius cultivar. The green stripe of P. tectorius cultivar leaves is in between the two yellow stripes (Eun-Jin et al., 2004). Mohsin et al. (2002) recently published antitubercular triterpenes and phytosterols from p. tectorius (megkuang).

Previously, (Belardi et al., 2006) reported that the taxonomy of the Pandanus species is difficult due to the lacking of correlation between male and female plants and extensive widely distributed of the species.

Pandanus amaryllifolius is still unexplored through more lately, it is noticeable in urban area as a landscaper. The extraction procedure of the leaves from this species will be reported elsewhere (Mohsin, *et al.* 2003). In this research, the chromatographic profile of the leaves extract is presented.

2.1.1 Application of 2-Acetyl-1-Pyrroline (2-AP).

2.1.1.1 Flavor and coloring for food

Pandan leaves have strong nutty flavor that adds a distinct aroma in the various dishes of different regions of Asia including Filipino, Thai, Malaysian, Vietnamese, Burmese and even some regions of India. Various forms of pandan leaves are used for culinary purposes. It can be used for wrapping certain dishes and it is also used in paste form to get that distinct vanilla like flavor. Pandan leaves essence and pandan leaves paste is available in the Asian markets, so that it could get easy for the people who find it difficult to get fresh pandan leaves.

2.1.1.2 Healing wound and diseases.

Pandan leaves are very beneficial for various health conditions. Pandan leaves consist of essential oils, traces of tannin, glycosides and alkaloids as well. As a matter of fact, whole pandan plant is considered to be diuretic and is extremely useful for healing various wounds and diseases like smallpox. Pandan leaves are said to be pain relievers and used that way to cure chest pain, headache, reduce fever, arthritis, earache, etc. Pandan leaves are also used as a healthy laxative for children.

Chewing pandan leaves is an easy way to get rid of gum pain. Pandan leaves are also effective in reducing stomach cramps and stomach spasm. They are also found to be effective in recovery of women with weakness after childbirth. It has a cooling effect and is excellent for the treatment of internal inflammations, urinary infections, colds, coughs, measles, bleeding gums and skin diseases.

2.1.1.3 Preventing Cancer.

Pandan leaves are also anti-carcinogenic, while benefits of pandan leaves for diabetes are also very significant. Pandan leaves are also useful for treating several skin disorders including leprosy. Pandan leaves are also used for preparation of various herbal teas, with other herbs like lemongrass, mulberry leaves, safflower, green tea and other such herbs. Bathing with water having boiled pandan leaves, is useful for treating skin diseases and sunburns.

2.1.1.4 Fragrance

Fragrant pandan fragrance is often used as food for such a distinctive aroma. In addition to the fragrances, cakes, pandan leaves are also used as a green source of food, and also as a sweetener for the flower arrangements for weddings and scent the room. In addition, pandan fragrance also has benefits for beauty. Plants that thrive in the tropics can be used to eliminate dandruff and hair black.

2.2 3-Hexanol

3-Hexanol (IUPAC name, also called ethyl propyl carbinol) is anorganic chemical compound. It occurs naturally in the flavor and aroma of plants such as pineapple and is used as a food additive to add flavor. 3-Hexanol can be synthesized by the hydroboration of unsaturated hexane compounds such as 3-hexyne (Bergman et al., 2000).

2.3 Solvent Extraction

Solvent extraction is usually used to recover a component from either a solid or liquid. The sample is contacted with a solvent that will dissolve the solutes of interest. Solvent extraction is of major commercial importance to the chemical and biochemical industries, as it is often the most efficient method of separation of valuable products from complex feedstocks or reaction products. Some extraction techniques in involve partition between two immiscible liquids, others involve either continuous extractions or batch extractions. Because of environmental concerns, many common liquid/liquid processes have been modified to either utilize benign solvents, or move to more frugal processes such as solid phase extraction. The solvent can be a vapour, supercritical fluid, or liquid, and the sample can be a gas, liquid or solid. There are a wide range of techniques used, and details can be found in Organic Vogel, Perry, and most textbooks on unit operations (William et al., 2007).

A solvent such as methanol, chloroform, ethyl acetate and hexane is placed in concentration distribution ratio in pandan leaves. When a distribution ratio is often quoted as a measure of how well-extracted a species is. The distribution ratio is equal to the concentration of a solute in the organic phase divided by its concentration in the aqueous phase. Depending on the system, the distribution ratio can be a function of temperature, the concentration of chemical species in the system, and a large number

of other parameters. The separation factor is one distribution ratio divided by another; it is a measure of the ability of the system to separate two solutes.

Solvent extraction is a mature technique in that extensive experience has lead to a good understanding of the fundamental chemical reactions. At the same time, compared to many other chemical separation processes like precipitation, distillation, pyrometallurgical treatment, the large-scale application of solvent extraction is nevertheless, a young technique. Considering such factors as demand for higher product purity, less pollution, and the need for recovering substances from more complex matrices and more dilute resources, the efficiency and high selectivity of solvent extraction should make it an increasingly competitive separation process both in research and in industry.

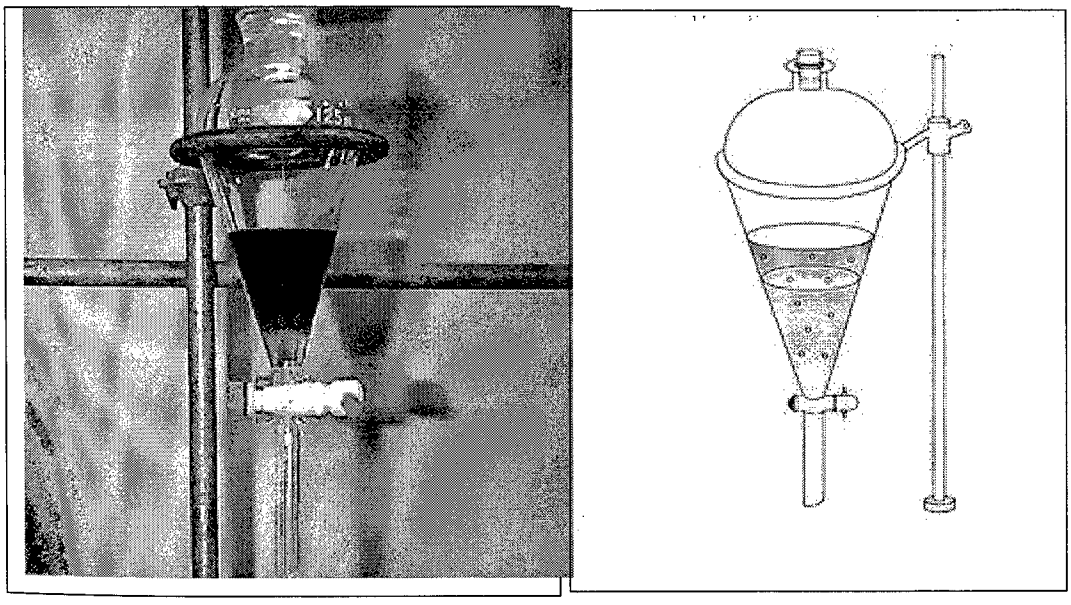

Figure 2.1 Method of solvent extraction.

2.4 Gas Chromatography - Mass Spectrometry (GC-MS)

The Gas Chromatography/Mass Spectrometry (GC/MS) instrument separates chemical mixtures (the GC component) and identifies the components at a molecular level (the MS component). It is one of the most accurate tools for analyzing environmental samples. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas such as helium. As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. A "library" of known mass spectra, covering several thousand compounds, is stored on a computer. Mass spectrometry is considered the only definitive analytical detector (Harold et al., 2009).

Gas chromatography is specifically gas-liquid chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

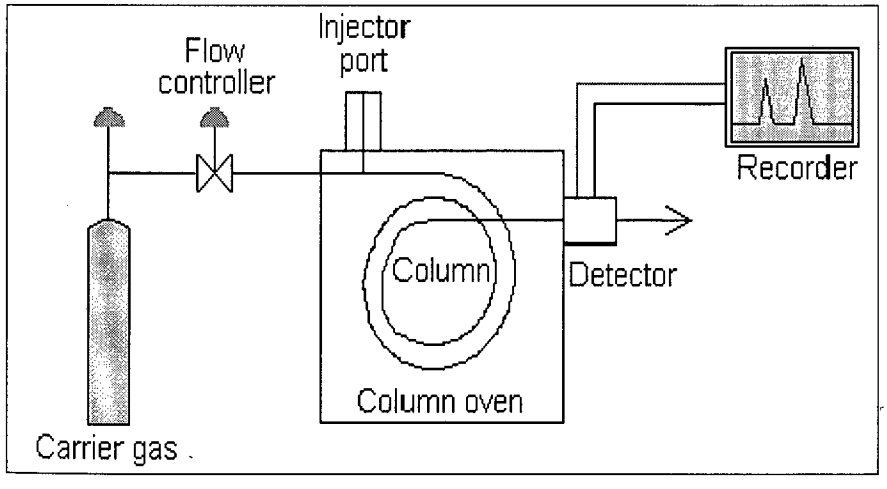

Figure 2.2 Schematic Diagram of a Gas Chromatography.

2.4.1 Theory of Operation

2.4.1.1 Mobile Phase

The mobile phase is the part of the chromatographic system which carries the solutes through the stationary phase. The mobile phases are either liquids or gases. The liquid mobile phases are used to adjust the chromatographic separation and retention in liquid chromatography and the temperature of the gas mobile phase is used to adjust the retention in gas chromatography.

This transfer is not instantaneous, time is required for the molecules to pass (by diffusion) through the mobile phase to reach the interface and enter the stationary phase. Those molecules close to the stationary phase enter it immediately, whereas those molecules some distance away will find their way to it sometime later. Since the mobile phase is continually moving, during this time interval, those molecules that remain in the mobile phase will be swept along the column and dispersed away from those molecules that were close and entered the stationary phase immediately.

2.4.1.2 Stationary Phase

The stationary phase is a high-boiling liquid. This is a viscous oil, or waxy substance. This high-boiling liquid is packed into a long, narrow glass or metal column. The mixture to be analyzed is loaded by syringe into the beginning of this column. The mobile phase is an inert gas which continuously flows through the column. The components of the mixture distribute between the stationary high-boiling liquid (these components are either condensed or absorbed on the high-boiling liquid) and mobile gas (vapor) phase moving through the column. The gaseous mixture flows