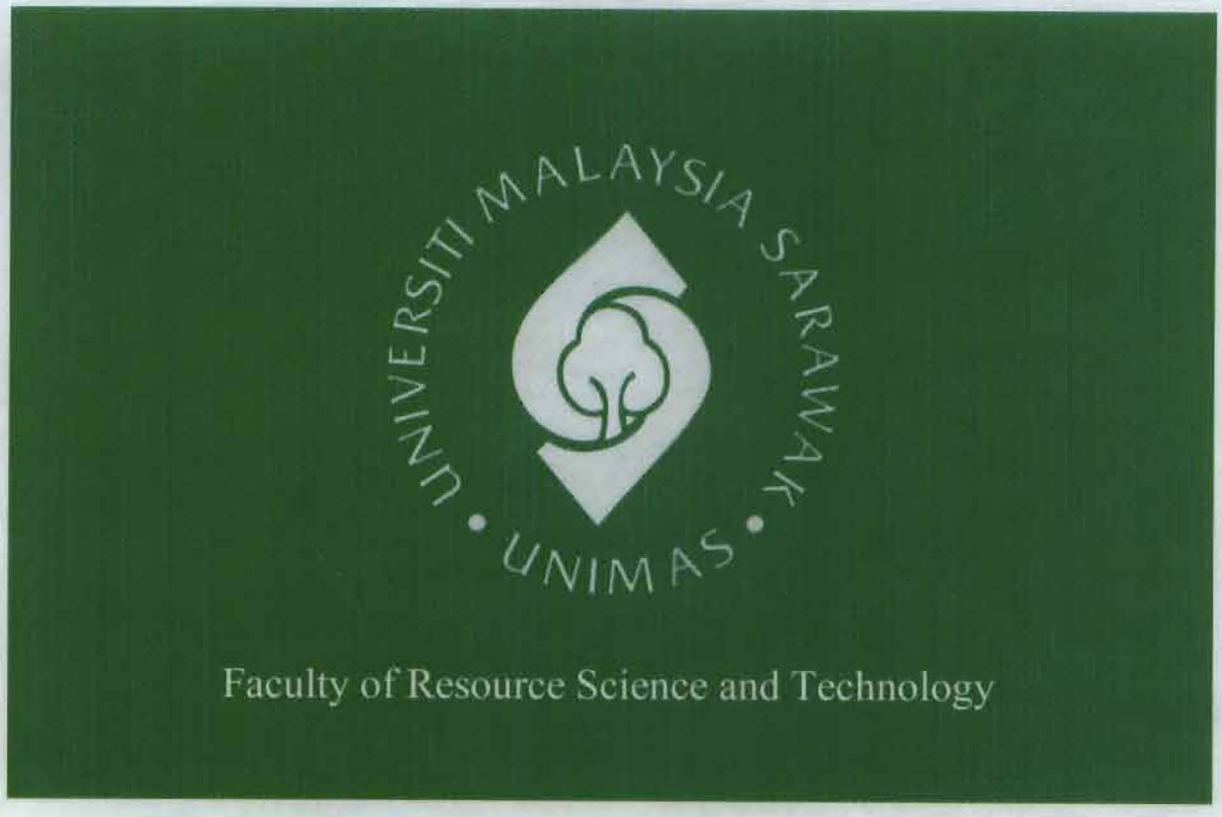

EXTRACTION OF WAX FROM PLANTS FOR POSSIBLE COMMERCIAL APPLICATION

Jackie Wong Teck Huat

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Extraction of Wax from Plants for Possible Commercial Application

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A thesis submitted in fulfilment of the requirement for the Bachelor of Science with Honour (Resource Biotechnology)

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DECLARATION

I, Jackie Wong Teck Huat, hereby declare that this thesis entitled "Extraction of Wax from Plants for Possible Commercial Application" is based on my original work except for quotations and citations, which have been dutifully acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UNIMAS or any other institutions.

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LIST OF ABBREVIATION

NaCl	-	Sodium chloride
KBr	-	Potassium bromide
DCM	-	Dichloromethane
GC	-	Gas chromatography
TLC	-	Thin layer chromatography
UV	-	Ultraviolet
GC-MS	-	Gas chromatography-Mass spectometry
IR	-	Infrared
wt	-	Weight

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Extraction of Wax from Plants for Possible Commercial Application

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ABSTRACT

Waxes are used in many industries such as cosmetics and personal care products. The market of wax industry is also expanding and growing at an encouraging rate. It is also noted that natural waxes are increasing in demand, especially plant-based waxes. The objective of this study is to extract wax from plants in order to determine its chemical composition and possible commercial applications. Banana (*Musa* sp.) and yam (*Alocasia* sp. and *Colocasia* sp.) were used in this study. The waxes from these species were successfully extracted using n-hexane and their chemical compositions were determined. Wax of *M. acuminate Colla* has a higher percentage of esters whereas wax of *A. macrorrhiza* has a higher percentage of hydrocarbons, mostly alkanes. The possible commercial applications of the waxes based on their chemical composition were also estimated.

Key words: Waxes, n-hexane, plant-based, commercial applications

ABSTRAK

Lilin digunakan dalam pelbagai industri seperti kosmetik dan produk penjagaan. Pasaran untuk industri lilin juga berkembang pada kadar yang memberansangkan. Lilin yang berasal dari sumber semula jadi semakin mendapat sambutan, terutamanya lilin yang berasaskan tumbuhan. Objektif kajian ini adalah untuk mengekstrak lilin daripada tumbuhan untuk mengkaji komposisi kimia dan potensi lilin ini untuk digunakan dalam aplikasi komersial. Kajian ini telah menggunakan daun daripada pisang (*Musa* sp.) dan keladi (*Alocasia* sp. dan *Colocasia* sp.). Lilin dari spesies-spesies ini telah berjaya diekstrak menggunakan n-hexana dan komposisi kimia lilin tersebut telah dianggarkan. Lilin *M. acuminate Colla* mengandungi lebih banyak ester manakala lilin *A. macrorrhiza* mengandungi lebih banyak hidrokarbon, kebanyakannya alkana. Potensi untuk lilin ini digunakan dalam aplikasi komersial berdasarkan komposisi kimia juga telah dijangka.

Kata kunci: Lilin, n-hexana, berasaskan tumbuhan, aplikasi komersial

INTRODUCTION

It has always been observed that plants, despite being exposed to contaminants and rain, remain clean and dry. This is due to the presence of an epicuticular wax layer and the plant leaf microstructures. This study will focus more on the chemical compositions of the wax layer.

Waxes generally refer to mixtures of long-chain apolar lipids and long-chain fatty alcohols found on the surfaces of plant leaves and fruits as well as in animals, with wax esters as their main constituent (Christie, 2011). Some waxes originate from minerals, while others are artificially manufactured. Plant surface wax or epicuticular wax is a mixture of hydroxy fatty acid polymer (cutin) and suberin. Cutin is the outer covering of plant surface wax; whereas suberin is the plant wax found on underground parts and healed wound surfaces of plants (Taiz & Zeiger, 2012). In general, wax layer is made up of long-chain hydrocarbon compounds such as alkanes, primary alcohols, aldehydes, secondary alcohols, ketones, esters and other derived compounds (Shepherd & Griffiths, 2006).

Currently, the four recognized classes of wax are animal waxes, plant waxes, mineral waxes and synthetic waxes (Kline & Company, Inc., 2010). Report by Kline & Company, Inc. (2010) indicates that global demand for waxes in that year was dominated by mineral waxes, followed by synthetic waxes and finally animal and plant-based waxes. Due to the amazing market of waxes (applied in candles, health industries, packaging and surface applications) (Kline & Company, Inc., 2010), it is to the best of interest if waxes from natural resources, such as plant waxes can be used to replace or compete with

current popular waxes. In fact, plant waxes have the natural ability that many synthetic and mineral waxes have. Thus, it is important to further study the possibility of the properties of these waxes in order to apply them commercially.

However, there are not much studies conducted on the properties of plant wax. In the agricultural industry, much of the vegetative parts which cannot be consumed are wasted and become pollutants. In order to solve these problems, this study was done to achieve the objectives as stated below:

i. To extract the wax from proposed plant samples

ii. To determine the chemical compositions of plant wax

iii. To study the possible applications of plant wax °

LITERATURE REVIEW

Properties and Constituents of Plant Leaf Wax

Christie (2011) listed the contents of waxes extracted from various plant species and from different sites of extraction. It shows that the plant wax contents differ greatly across species and parts of the plants (Christie, 2011). This is summarized in the following Table 1. Other components that are not listed include various diol types and triterpenoid acids.

Table 1: Relative Proportions (wt %) of the Common Wax Constituents in Some Plant Species

	Grape leaf	Rape leaf	Apple fruit	Rose flower	Pea leaf	Sugar cane stem
Hydrocarbons	2	33	20	58	40-50	2-8
Wax esters	6	16	18	11	5-10	6
Aldehydes	6	3	2	-	5	50
Ketones	-	20	3	-	-	-
Secondary alcohols	-	8	20	9	7	-
Primary alcohols	60	12	6	4	20	5-25
Acids	8	8	20	5	6	3-8

Adapted from Christie, 2011.

The major constituents of plant leaf are also listed by Christie (2011), as shown in Table 2.

Table 2: Major Constituents of Plant Leaf Waxes

Compound	Structure	
n-Alkanes	CH3(CH2)xCH3	21 to 35C – odd numbered
Alkyl esters	CH3(CH2)xCOO(CH2)yCH3	34 to 62C – even numbered
Fatty acids	CH3(CH2)xCOOH	16 to 32C – even numbered
Fatty alcohols (primary)	CH3(CH2)yCH2OH	22 to 32C – even numbered
Fatty aldehydes	CH3(CH2)yCHO	22 to 32C – even numbered
Ketones	CH3(CH2)xCO (CH2)yCH3	23 to 33C – odd numbered
Fatty alcohols (secondary)	CH3(CH2)xCHOH (CH2)yCH3	23 to 33C – odd numbered
β-Diketones	CH3(CH2)xCOCH2CO (CH2)yCH3	27 to 33C – odd numbered
Triterpenols	Sterols, *alpha*-amyrin, *beta*-amyrin, uvaol, lupeol, erythrodiol	
Triterpenoid acids	Ursolic acid, oleanolic acid, etc	

Adapted from Christie, 2011.

Besides that, plant leaf waxes may also contain hydroxy-β-diketones, oxo-β-diketones, alkenes, branched alkanes, acids, esters, acetates and benzoates of aliphatic alcohols, methyl, phenylethyl and triterpenoid esters (Christie, 2011).

Yam Species

In Malaysia, the local name for yam species is "keladi". It refers to herbaceous plants which have elephant ear-like leaves, regardless of the family or genus of the species. In short, the local naming of yams as "keladi" is purely based on the phenotype of the leaves. Typically, yams (in the context of Malaysia) include genera such as *Colocasia*, *Caladium* and *Alocasia* (International Aroid Society, Inc., 2012). These genera are in the same family of Araceae (International Aroid Society, Inc., 2012), thus have similar phenotypes, the most notable being the shape and size of the leaves.

This particular family of plants was used for wax analysis because of the large size of its leaves. Moreover, the leaves are good water repellent, thus is further physical evidence that a substantiate amount of wax is present. The cultivation method is also fairly easy. According to an article by Carey and Avent (2010), the cultivation of *Colocasia* utilizes only water and fertilizers. Besides that, yams are common in areas around Kuching as they are cultivated for human consumption, as well as for animal feed (Moore, 2003), such as *Colocasia esculenta*. *Alocasia macrorrhiza* can be found easily in, for example, drains and plantation areas, where plenty of water is available.

Banana Species

Banana species is under the family of Musaceae (Ploetz, Kepler, Daniells, & Nelson, 2007). Although banana refers generally to the genus *Musa*, this genus actually has two distinct plants, namely bananas and plantains (Nelson *et al.*, 2006). This family of plant originated from Southeast Asia (Ploetz *et al.*, 2007). Although bananas and plantains are widely used in products such as medicine, beverages, fibers, dyes, etc, the best known use of bananas and plantains are as edible fruits (Ploetz *et al.*, 2007). Most of these edible varieties originated from two species, namely *M. acuminata* and *M. balbisiana*, with modern species usually hybrids of the two species, or between subspecies of *M. acuminata*, resulting in hybrids that are either diploids, triploids or tetraploids (Ploetz *et al.*, 2007).

To denote the genome of different cultivars, lettering system is used, such as AA and BB to denote *M. acuminata* and *M. balbisiana* which are diploid (Ploetz *et al.*, 2007). The other hybrids are given letters indicating their genomic compositions, with A representing genomes originating from *M. acuminata* and B representing genomes originating from *M. balbisiana* (Nelson *et al.*, 2006). Examples are diploids with letterings of AA, AB and BB, triploids (most common cultivar) such as AAA, AAB and ABB, as well as tetraploids such as AAAB, AABB, AAAA and ABBB (Nelson *et al.*, 2006; Ploetz *et al.*, 2007; Abdullah *et al.*, 2011).

This particular species was used as the leaves of the plants are large and have a layer of visibly thick wax. Moreover, bananas grown in Malaysia covers a total land area of approximately 27500 hectares as of early 2011, with most of the plantations located in

the states of Johor, Pahang and Sarawak (Mokhtarud-din, 2011). According to Mokhtarud-din (2011), large companies are beginning to invest in banana cultivation, thus promising that more banana plantations will be opened up in the near future. With cultivars such as *M. acuminata Colla* (Pisang Mas), *M. acuminata×balbisiana Colla* (Pisang Rastali) and *M.×paradisiaca* 'Horn' (Pisang Tanduk) which can be found locally in areas near or around Kuching, samples from these cultivars are fairly easy to obtain.

Commercial Uses of Wax

To date, waxes are used commercially in many fields, which include the field of dentistry, cosmetics, automobile and commercial applications. For example in cosmetics, waxes such as candelilla wax, beeswax and carnauba wax are used as ingredients for producing varnish, lipsticks, lotions and creams (All Natural Cosmetics 4u, 2012). In dentistry, applications of waxes include baseplate wax, bite registration wax, indicator wax, sticky wax, utility wax and diagnostic wax-up (Integrated Publishing, n.d.). As for commercial applications and automobile industry, waxes are applied in the production of candles, car polish, lubricants and dyes.

Waxes are also used in paper, textile and match impregnants, fruit and food preservatives, insulators for electrical wires, manufacture of polishes and pharmaceuticals (Ivanovszky, 1955). Besides the commercial industries, waxes are used as starting materials for chemical conversions such as chlorination, oxidation, cracking and polymerization (Ivanovszky, 1955).

MATERIALS AND METHODS

Materials

The materials for this study were the local yam and banana species. A total of 3 different yam and banana species respectively were used. Both plant varieties were readily available as they are cultivated as food crops. For example, banana plantation area increased by 24% to 27453 hectares from 2008 to 2009 (TFNet, Mardi, & DOA, 2011). The species of plants used in this study are as follow:

Table 3: Species of Plants Used

Yam Species	Banana Species
1. *Colocasia esculenta* (Taro) 2. Keladi Pinang 3. *Alocasia macrorrhiza* Borneo Giant	1. Pisang Pisang 2. *Musa acuminata x balbisiana Colla* (AAB Group) (Pisang Rastali/Restali) 3. *Musa acuminata Colla* (AA Group) (Pisang Mas)

The chemicals used in this study are n-hexanes, absolute methanol, acetone, sodium chloride (NaCl), potassium bromide (KBr) and dichloromethane (DCM). Other apparatus used are beakers, conical flasks, knives, separating funnels and gas chromatography (GC) vials.

Sample Collection and Preparation

Samples of yam and banana were collected either from plantations, farms or their natural habitat. All samples were cleaned with running tap water to remove dusts, soil particles and other contaminants. The samples were then left to dry under the fan to minimise contamination. Samples which were not used immediately were stored at 4°C.

Wax Extraction Protocol

(i) Extraction via scraping

Waxes from samples of Pisang Pisang and *M. acuminata x balbisiana Colla* were extracted on-site by scraping. This procedure was used for samples SB1, SB2, SB3, SB4, SB5, SB6 (a), SB6 (b) and SB7 (a). A sharp knife was used to carefully scrap the visible wax layer from the stem and also the abaxial surface of the leaves. The scraped waxes were stored in separate beakers and labelled accordingly. The wax samples were brought back to the laboratory and stored under -20°C if it was not used for further analysis or process immediately.

The wax samples were dissolved in n-hexane. The amount of n-hexane used was just enough to cover all the wax. The setup was left to stand for 24 hours under room temperature to allow all the wax to dissolve in the n-hexane. After that, the undissolved sediments were removed and fresh hexane was added to it. The n-hexane containing the dissolved wax was collected and stored under -20°C. This process was repeated for a total of three times and the dissolved wax was pooled. Then, the pooled n-hexane containing

the dissolved wax was left to dry under room temperature in a fume hood. The dried layer of wax was stored under -20°C until further analysis.

(ii) Extraction using n-hexane

This procedure was used for samples SY2, SY3, SY4, SB9 (a) and SB9 (b). Leaf samples were weighed and the readings were recorded. The samples were cut into small pieces and were placed in a beaker of appropriate size. After that, the leaf pieces were immersed in n-hexane for 24 hours. The amount of n-hexane used was just enough to cover all the leaf pieces. The beaker was covered with aluminium foil to minimise the evaporation of the n-hexane. The setup was left in a fume hood for 24 hours. After 24 hours, the n-hexane was collected in a new beaker. The beaker with the collected n-hexane was covered with aluminium foil and secured with tapes to prevent evaporation. The collected n-hexane was stored at -20°C. Fresh n-hexane was used to immerse the leaves for another 24 hours. This process was repeated for a total of three times and the collected n-hexanes were pooled. At this stage, the collected n-hexane was greenish in colour, thus suggesting contaminations by chlorophylls.

Next, absolute methanol was added to the pooled n-hexane. This is because polar substances such as chlorophylls dissolve in alcohols; whereas wax will remain dissolved in the n-hexane. The mixture was mixed thoroughly by swirling actions. Two distinct layers will be formed, with n-hexane as the top layer (lower density) and absolute methanol as the bottom layer. A separating funnel was used to separate the two layers. The n-hexane layer was retained and the separation steps were repeated for a total of three times. After three rounds of separation, the n-hexane containing extracted wax was

collected in a beaker. At this stage, the n-hexane was much clearer than before separation. A few drops of the sample were used for thin layer chromatography (TLC) to test for the presence of wax and other possible contaminants.

2 ml from each samples SY4 and SB9 (a) were collected in GC vials for GCMS procedure. The remaining sample was left to dry in a fume hood under room temperature. For samples SY2, SY3 and SB9 (b), no n-hexane were collected before drying. The layer of dried wax was weighed, collected and labelled accordingly. All data was recorded.

TLC Protocol

Using a sharp pencil, two straight lines were drawn on the TLC plate; one on each end of the plate. After that, a capillary pipette was used to spot 1 – 2 µl of the sample on one end of the TLC plate on the drawn line. The spot was then left to dry. Next, the TLC plate was placed into a TLC tank with the side spotted with the sample at the bottom of the tank. DCM was added as solvent into the TLC tank. The level of DCM added was maintained below the drawn line. The setup was closed with a cover and left to stand to allow the solvent front to reach the upper line. Then, the TLC plate was taken out using forceps and placed in the ultraviolet (UV) chamber. The sample was separated according to the compound type. A photo of the TLC plate was taken as evidence.

Gas Chromatography-Mass Spectrometry (GC-MS) Protocol

The wax samples labelled SY4 and SB9 (a) were analysed using GC-MS. The 2 ml of each sample collected earlier in GC vials were used. After the standard for the analysis was determined, the samples were injected into the GC-MS analyser. The analyser was put on automatic mode. The GC-MS graphs generated from this analysis were saved and interpreted to determine the possible classes of compounds present in each sample. The GC standard used for this analysis was as follow:

Table 4: GC-MS Standard Used

Injector temperature	: 250°C
Injection mode	: split (20)
Initial temperature	: 50°C
Ramp rate	: 20.00
Final temperature	: 280°C
Interface temperature	: 300°C
Column	: BPX-5
Initial time	: 0.00 min
Final time	: 30.00 min