EXTRCATION AND CHARACTERIZATION OF MALAYSIA PANDAN LEAVES BY SOXHLET METHOD

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ABSTRACT

Research in finding more sources for specialty oils have been actively conducted nowadays . In this study, Soxhlet Extraction method is one of the extraction technique can be used to extract a soluble fraction from solid medium into an organic solvent such n-hexane and ethanol. In this research, the soxhlet extraction method used to extract the pandan leaves (*Pandanus Amaryllifolius Roxb.*) . This leaves was selected because of it is easily find in our local market. To produce high extract yield of the leaves, parameters that affect the performance of the soxhlet extraction method were investigated. The manipulated parameters were pandan leaves surface area and the solvent extractant. The yield was observed. Grind leaves produced higher yield compared to non-grinding leaves. In addition, ethanol was found to be better solvent than n-hexane. Both extraction were using ethanol and n-hexane.

ABSTRAK

Penyelidikan untuk mencari sumber baru terutama dari minyak istemewa semakin banyak dilakukan pada masa ini. Dalam kajian ini, proses pengekstrakkan menggunakan soxhlet merupakan teknik pengekstrakkan untuk mengekstrak pecahan bahan terlarut dari medium pepejal menggunakan bahan pelarut seperti n-heksana dan etanol. Dalam menjalankan kajian ini, proses pengekstrakkan menggunakan soxhlet untuk mengekstrak daun pandan (*Pandanus Amaryllifolius Roxb.*). Bahan asas ini dipilih berdasarkan keadaannya yang mudah diperolehi dipasaran tempatan. Untuk memastikan sama ada hasil pengekstakkan mempunyai kualiti yang baik, parameter-parameter yang mempengaruhi produktiviti menggunakan soxhlet sebagai alat pengekstrakkan dikaji. Antara parameter- parameter yang dikaji ialah luas permukaan daun pandan yang digunakan dan jenis pelarut yang digunakan. Penghasilan ekstrak dikaji. Daun pandan yang dikisar menghasilkan lebih banyak ekstarak daripada daun pandan yang tidak dikisar. Tambahan pula, penggunaan etanol sebagai pelarut adalah lebih baik daripada n-heksana. Dalam proses pengekstrakan ini, kedua- dua pelarut iaitu etanol dan heksana digunakan.

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LIST OF SYMBOLS/ABBREVIATIONS

g	-	Gram
h	-	Hour
L	-	Liter
^{o}C	-	Degree Celcius
T	-	Temperature
%	-	Percentage

CHAPTER 1

INTRODUCTION

1.1 Background

Pandan leaf (Pandanus amaryllifolius Roxb.) is a tropical plant in a screw pine genus. It is an erect green plant with fan-shaped sprays of long, narrow, bladelike leaves and woody aerial roots which approximately 4 inches (10 cm) long. It is a source of natural flavoring that is widely used in various parts of Asia including India, Thailand, Indonesia and Malaysia. In south-east Asia, pandan leaves are commonly used when preparing rice dishes as means of enhancing flavor. For example, non-aromatic rice varieties are often cooked with pandan leaves to stimulate the flavor of the expensive and aromatic type rice like basmati and jasmine rice. Fresh or slightly withered pandan leaves are typically torn into strips, tied in bunch to facilitate removal than placed in the cooking pot and finally removed at the end of cooking. Commonly used to wrap foods like fish or shrimp, pandan leaf paste can imbue a dessert with sweetness and bright green coloring.

When used in cooking and baking, the pandan leaf is often pounded into a sweet paste that is diluted with water. The paste adds citrus and pine overtones and a green tint to cakes, crepes, ice cream, and curry sauces. When making rice, panda leaves can be placed on top of the boiling grains to add flavor and sweetness. It is also traditional to

deep fry or grill chicken wrapped in pandan leaves. Pandan leaves have been used to make thatched roofs, baskets and grass skirts. Additionally, this plant is believed to have medicinal properties and is an effective and natural cockroach repellent.

Traditionally, pandan leaves are widely use to prevent some insect. It is said that taxi drivers in Singapore and Malaysia keep bunches of pandan leaves (*Pandanus amaryllifolius* Roxb.) in their taxis to ward off cockroaches.

The major component contributing to the flavor characteristic of pandan is 2-acetyl-1-pyrroline (2AP) .This hydrophilic compound has an odor threshold value as low as 0.1 ppb in water. 2AP is also significantly contributing to the flavor of rice varieties such as basmati and jasmine rice. It has been reported by Buttery et al. that quantities of 2AP present in pandan leaves (of the order of 1ppm) is more than 10 times of those found in scented milled rice like basmati and 100 times of those found in common rice. According to Wongpornchai *et.al* ,2AP is also occurs naturally in fresh Vallaris glabra Ktze (bread flower) leaves with a concentration of 0.53 ppm. However, it is important to note that pandan leaf is one of the best natural sources of 2-AP.

Figure 1.1 : Image of Pandan leaves

Figure 1.2 : Image of pandan leave

Figure 1.3 : Use of Pandan leaf in dishes

1.2 Problem Statement

Higher demand of essential oil as pharmaceutical, aromatherapy aid and cosmetic ingredients give large opportunities for global marketing. The world wide market for essential oil growth rapidly. A number of scientific researches presently focused on the industrial development together with environmental preservation. In this contact, it's necessary to find the most appropriate technique or method to improve the quality of essential oil.

Traditional extraction method used to obtain essential oil have several drawbacks which are longer time consuming, have low extraction yields, laborious at higher operational costing.

In this study, Soxhlet method will be used for extraction of essential oil from Pandan leaves to overcome drawbacks mentioned earlier.

1.3 Research Objectives

The objective of this study is to investigate the performance of potential of Soxhlet method in extraction of essential oils from Pandan leaves.

1.4 Scope of Study

In order to achieve the objectives of this study, the following scopes have been identified:

1. Study the effect of different solvent use in extraction process towards the yield Extract.

2. Study the effect of particle size of leaves towards the yield extract.

3. Study the performance of Soxhlet in extraction of essential oil.

CHAPTER 2

LITERATURE REVIEW

2.1 Pandan Leaves Properties

2.1.1 History of Pandan Leaves

The leaves are used medicinally in South East Asia to refresh the body, reduce fever, and relieve indigestion and flatulence scented (MacLeod *et al*.,1982). The previous study reports the beneficial effects of P. amaryllifolius leaf extract in palm olefin during accelerated oxidation and frying in order to understand its potential use as an antioxidant in the food industries. The oil of the leaf is described as stimulant and antispasmodic and is effective against headaches, rheumatism, and epilepsy and as a cure for sore throats (Quisumbing, 1951). The leaf contains essential oils, carotenoids, tocopherols and tocotrienols (Lee, Su, & Ong, 2004), quercetin (Miean & Mohamed, 2001), alkaloids (Busque, March, Figueredo, Font, & Sanfeliu, 2002), fatty acids and esters (Zainuddin, 2004) and non-specific lipid transfer proteins (Ooi, Wong, Sun, & Ooi, 2006). Spectroscopic analysis of the extract revealed that the flavor was an oxidative degradation product of a yellow carotenoid pigment that develops only when the plant withers; the fresh, intact plants hardly have this odor.

The leaves yield traces of essential oil up on distillation and the flavor component, 2-acetyl-1-pyrroline (2-AP) (popcorn-like aroma, as described by non-oriental, and pandan-like aroma, as described by oriental) was identified as a major component of the volatile oil of freeze-dried pandan leaves .This is also the principal aroma component of aromatic rice varieties such as Basmati and Jasmine. It is present in the rice endosperm at ten time's greater concentration in scented rice than in non-aromatic rice. There is a controversy regarding the amount of 2-AP present in pandan leaves.

Continuous steam-distillation extraction of freeze-dried fresh leaves of Pandanus yields 12 ppm (based on dry weight of leaves) of steam-volatile oil. Gas chromatography-mass spectrometry (GC-MS) analysis showed 1 ppm of 2-AP in the volatile oil. The concentration of 2-AP in pandan leaves is reportedly 10 times greater than that found in milled, scented rice varieties, and 100 times greater than that found in common non-aromatic milled rice, where it is present at0.04–0.06 ppm (Buttery *et al*., 1986).

However, the other alkaloids (such as, pandanamine, pandamerilactones) with pyrroline-derived structures are also found in the leaves (Nonato et al., 1993; Takayama *et al*.,2001).

2.1.2 2-Acetyl-1-Pyrroline (2AP)

2-Acetyl-1-pyrroline (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), and bread flowers (Vallaris glabra) 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. Both compounds have odor thresholds below 0.06 ng/l. (Harrison and Dake,2005).

Extraction of 2-AP by solvent extraction and simultaneous Likens–Nickerson steam-distillation-solvent extraction has been previously reported (Yoshihashi and T, 2002). However, they were unable to quantify 2-AP in the pandan oil extract by GC-MS.

In the present work, an attempt has been made to extract the flavor compound from the scented leaves of P. amaryllifolius Roxb., by using Soxhlet extraction. P. amaryllifolius are reported to contain lower epidermal papillae and through histochemical studies we have reported that these papillae are the sites of storage of the aroma principal 2AP. However, developmental pattern of the papillae are not reported so far. Figure 2.1 below show the chemical structure of 2-AP.

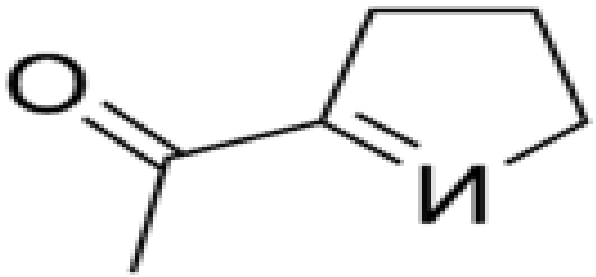

Figure 2.1 : Structure of 2-acetyl-1-pyrroline

2.1.3 Pandan Description and Cultivation

Plants belonging to the genus Pandanus (Pandanaceae) are palm-like evergreen trees or shrubs, widely distributed in the moist tropics from Africa to the Pacific Islands. Among the 36 species that have been recorded in India, Pandanus odoratissimus Linn. and Pandanus amaryllifolius Roxb. are of commercial interest to the flavor industry. In P. odoratissimus, the flowers are the scented part of the plant, while, in P. amaryllifolius, the leaves are scented (Zaheer *et al*.,1966). The flavour components of Pandanus leaves are not very well known. Spectroscopic analysis of the extract revealed that the flavor was an oxidative degradation product of a yellow carotenoid pigment that develops only when the plan withers; the fresh, intact plants hardly have this odor.

Pandan also used for handicrafts. Craftswomen collect the pandan leaves from plants in the wild. Only the young leaves are cut so the plant will naturally regenerate. The young leaves are sliced in fine strips and sorted for further processing. Weavers produce basic pandan mats of standard size or roll the leaves into pandan ropes for other designs. This is followed by a coloring process, in which pandan mats are placed in drums with water-based colors. After drying, the colored mats are shaped into final products such as place mats or jewelry boxes. Final color touch-ups are applied to assure a product of high quality. The whole process from harvesting of raw materials to finished product is handled by craftswomen, making this a truly community-based handicraft product

2.1.4 Spice Description

Screw-pine leaf is long, thin, narrow, and green. It is sold fresh, frozen, or dried. The leaves and flowers also come as bright green extracts. Properties: the dried leaves are less fragrant than the fresh leaves. The leaves have to be bruised or boiled in order to release their flavor. The leaves have a roselike, almondy, and milky sweet, vanilla-like flavor. The dried leaves have no flavor. The flowers are golden yellow and have a fragrant, strong, and sweet aroma.Because color is one of the key attributes of pandan leaf, it must be carefully dried to retain its bright-green appearance and unique fragrance. The leaves are then chopped into pieces large enough to remove from a dish after cooking, or powdered finely so the texture is no longer reedy and fibrous. Fresh whole leaves are either crushed or boiled to make an extract that is used to color cakes or confectionery.

2.1.5 Pandan Leaves Culinary Uses

The leaf is used in curries of Sri Lanka and in Malaysian, Balinese, and Thai cooking. It is commonly used as a flavoring and coloring in Malaysian and Singaporean cooking, especially in Malay dishes. The screw-pine or pandan leaves are tied in a knot and placed in soups or stews that are being cooked. The leaf is also bruised or raked with the tines of a fork to release its aroma, pounded to release its aromatic juice, or even boiled to obtain its flavor.

Pandan leaves are used as wrappers in Southeast Asian cooking to provide a distinct flavor to the foods. They are wrapped around chicken, pork, glutinous rice, fish, and desserts before grilling, roasting, barbecuing, or steaming. Pandan leaves also

enhance the flavor of seasoned rices, puddings, beverages, and curries. Nasi lemak, nasi kuning, and nasi padang are some of the fragrant pandan-flavored rices eaten in Malaysia and Indonesia. It pairs well with coconut milk, glutinous rice, lemongrass, milk, brown sugar, and turmeric. It also provides color to Indonesian, Thai, Malay- and Nonya-style glutinous rice-based desserts, candies, puddings, soups, and coconut drinks.

Screw-pine flower, which is more delicate and fragrant than the leaf, is used in North India to perfume biryanis. It goes well with rices, coconut, lemongrass, brown sugar, star anise, cumin, and nutmeg. Its extract, called kewra, is also commonly used to flavor Indian desserts such as rasgulla (cottage cheese in syrup), gulab jamun (fried cottage cheese in syrup), rasmalai (cottage cheese with condensed milk), cakes, and beverages.

The commercially available pandan leaf extract is much too bright green and does not totally capture its true flavor and color profiles.

2.1.6 Others Name of Pandan Leaves

- Arabic : kathey
- Japanese : takonoki
- Portugese : Pandano
- French : Pandanus

2.1. 7 Attributed Medicinal Properties

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. Though rare, pandan flowers are said to be aphrodisiac , which means they stimulate sexual desire. 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.

2.2 Introduction of Separation

In chemistry and chemical engineering, a separation process is used to transform a mixture of substances into two or more distinct products. The separated products could differ in chemical properties or some physical property, such as size, or crystal modification or other separation into different components. Almost every element or compound is found naturally in an impure state such as a mixture of two or more substances. Many times the need to separate it into its individual components arises.

Separation applications in the field of chemical engineering are very important. A good example is that of crude oil. Crude oil is a mixture of various hydrocarbons and is valuable in this natural form. Separation processes can be termed as mass transfer processes. The classifications of separation process are:

- Evaporation : Evaporation of a volatile solvent from a nonvolatile solute.
- Drying : Volatile liquid are removed from solid materials.
- Distillation : Separation of component liquid mixture base on their boiling point because of their differences in vapor pressure.
- Absorption : Removal of component from a gas stream by treatment with a liquid.
- Membrane separation : Separation of a solute from a liquid
- Liquid-liquid extraction : Removal of solute in liquid solution by contacting with another liquid solvent that is relatively immiscible with the solution.

- Adsorption : Removal and adsorption of component gas or liquid stream by a solid adsorbent.
- Ion exchange : Removal of certain ions in solution by an ion-exchange solid from a liquid.
- Liquid-solid leaching : Treating finely divided solid with a liquid that dissolves out and removes a solute contained in the solid.
- Crystallization : Removal of a solute from a solution by precipitating the solute from the solution.
- Mechanical-physical separations: Separation of solids,liquids, or gas by mechanical means such as filtration,settling,centrifugation, and size reduction.

2.3 Introduction of Extraction

In order to separate one or more of the components in a mixture, the mixture is brought into contact with another phase. The two-phases can be gas-liquid, vapor-liquid, liquid-liquid, or fluid-solid.

2.4 Types of Extraction

There are many types of extraction process. The example of extraction processes are liquid-liquid extraction, solid-liquid extraction, solid-liquid and soxhlet extraction.

2.4.1 Liquid-liquid extraction

Liquid-Liquid extraction is a mass transfer operation in which a liquid solution (the feed) is contacted with an immiscible or nearly immiscible liquid (solvent) that exhibits preferential affinity or selectivity towards one or more of the components in the feed. Two streams result from this contact are the extract, which is the solvent rich solution containing the desired extracted solute, and the raffinate, the residual feed solution containing little solute.

It is possible in non-aqueous systems. In a system consisting of a molten metal in contact with molten salt, metals can be extracted from one phase to the other. This is related to a mercury electrode where a metal can be reduced. Hence, the metal will then dissolve in the mercury to form an amalgam. Amalgam can modifies its electrochemistry greatly.

Liquid-liquid extraction also known as solvent extraction. Solvent extraction always been used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries

.2.4.2 Solid-liquid extraction

Solid-liquid extraction allows soluble components to be removed from solids using a solvent. Applications of this unit operation include obtaining oil from oil seeds or leaching of metal salts from ores. It also may be considered as the dissolving of one or more components in a solid matrix by simple solution, or by the formation of a soluble form by chemical reaction. The largest use of liquid/solid extraction is in the extractive metallurgical, vegetable oil, and sugar industries.

The field may be subdivided into the following categories: leaching, washing extraction, and diffusion extraction. Leaching involves the contacting of a liquid and a solid (usually an ore) and the imposing of a chemical reaction upon one or more substances in the solid matrix so as to render them soluble. In washing extraction the solid is crushed to break the cell walls, permitting the valuable soluble product to be washed from the matrix. In diffusion extraction the soluble product diffuses across the denatured cell walls (no crushing involved) and is washed out of the solid.

2.4.3 Soxhlet extraction

In 1879, von Soxhlet developed a new extraction system (Soxhlet extractor) which has for a long time been the most widely used leaching technique (F. Soxhlet, Dinglers' Polyt,1879). In fact, Soxhlet extraction has been a standard technique for over a century and the methods based on it remain the primary references against which performance in new leaching methods is measured. The advantages and shortcomings of Soxhlet extraction have been used as starting points for the development of a variety of modifications intended to alleviate or suppress the latter while keeping or even improving the former. Most of the modifications reported over the last few decades have been aimed at bringing Soxhlet closer to that of the more recent techniques for solid