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MOLECULAR SIEVE APPLICATION IN THE RECOVERY OF GAHARU ACTIVE MARKER COMPOUND FROM WATER MIXTURE

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ABSTRACT

The essential oil of agarwood, which is known as Gaharu in Malaysia, is highly prized for its value for luxury fragrances and incense. However, the complexities of the chemical composition of agarwood are the primary challenge facing the establishment of an effective recovery method that can ensure uniform qualities and standards for each batch of essential-oil production. Although agarwood oil can be easily retrieved using the present hydro-distillation method, the high water solubility properties of a number of their key markers are the limiting factor for a proficient retrieval process. Regarding this problem, an elementary chemistry resolution study was performed on commercial agarwood essential oil-water mixture with the application of high performance liquid chromatography (HPLC) and fourier transforms infrared spectroscopy (FTIR). Interpretation of the results leads to a theoretical postulation that the agarwood water mixture consists of agarospirol, jinkohol, jinkoh eremol and kusenol. This study serves to identify the chemical characteristics of water-soluble agarwood compounds and to provide insight for researchers to develop a more strategic technique to improve their extraction process. In addition, this study is the groundwork on the ability of nano-sieve technique in the recovery of water soluble agarwood marker compounds from agarwood hydrosol. To achieve this purpose, a polypiperazine (PPA) and polyethlenesulfone (PES) based nanofiltration (NF) membrane were used. The effectiveness of this membrane on the separation of agarwood marker molecules was analysed and FTIR results showed that most of marker compounds (i.e. agarospirol, jinkohol, jinkoh eremol and khusenol) has been successfully separated from the aqueous agarwood. However, the performance of the membrane in terms of flux and permeability is quite low, which is 23.30 L/m2.h, with 6.76 L/m2.h.bar, respectively. The recovery of gaharu marker compound using membrane would help the local gaharu industry to improve the quality of gaharu essential oil.

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ABSTRAK

Minyak pati gaharu, dikagumi untuk nilai wangian mewah dan sebagai bauan. Walau bagaimanapun, kerumitan komposisi kimia minyak gaharu adalah cabaran utama dalam mewujudkan kaedah pengekstrakan semula yang berkesan yang boleh memastikan kualiti dan standard seragam bagi setiap kelompok minyak. Minyak gaharu mudah diekstrak menggunakan kaedah hidro penyulingan ini. Sifat-sifat keterlarutan di dalam air yang tinggi beberapa sebatian penanda utama minyak gaharu adalah faktor yang mengehadkan untuk proses mendapatkan semula sebatian-sebatian ini. Bagi mengatasi masalah ini, satu kajian resolusi kimia asas telah dilakukan ke atas gaharu komersil yang mempunyai campuran sebatian penanda dengan penggunaan kaedah pencirian kromatografi cecair berprestasi tinggi (HPLC) dan fourier pengubah spektroskopi inframerah (FTIR). Hasil pencirian HPLC menunjukkan bahawa campuran air gaharu terdiri daripada agarospirol, jinkohol, eremol jinkoh dan kusenol. Kajian ini berfungsi untuk mengenal pasti ciri-ciri bahan penanda utama sebatian gaharu yang mudah larut air. Ini akan membantu penyelidik untuk membangunkan teknik yang lebih strategik untuk meningkatkan proses pengekstrakan mereka. Di samping itu, kajian ini akan menggunakan keupayaan teknik nano-ayak dalam pengekstrakan sebatian penanda gaharu yang larut air seperti hydrosol. Untuk mencapai tujuan ini, polypiperazine yang nanoayak (PPA) dan polyethylenesulfone nanoayak (PES) berdasarkan (NF) membran telah digunakan. Keberkesanan membran memisakan molekul penanda gaharu dianalisis dan keputusan FTIR menunjukkan kebanyakan sebatian penanda (iaitu agarospirol, jinkohol, jinkoh eremol dan khusenol) telah berjaya diasingkan daripada gaharu akueus. Walau bagaimanapun, prestasi membran dari segi fluks dan kebolehtelapan adalah agak rendah, iaitu 23.30 L / m2.h, dengan 6.76 L / m2.h.bar, masing-masing.

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LIST OF SYMBOLS

Da	Dalton
H	Hour
kV	kilo Volt
L	Liter
m	meter
mL	mili Liter

LIST OF ABBREVIATIONS

AW	Agarwood Water
FTIR	Fourier Transform Infra Red
GC	Gas Chromatography
HPLC	High Performance Liquid Chromtography
Jy	Flux
MW	Molecular Weight
NF	Nanofiltration
P_m	permeability coefficient
PPA	Polypiperazine
PVA	Polyvinil Alcohol
OH	Hydroxyl functional group
SEM	Scanning Electron Microcope
TFC	Thin Film Composite
TMPs	Transmembrane pressure
V	Volume

CHAPTER 1

INTRODUCTION

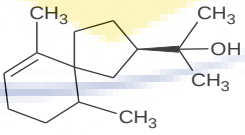
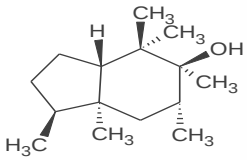
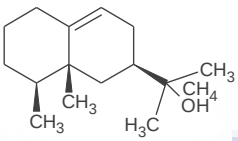
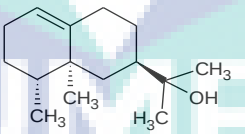
1.1 Background of the Study

Agarwood, or 'gaharu/karas' in Malay is the resin produced by *Aquilaria Malaccensis* tree, results from their defence mechanism against injuries, either natural or artificial. The oil of agarwood is significantly prized, especially for perfume, incense, religious rituals and medicinal purpose. Sesquiterpenoids compounds in Table 1 are the main marker compounds which are responsible for the aromatic scent of agarwood oil and highly prized for the determination of market price of this essential oil. At present, hydro distillation technique, utilizing water as solvent is commercially being used to extract the agarwood essential oil from the agarwood trees. Although this method is widely applied in agarwood industry, this technique however could not recover most of sesquiterpenoids compounds of agarwood. These compounds possess hydroxyl (OH) moieties, and have the ability to form hydrogen bonding with water. This reflects to their possibility to dissolve in water during processing. As a consequence, it is hypothesized that, they will be '*left-out'* in the hydrosol/distillate. Thus, the essential oil product quality and price is low.

Considering the demand and high value of these compounds, a new further purification approach needs to be explored. As an elucidation to this problem, a polymeric nanofiltration (NF) membrane is proposed to be the approach for the complete recovery of these sesquiterpenoids molecules from the hydrosol fraction. The molecular property of nanofiltration membrane surface plays an important role in driving and controlling the mass transfer during the separation of sesquiterpenoids marker compounds from water.

The type of suitable functional groups in the membrane material would provide and form the intermolecular interaction and particular pore size with the marker solute molecules.

Table 1.1 : Characteristics of agarwood sesquiterpenoids marker compounds

Chemical compounds	Chemical Formula	Molecular structure	Functional groups	Molecular weight (Da)
Agarospirol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane	222.36
Jinkohol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkane	222.36
Jinkoh eremol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane	222.36
Kusenol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane	222.36

In this work, the molecular dynamic simulation will be applied to recognize and understand the intermediate chemical interaction between the solvent (water), solute (sesquiterpenoids) and membrane material at the molecular level. By the appropriate recognition of their molecular chemistry relation, a formulation of NF membrane with an optimum performance could be customized. Once an efficient separation technique of these sesquiterpenoids molecules has been established, an insight to the product ion of agarwood essential oil with optimum quality could be anticipated. In the end, the research

does not only expect to enhance the profits of local licensed agarwood traders, but also proliferates country's income.

1.2 Objectives

1. To chemically characterize and analyse the main marker gaharu compound which is soluble in water solution using HPLC and FTIR
2. To design and develop the affinity nanofiltration membrane prototype for active gaharu marker compound recovery from water solution mixture.
3. To optimize the separation process in order to achieve the optimum yield.

1.3 Scope of the Study

In this study, there are a few parameters that are needed to be controlled. The parameters include permeability and selectivity of the membrane, permeate flux and the pressure where these parameters will affect the membrane's performance in the separation process.

During the experiment, the membrane performance can affect how the marker compounds of gaharu are being separated and filter out. Therefore, it is important to know the concept of permeability, selectivity, mass transfer and diffusion process in a membrane system.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

One of the objectives of this chapter is to explain the gaharu and its marker compound. Explanation on gaharu will be more detailed in the Aquilaria Malaccensis species which are found in Peninsular Malaysia. Then this chapter will explain the types of membrane that can be used in membrane separation.

The literature review is done based on the journals that are related Aquilaria Malaccensis species of gaharu. The literature review will cover the origins, types and characteristics of Aquilaria Malaccensis. This chapter will also cover the gaharu marker compound in its essential oils.

The membrane application will also be covered in the scope of this chapter. Types of membranes, properties and their application will be explained in this chapter. There are three types of membrane that will be discussed here which are ultrafiltration, microfiltration and nanofiltration.

2.2 Agarwood (Aquilarria Malaccensis)

Gaharu is a fragnant wood that is derived from the diseased timber of the genus Aquilaria. It is often occurs as dark coloured patches or streaks in the tree. Gaharu oil is greatly valued as perfume ingredient and incense because of its strong, unique scent and medical properties (Nor Azah et al. 2008).

Aquilaria genus is an aromatic plant that is commonly known as "Gaharu Wood" in South East Asia. The Aquilaria species is found mostly in Malaysia, Indonesia, India, Iran, Singapore, Bangladesh, Myanmar, Philippines and Thailand. One of the species, Aquilaria Malaccensis produces valuable resin marinate in the heartwood (Ibrahim et al. 2011).

Figure 2.1: Aquilaria Malaccensis Tree

Gaharu are classified into various grades such as Grade A, Grade B, Grade C and Grade D. Gaharu is graded according to their physical properties, formation and their unique scent (Nor Azah et al. 2008). Aquilaria Malaccensis produces valuable resin marinate in the heartwood which originates as a consequences of natural immune response towards fungal attack. One of

the fungus, endophyte lives inside healthy plant tissues. Some of these endophytes have found to have anticancer and antidiabetic properties. Essential oils of Aquilaria Malaccensis is safe and simple to use. It is produced and commonly used in traditional medicine to relieve pain, fever, rheumatism, and asthma (Ibrahim et al. 2011).

2.3 Gaharu Marker Compound

The lower grades of gaharu such as Grade C are often distilled to get gaharu oil (Nor Azah, 2008). In an extraction process, the presence of main components such as agarospirol, jingkohol-eremol, jingkohol and eremol are found in gaharu essential oils. In this study, it is desired to separate four compounds from the gaharu water mixture using membrane separation. The five compounds are agarospirol, jinkohol, guainene and selina.

2.3.1 Agarospirol

Agarospirol is a chemical compound that consists of fifteen carbon atoms, twenty six hydrogen atoms and one oxygen atom. The molecular weight and volume are 222.366 and 183.5 respectively. This compound has five methyl groups and a functional group of hydroxyl.

Agarospirol is reported as one of the main marker compound that contribute to the scent of gaharu. Figure 2.2 represent the molecular structure of agospirol. Table 2.1 shows the chemical formula and molecular weight of agarospirol.

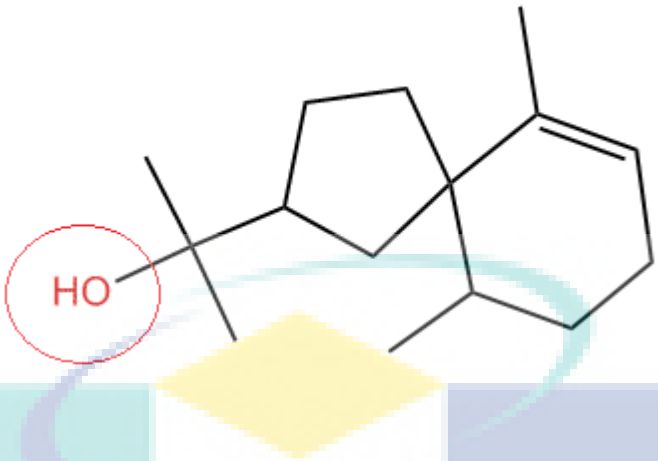


Figure 2.2: Molecular Structures of Agarospirol

Table 2.1 : Properties of Agarospirol

Chemical Formula	Molecular Weight
$C_{15}H_{26}O$	222.366

2.3.2 Jinkohol II

Jinkohol II is a chemical compound that consists of fifteen carbon atoms, twenty six hydrogen atoms and one oxygen atom. The molecular weight and volume are 222.366 and 176.64 respectively. It has three methyl group and the functional group of one hydroxyl group.

Similar with agarospirol, jinkohol II is classified as the main marker compound in gaharu. Jinkohol II also contributes to the characteristic aroma of gaharu. Figure 2.3 represent the molecular structure of jinkohol II. Table 2.2 lists the chemical formula and molecular weight of the compound.

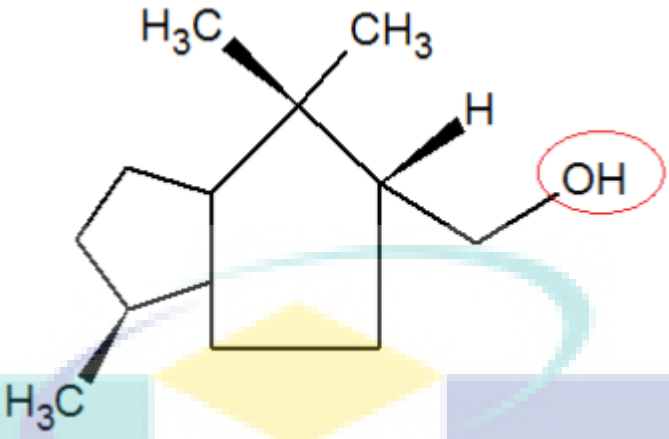


Figure 2.3 : Molecular Structure of Jinkohol II

Table 2.2: Properties of Jinkohol II

Chemical Formula	Molecular Weight
$C_{15}H_{26}O$	222.366

2.3.3 α-guaiene

α-guaiene is a chemical compound that consists of five carbon atoms, five hydrogen atoms, five nitrogen atoms and an oxygen atom. The molecular weight and volume are 151.126 and 83. It has three double bonds.

α-guaiene is one of the main marker compound in the gaharu water mixture. Therefore it is one of the most important compound that present in the gaharu water mixture. Figure 2.4 represent the molecular structure of α-guaiene. Table 2.3 lists the chemical formula and molecular weight of α-guaiene.

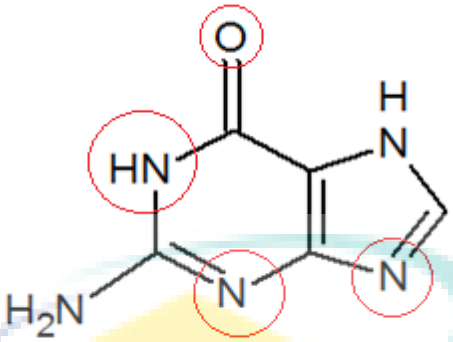


Figure 2.4: Molecular Structures of α-guaiene

Table 2.3: Properties of α-guaiene

Chemical Formula	Molecular Weight
$C_5H_5N_5O$	151.126

2.3.4 Selina-3,11-dien-9-al

Selina-3,11-dien-9-al is a chemical compound consists of hydrogen bonds and methyl groups. Similarly with other compounds that were explained above, selina-3,11-dien-9-al is also one of the main marker compound that present inside the gaharu.

The molecular structure of selina-3,11-dien-9-al has the functional group of hydroxyl. Figure 2.5 represent the molecular structure of selina-3,11-dien-9-al. Table 2.4 lists the chemical formula and molecular weight of selina-3,11-dien-9-al.

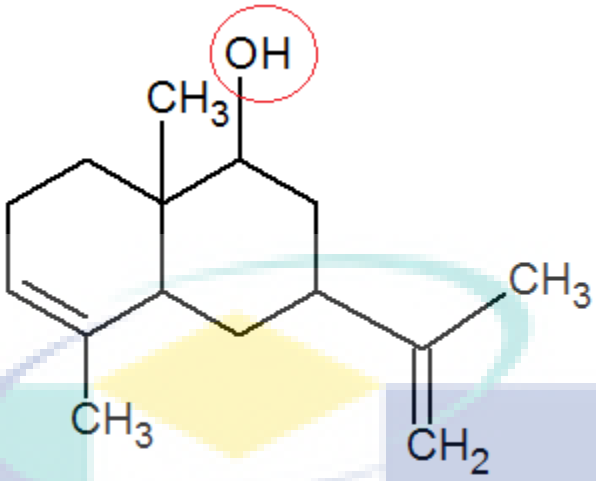


Figure 2.5 : Molecular Structure of Selina-3,11-dien-9-al

Table 2.4: Properties of Selina-3,11-dien-9-al

Chemical Formula	Molecular Weight
$C_5H_5N_5O$	151.126

2.3.5 Kusenol

Kusenol is also a marker compound in gaharu essential oil. It posses a hydrogen bond donor OH (hydroxyl) functional group and a polar compound. Figure 2.6 represent the molecular structure of Kusenol and Table 2.5 lists the chemical formula and molecular weight of kusenol.

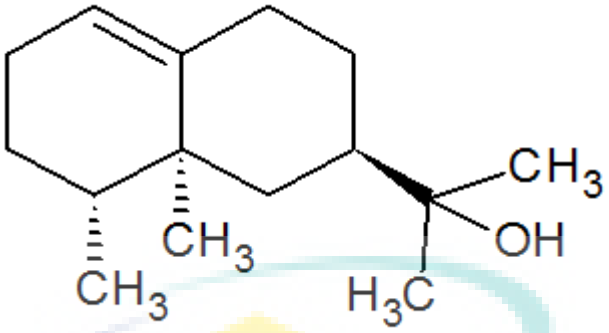


Figure 2.6 : Molecular Structure of Kusenol

Table 2.5: Properties of Kusenol

Chemical Formula	Molecular Weight
$C_{26}H_{32}O_8$	472.527

2.4 Membrane Separation

2.4.1 Introduction

Membrane separation is becoming increasingly important in process industries. In this process, the membrane acts as a semi permeable barrier. The separation process occurs by the membrane controlling the rate of the movement of various molecules between two liquid phases, two gas phases, or a liquid or a gas phase (Geankoplis et al. 2003). In this study, three types of membrane separation process will be discussed which are ultrafiltration, nanofiltration and microfiltration.

2.4.2 Advantage of Membrane Separation

Membrane separation can be used in a number of applications. One of them is the separation of two chemical compounds in a mixture that have narrow boiling points. In the field of hydrocarbon separations, the separation of condensed olefin from corresponding paraffin is complicated process. Separation of paraffin and olefin by conventional distillation is expensive and difficult as the two substances has a narrow temperature ranges.

Therefore, membrane separation can be offered as an alternative for the separation process. In the polymeric matrix membranes, the introduction of chemically active sites can offer increase membrane selectivity and permeability.

This can therefore improve in the efficiency of olefin and paraffin separations (Bessarabov et al. 1999). Besides that, membrane separation can also be applied in the water and waste water treatment plant. A number of advantages can be offered by membrane processes over conventional water and waste water treatment processes. The advantages include reduced environmental impact of effluents, land requirement reduce, higher standards of final product and the potential of the mobile treatment units of membrane process.

In the water and waste water treatment, membranes can used in a few applications. The applications include removal of colour, trihalomethanes and other disinfection by product removal and also iron removal.

Membrane processes are also reported to be cost effective in a few situations and applications. It can be used as an alternative to conventional softening plant and as a pretreatment plant for reverse osmosis (Owen et al. 1995).

2.4.3 Nanofiltration

Nanofiltration membrane has been largely developed and commercialized for the past decade. It is one of promising technology for the separation of neutral and charged solutes in aqueous solution (Wang et al. 2009).

Nanofiltration membrane pass low relative to high molecular weight solutes (300 to 2000 Daltons). The typical applications of nanafiltration are concentrating and desalting food products, separating organic and multivalent ions from water, removing edible oils from organic extraction solvent, separating amino acids, peptides, proteins in bioprocessing, and recovering organic solvents in the production of pharmaceuticals (Gupta et al. 2007).

According to Jahanshashi & Peyravi (2010), the performance characteristics of the nanofiltration membrane stands at between those of reverse osmosis and ultrafiltration membrane. The main advantages of nanofiltration process are low operating pressure, high fluxes, high rejection of multivalent salt, low investment and operation cost. Nanofiltration membrane is also used for treatment process such as water softening, waste water treatment, color removal, chemical oxygen demand and biological oxygen demand reduction, pharmaceutical and biochemical industries.

2.4.4 Ultrafiltration

An ultrafiltration filter has a pore size of 0.01 micron. Ultrafiltration is currently used for the concentration of a wide range of protein products (Mehta and Zhydney, 2005). The effects of pore size on the performance of ultrafiltration membranes are fairly understood. Membrane with large pores tends to have high filtrate flux but low protein retention, with the reverse being true for membranes with small pores.

Ultrafiltration process is a very versatile and widely employed separation process whose nature is between reverse osmosis and microfiltration. The membranes for ultrafiltration have pore size typically ranging from $1x10^{-9}$ to $5x10^{-8}$m. They are capable of retaining species in the molecular weight range of 300-500000 Da (Hamza et al. 1997).

In the ultrafiltration process, pressure is used to obtain a separation of molecules, by means of a semipermeable polymeric membrane. The membranes discriminate on the basis of molecular size, shape, or chemical structure and separates relatively high molecular weight solutes. The solutes or molecules to be separated generally have molecular weights greater than 500 and up to 1000000 (Geankoplis, 2003).

According to Hamzah et al, macromolecules, targeted for separation by the ultrafiltration membrane, tend to be adsorbed, on to the surface and into the pores of the membrane material, which is mostly polymeric. The extent of adsorption depends on the different type of solute macromolecule-membrane polymer interaction. The polymer interactions are such as hydrophobic/hydrophilic interaction, hydrogen bonding, van der Waals interactions and electrostatic effects.

2.4.5 Microfiltration

Microfiltration has been widely used for separating fine particles, colloids, and microbes in chemical, biotechnological, ceramic and material processes. Microfiltration process is economical and efficient but its development is hindered by membrane fouling due to membrane blocking, cake formation or concentration polarization (Hwang et al. 2007).

Microfiltration membranes are typically used to remove particles. The range of the particles is from 0.1 to 10 micrometer from a suspension. Crossflow microfiltration is a

pressure driven separation process, which is widely used in concentrating, purifying or separating macromolecules (Wakeman and Williams, 2002).

Crossflow microfiltration in polymeric membranes is widely used as a final refining step in commercial processes. The processes include drinking water production, wastewater treatment, food processing, pharmaceutical industry, biotechnology and biomedicine. However due to membrane fouling, remains a restriction (Seminario et al. 2002).

In the microfiltration process, pressure driven flow through a membrane is used to separate micron-size particles from fluids. The particle size ranges from 0.02 micrometer to 10 micrometer. The particles are usually larger than the solutes in reverse osmosis and ultrafiltration. These membranes retain bacterias and other microorganisms. Many different geometries of membranes are used. These include spiral-wound, plate and frame, hollow fiber, cartridge fiber with pleated membranes and so on (Geankoplis, 2003).

Table 2.6 : Comparison properties of Nanofiltration, Ultrafiltration and Microfiltration Membrane

	Nanofiltration	Ultrafiltration	Microfiltration
Membrane	Asymmetrical	Asymmetrical	Symmetrical Asymmetrical
Thickness Thin film	150 µm 1µm	150 µm -250 µm 1µm	10-150µm
Pore size	<0.002 µm	0.2-0.02µm	4-0.02µm

Rejection of	HWMC Mono- , di- and Oiligosaccharides Polyvalent neg. ions	Macro molecules, Proteins, Polysaccharides Vira	Particles, Clay, bacteria
Membrane material	CA Thin film	Ceramic PSO, PVDF, CA Thin Film	Ceramic PP,PSO,PVDF
Membrane module	Tubular Spiral Wound Plate and Frame	Tubular Hollow Fiber Spiral Wound Plate and Frame	Tubular Hollow Fiber
Operating pressure	5-35 bar	1-10 bar	< 2 bar
Molecular weight	100	200-400	500

2.4.6 Factors that Affect the Membrane Performance

In nanofiltration separation, the separation process occurs primarily due to steric hindrance and the membrane solute interaction. For the retention of uncharged molecules, non-electrostatic membrane-solute interaction such as Van der Waals forces and steric hindrance play a major role.

The transport of the uncharged molecules takes place by convection. The transport occurs due to the pressure difference and by diffusion due to a concentration gradient across the area of the applied membrane (Teixeira et al., 2005).

The neutral molecules also interact with membrane charge. The interaction is mainly through the polarity effects. Polarity can decreases the retention, which can be explained by electrostatic interaction directing the dipole towards the membrane.

Both steric hindrance and electrostatic interactions are affecting and responsible for the separation of charged compounds. The membrane charge along its surface and through the pores is also one of the important parameter in the transport process.

Membranes which are in contact with an aqueous solution can acquire electric charges by a few mechanisms. The mechanisms include dissociation of surface functional groups, adsorption of ions from the solutions and the adsorption of polyelectrolytes, ionic surface and macromolecules.

The charging mechanism can occur on the exterior membrane surface. It also can occur on the interior pore surface of the membranes. This is due to the distributions of ions in the solution to maintain the electroneutrality of the system (Teixeira et al. 2005).

2.5 Mass Transfer in Membrane

Mass transfer process in membrane is an important element. Mass transfer process shows how the marker compounds in the gaharu are attracted to the surface of membrane. Therefore, the membranes used in this research are chosen based on the interaction of membrane and the marker compounds inside the gaharu water mixture (UIC,2013).

The mass transfer process includes the diffusion of particles on to the membrane. Other than that, permeability and selectivity of the membranes also affect the diffusion pattern and rate of the membrane processes. In this section, the diffusion theory of membrane and the concept permeability and selectivity of membrane will be explained. (UIC,2013).

2.5.1 Diffusion

Diffusion can be defined as the spontaneous movement of particles from an area of high concentration to an area of low concentration. The diffusion process does not require energy as it is a exergonic and spontaneous process.

In the diffusion process, the random kinetic movement is present and exhibit around the mass transfer interaction. When the concentration on both sides and area of the membrane are equal, the net diffusion will stop. The same condition of stopping the net diffusion can also be achieved when there is already a uniform distribution of the particles (University of Arizona, 2013).

Net diffusion also stops whenever the equilibrium is reached. After the net diffusion stops, the molecules around the membrane continues to move, but there is no net change in the concentration in the area surrounding the membrane.

Molecules or particles can move through the membranes by two ways. The two ways are passive transport and active transport. Diffusion is the principle means of passive transport. The passive type of transport does not require energy and the transport process occurs spontaneously (University of Arizona, 2013).

The motion of the molecules affects the diffusion process and will continue until the system reaches the equilibrium state. The speed and how fast a molecule can diffuse can be affected by several factors. One of the factors is the kinetic energy of the molecule. The kinetic energy of the molecule is frequently measured as the temperature of the system.

At higher temperatures, molecules in a system have more energy compared with the molecules that present in lower temperatures. Therefore, the molecules in higher temperature will have higher amount of energy, thus will move faster and diffuse faster compared with molecules in lower temperatures (University of Arizona, 2013).

2.5.2 Permeability and Selectivity

Due to increasingly widely use of membrane, permeation through a membrane is an area of great interest. Permeability is an important parameter for the fundamental understanding the membrane extraction process. Permeability is also an important parameter to correctly selecting a membrane material for different types of applications.

It is important for an analyst to measure the permeabilities of their targeted analyst through a membrane. The permeability data for a specific membrane for different analytes are also available in the literature (Liu, 2006).

For an example, the reverse osmosis (RO) membrane desalation is a mature and practical process for the production of potable water from seawater. The current and newer RO membranes are available in high permeability. The high value of permeability must be sufficient enough to enable desalting in which the operational feed pressures can approach the thermodynamic osmotic pressure of the produced concentrated stream. The current high permeability membranes can enable equivalent and higher permeate productivity at lower pressure (Zhu et al. 2009).

In a membrane reactor, the chemical reaction and the separation take place simultaneously. The permeability and the selectivity of the membrane is a great concern in the performance of a membrane reactor and the membrane separation process.

It is known that the permeability and selectivity have an inverse relationship for a polymeric membrane. In the polymeric membrane, the selectivity has an upper bound at a given permeability.

Many efforts have been proposed and made to improve the selectivity of porous inorganic polymeric membrane at the same permeability by pore modifications (Won and Seung, 2000). Permeate flux was calculated according to the following equation:

$$Jv = V/ (t \times A) \qquad \text{Eq. 1}$$

Where Jv is flux (L/m2.h), V is volume of permeate in liter, t is time in hour and A is the effective area of the membrane, which is 0.00287 m2.

CHAPTER 3

METHODOLOGY

3.1 Introduction

In this study there are two main activities which are membrane separation experiments and molecular dynamic (MD) simulation run. The methodology for each activities are shown in Figure 3.1 and Figure 3.2.

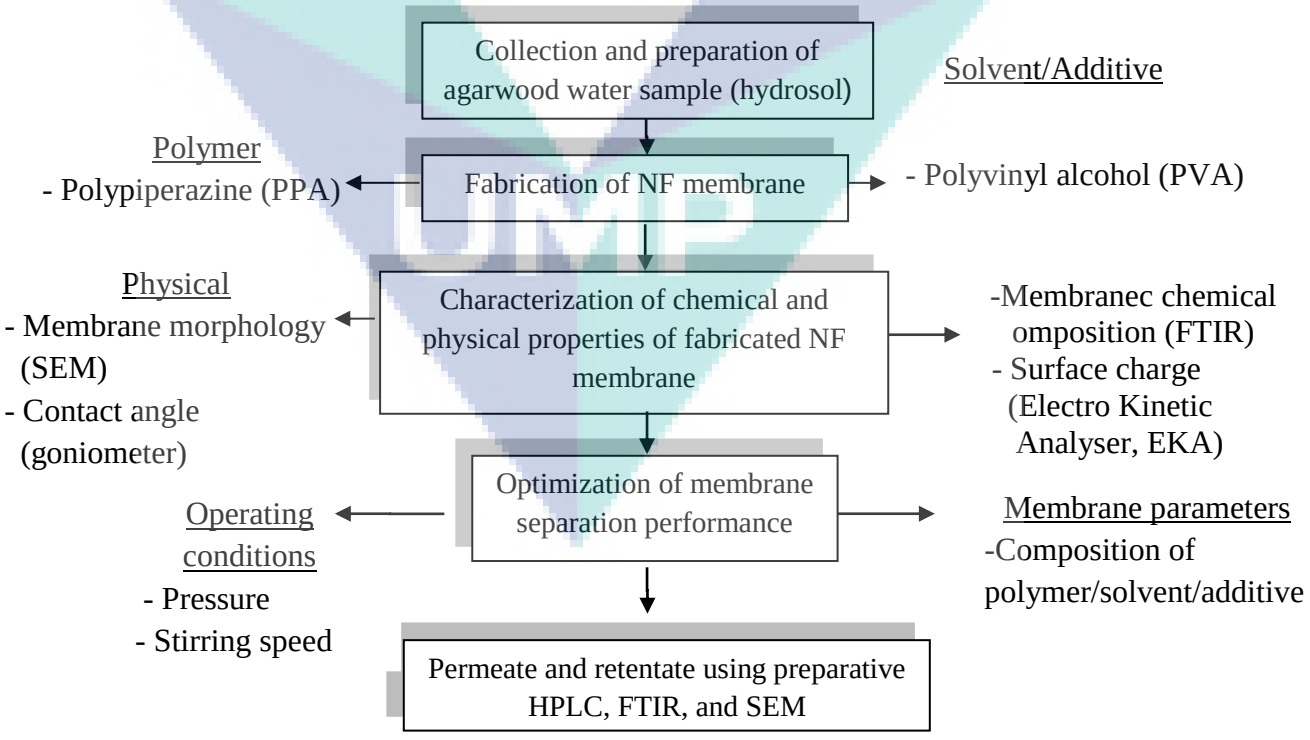


Figure 3.1: Membrane separation experiment

3.2 Separation Experiment

Agarwood essential oil was supplied by Kedaik Gaharu Sdn. Bhd., Rompin, Pahang, Malaysia, which is a local industry certified by the Forestry Department of Malaysia. Other chemicals and solvents (acetone and methanol, both of which were from Fischer Scientific (M) Sdn. Bhd.) for the analysis were of reagent grade.

The sample was prepared at two different temperatures: 30°C (room temperature) and 100°C (water boiling temperature). Test tubes were filled with a mixture of agarwood essential oil and distilled water (1:10). These tubes were stirred using a thermo mixer for 16 hours until the equilibrium mixture was obtained. After completion, the mixture was left for another eight hours to cool. Two layers (oil and water) were formed in each tube (Figure 6). The oil was separated from the water using a syringe. Figure 7 shows the separated solutions.

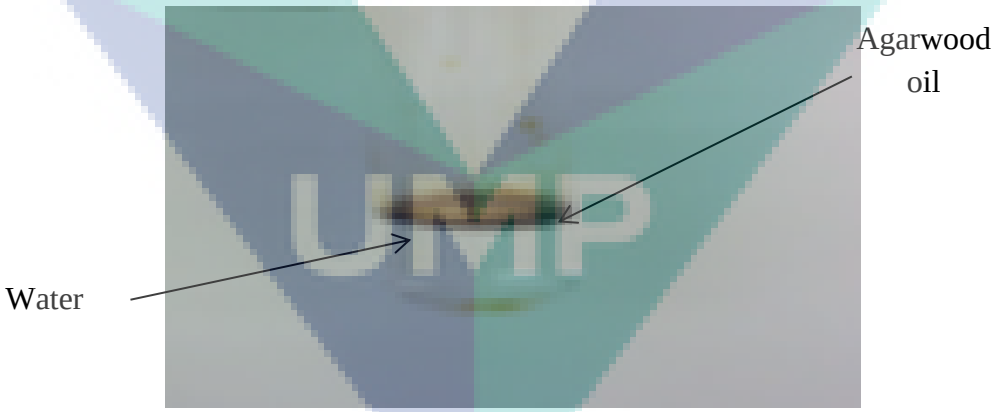


Figure 3.2: Mixture of agarwood essential oil-water

Figure 3.4 illustrates the molecular structure of polypiperazine amide which has functional groups that can attract the gaharu marker compounds due to the polarity nature of the molecules. Therefore, the marker compounds of gaharu can diffuse into the membrane surface and thus separated from the gaharu water mixture.

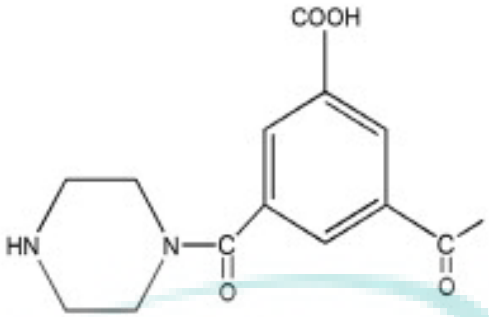


Figure 3.3: Molecular Structure of polypiperazine amide

3.2.1 Milipore Stirred Ultrafiltration Cell Model 8200

The experiment was conducted in a set of Milipore Stirred Ultrafiltration Cell Model 8200 or normally known as AMICON 8200 (Figure 3.5). AMICON 8200 has the ability for rapid concentration or purification of macromolecular solution in the volume of 200ml.

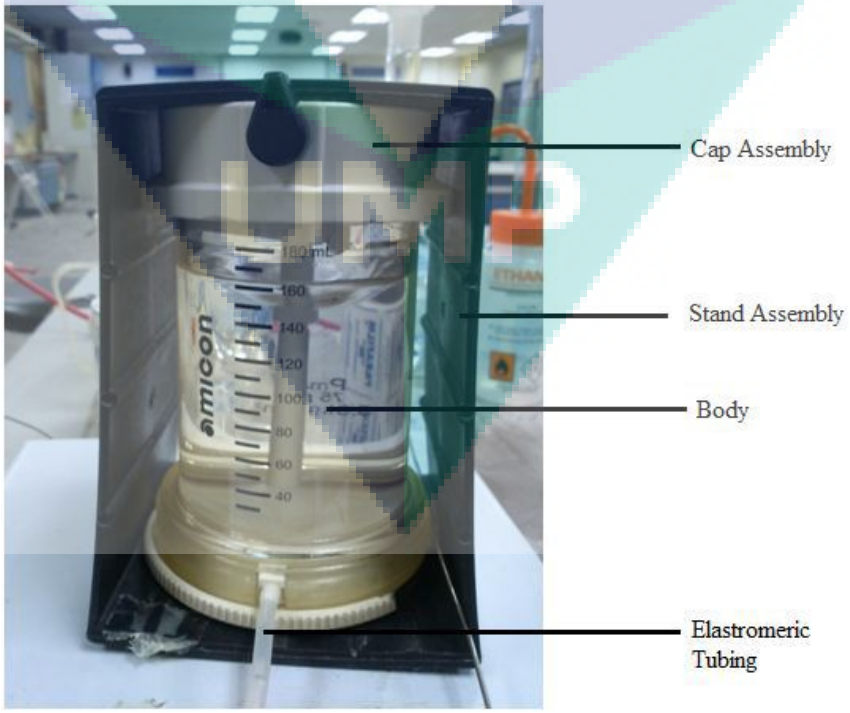


Figure 3.4: AMICON 8200 Model

The stirred cell model was pressurized by the supply of nitrogen gas. Nitrogen gas was chosen because of its inert nature and won't react with the solution inside the stirred cell model. In this experiment, the pressure applied to the stirred cell model was between 1 bar to 4 bar. From the instruction manual of the stirred cell model, the maximum pressure that can be applied is 5.3 bar while the recommended operating pressure is 3.9 bar.

The stirred cell model was operated under the concentration mode. In this concentration mode, the gas pressure was applied directly to the stirred cell model. The solutes above the membrane molecular weight cut-off are retained in the cell. On the other hand, the water and solutes below the cut-off pass into the filtrate and out of the cell.

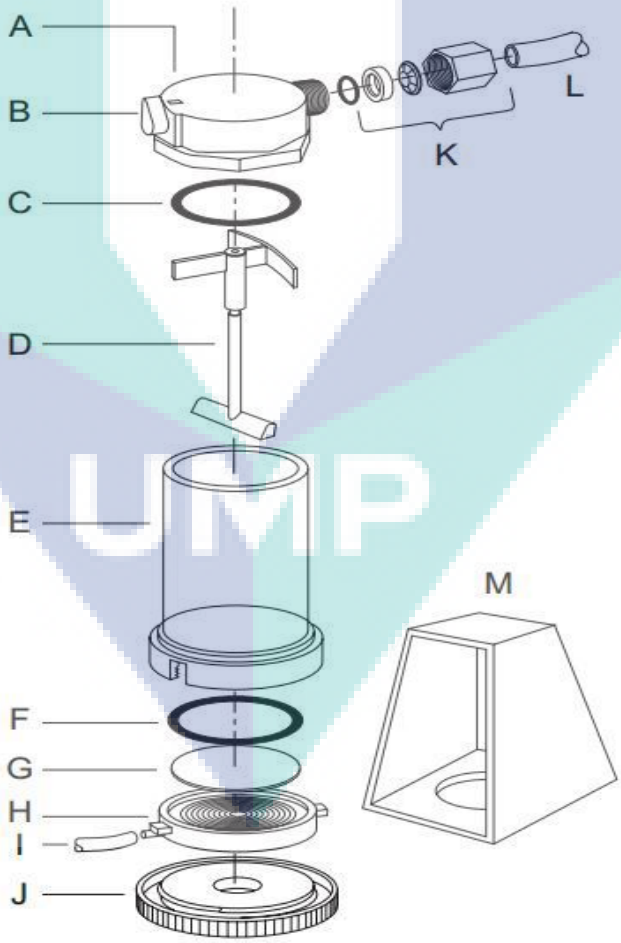


Figure 3.5: Parts List for AMICON 8200

Table 3.1 : Parts List for AMICON 8200

Label	*Item Description*
A	Cap Assembly
B	Pressure Relief Valve
C	O-Ring
D	Stirrer Assembly
E	Body
F	O-Ring
G	Membrane
H	Membrane Holder
I	Elastomeric Tubing
J	Base
K	Tube Fitting Assembly
L	Tubing, Plastic
M	Stand Assembly

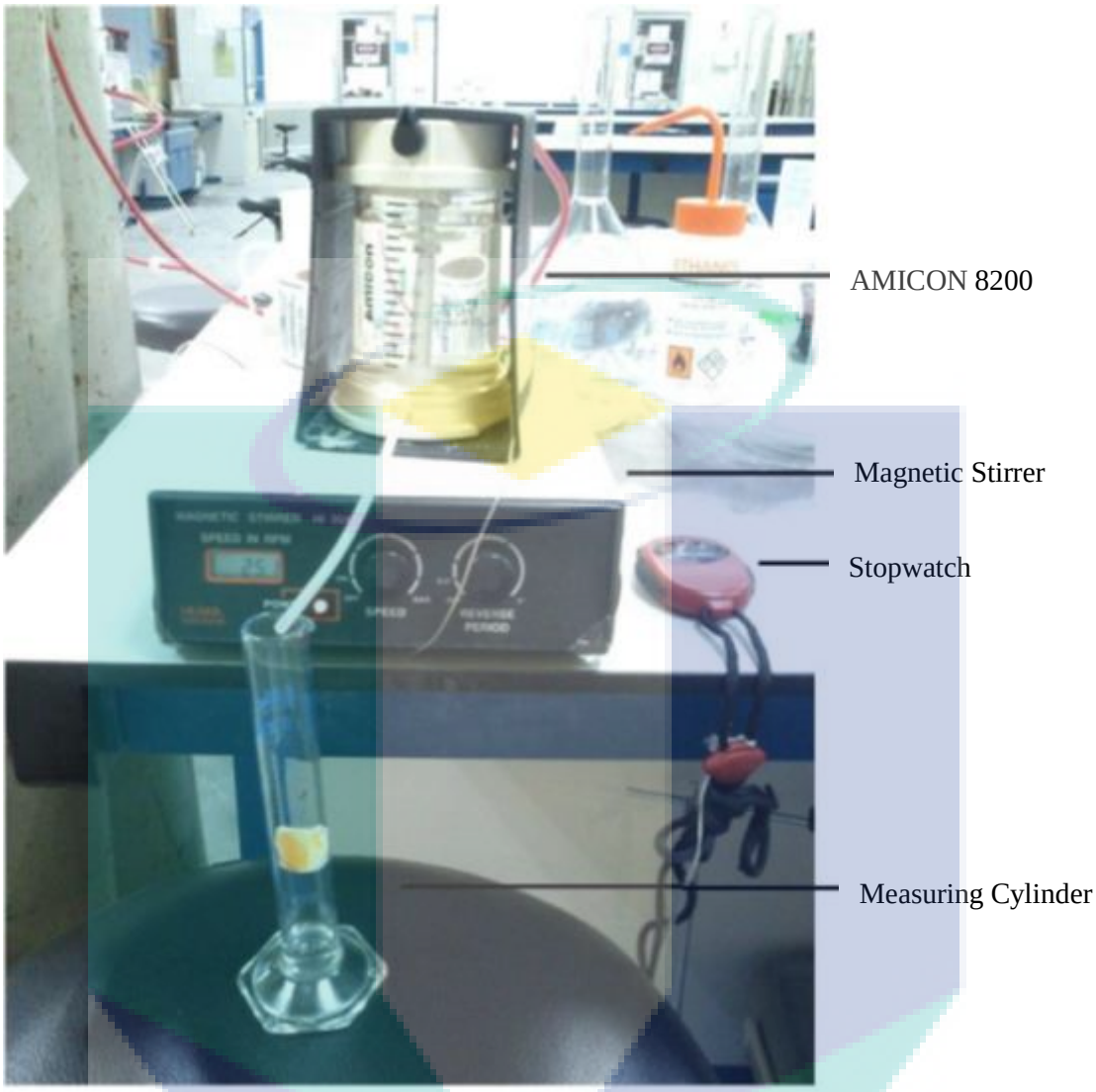


Figure 3.6 : Experimental Set Up of Membrane Separation

For the preparation, the membrane was cut to a circular size of 31.67 cm2 with the radius 31.67 mm. During the experiment, the effective membrane area will be 28.7 cm2.

3.3 Characterizations

In this work the characterisation of gaharu water solution and the membrane material used is explained in the next section. Characterisation of chemical composition

and physical of both solution and membrane were conducted using HPLC, FTIR and SEM.

3.3.1 Prep-High Performance Liquid Chromatography

The High Performance Liquid Chromatography (HPLC) was performed to quantitatively analyze the compounds in agarwood essential oils and agarwood water mixture. The HPLC in this experiment was the Waters Auto Purification System, and the column was Zorbax Eclipse C18 (4.6 x 150 mm). The mobile phase was an isocratic combination of methanol (MeOH):H_2O with a flow rate of 1 mL/min. The operating volume for all samples and control was 40 µl. The runtime was 40 minutes, and all samples were dissolved in methanol prior to the sample injection.

3.3.2 Permeation Flux Study

Permeation process: Optimization of permeation process was performed by applying different transmembrane pressure (TMPs). 20 mL of feed solution (agarwood hydrosol) was poured into permeation apparatus and the volume of permeate collected in five minutes was measured. Permeate flux was calculated according to the following Eq. 1.

The filtration was carried out at different pressure to calculate the permeability coefficient (Pm), which is determined from the slope of the graph. Permeate and retentate stream were both analysed by Fourier Transform Infrared (FTIR).

3.3.3 Fourier Transform Infra Red

The FTIR measures the frequencies at which the sample absorbs, and also the intensities of these absorptions. The frequencies are helpful for the identification of the sample's chemical make-up due to the fact that chemical functional groups are responsible for the absorption of radiation at different frequencies. FTIR is very helpful for the predictive assignment of chemical compounds. The spectrum of all samples (agarwood hydrosol, retentate and permeate) was recorded at range of 4000 – 400 cm-1 (mid infrared spectroscopy) at 4 cm-1 resolution (FTIR model: Nicolet Avatar 370 DTGS).

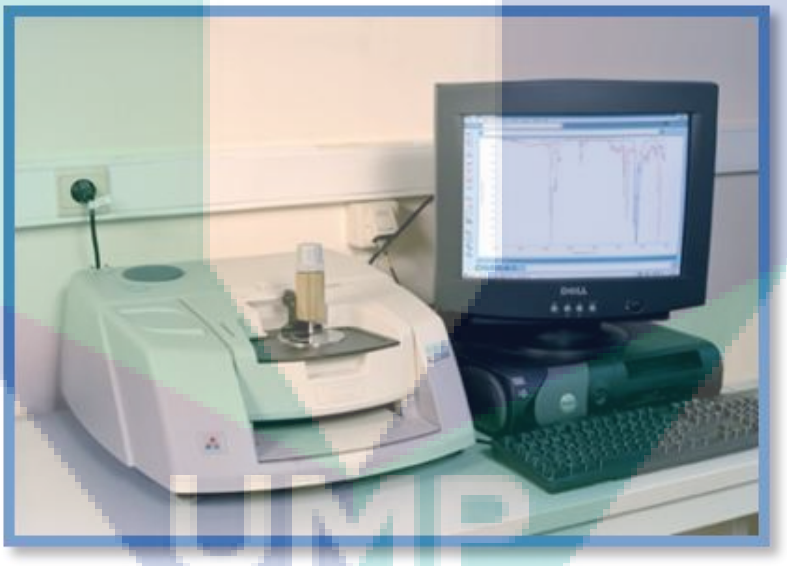


Figure 3.7: Sample placed on the FTIR Sample Holder

3.3.4 Scanning Electron Microsopy

Scanning Electron Microscopy (SEM) is a scientific instrument that uses a principle of a narrow beam of the electron with kinetic energies hits (1 to 25kV) the membrane sample.

It allows a clear and concise view of the overall membrane structure. For this work, a scanning electron microscopy (Model: Zeiss Evo 50) was used to probe the morphological features of membranes. Small pieces of membrane were fractured cryogenically in liquid nitrogen, and sputtered by platinum, prior to viewing under SEM.

Figure 3.8 : Scanning Electron Microscope (Carl Zeiss EVO50)

CHAPTER 4

RESULTS AND DISCUSSION

4.1 A Fundamental Study on the Molecular Chemistry of Agarwood Water Mixture

The analyzed HPLC chromatogram of the pure agarwood oil is shown in Figure 4.2. Six compounds were detected at the wavelengths of 210 cm-1, 213 cm-1, 240 cm-1, 273 cm-1, 274 cm-1 and 387 cm-1. Because the commercial agarwood marker compounds were not available for standard comparison, this chromatogram (Figure 4.2) was considered the reference and was compared to the chromatogram of sample AW30 and AW100 (Figure 4.1). Both chromatograms AW30 and AW100 (Figures 4.3 and 4.4) show that an identical wavelength was generated at 210 cm^{-1}.

Figure 4.1: (From left to right) Agarwood water at 30°C (AW30), agarwood water at 100°C (AW100) and pure agarwood essential oil.

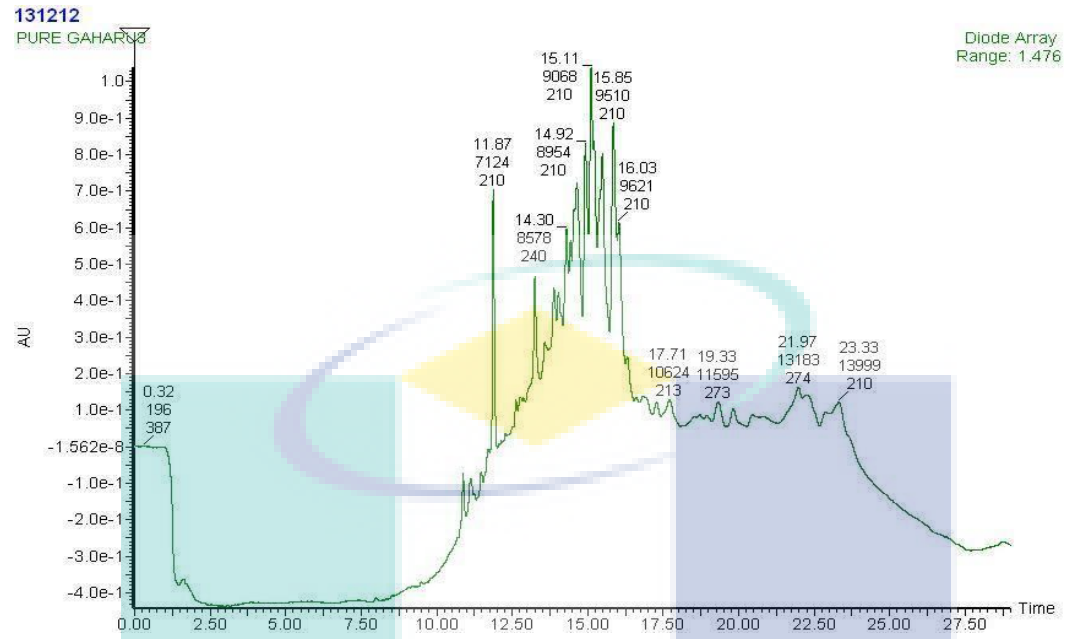


Figure 4.2: HPLC chromatogram of pure agarwood oil

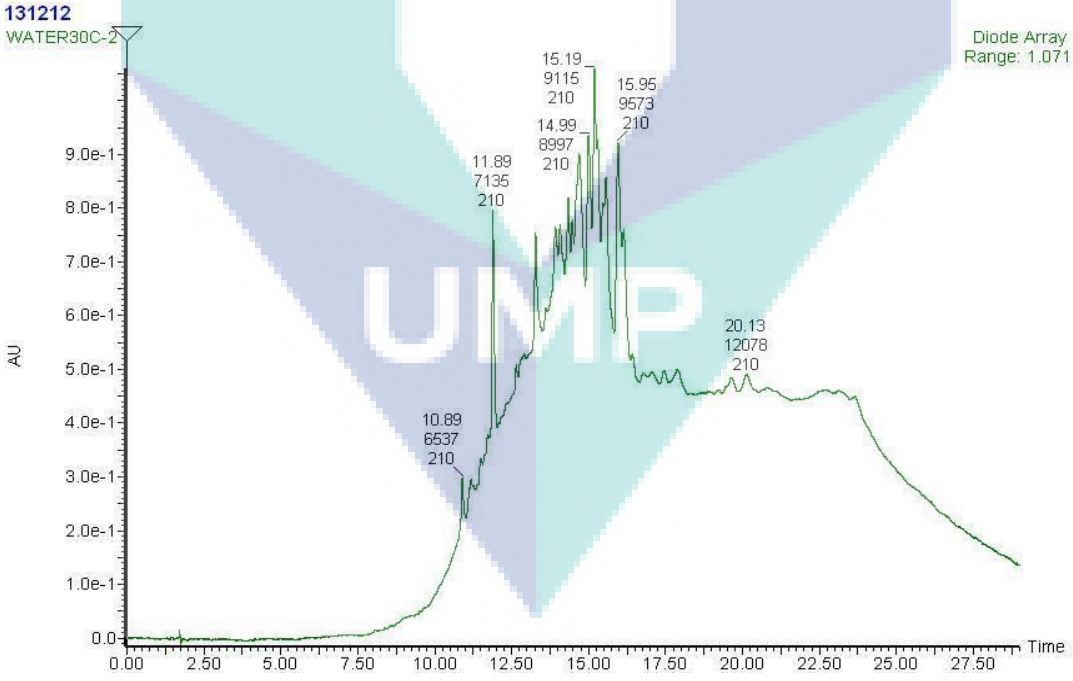


Figure 4.3: HPLC chromatogram of agarwood water at 30°C (AW30)

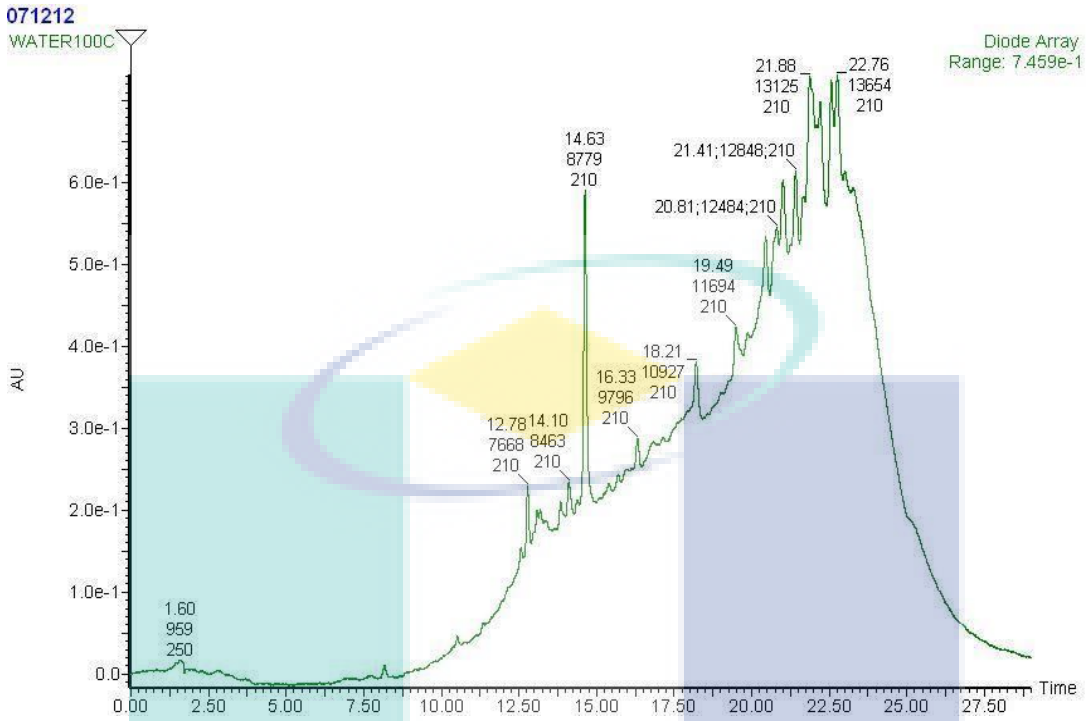


Figure 4.4: HPLC chromatogram of agarwood water at 100°C (AW100)

Hence, fraction at this point was hypothesized to possibly contain the water-soluble agarwood markers. The physically pleasant smell of this aqueous part also supports this assumption. These findings were further evaluated using FTIR, and the results are compiled in Table 4.1 and Figure 4.5. Both spectra were compared with the spectrum of pure water (Figure 4.6). According to these data, the major functional elements in the agarwood water mixture, which were recorded using FTIR, are hydroxyl constituents. This result is practically accepted because the sesquiterpenoid components of agarwood are the OH-bearing compounds (Table 4.1). In addition to OH, the other detected groups are alkenes (C-H), which theoretically also resemble the sesquiterpenoid components. The most significant differences between the agarwood water mixture and pure water is the presence of H-O-H stretching in pure water [23 -24]. Nevertheless, this study is only an elemental review, and a more advanced study on agarwood hydrosol must be conducted with these results as the preliminary references. Another significant finding is that most compounds were detected using the preparative HPLC at 100°C instead of 30°C. This result may also be considered to hypothesize the range of ideal extraction temperature for these markers in future work.

Table 4.1: FTIR of agarwood hydrosol.

IR absorption (cm^{-1})	Functional groups	Molecular motion	Potential Components (Theoretical prediction)
~ (3200-3600)	Hydroxyl	O-H stretch	Agarospirol Jinkohol Jinkoh eremol Kusenol
~ (3000-3100)	Aromatic	C-H stretch	Agarospirol Jinkohol Jinkoh eremol Kusenol
~ (1600-1680)	Alkenes	C=C stretch	Agarospirol Jinkoh eremol Kusenol
~ (1400 – 1500)	Aromatic	C-C stretch (in ring)	Agarospirol Jinkohol Jinkoh eremol Kusenol
~ (1350 – 1370)	Alkane	C-H	Agarospirol Jinkoh eremol Kusenol
~ (650-1000)	Alkene	= C-H bend	Agarospirol Jinkoh eremol Kusenol

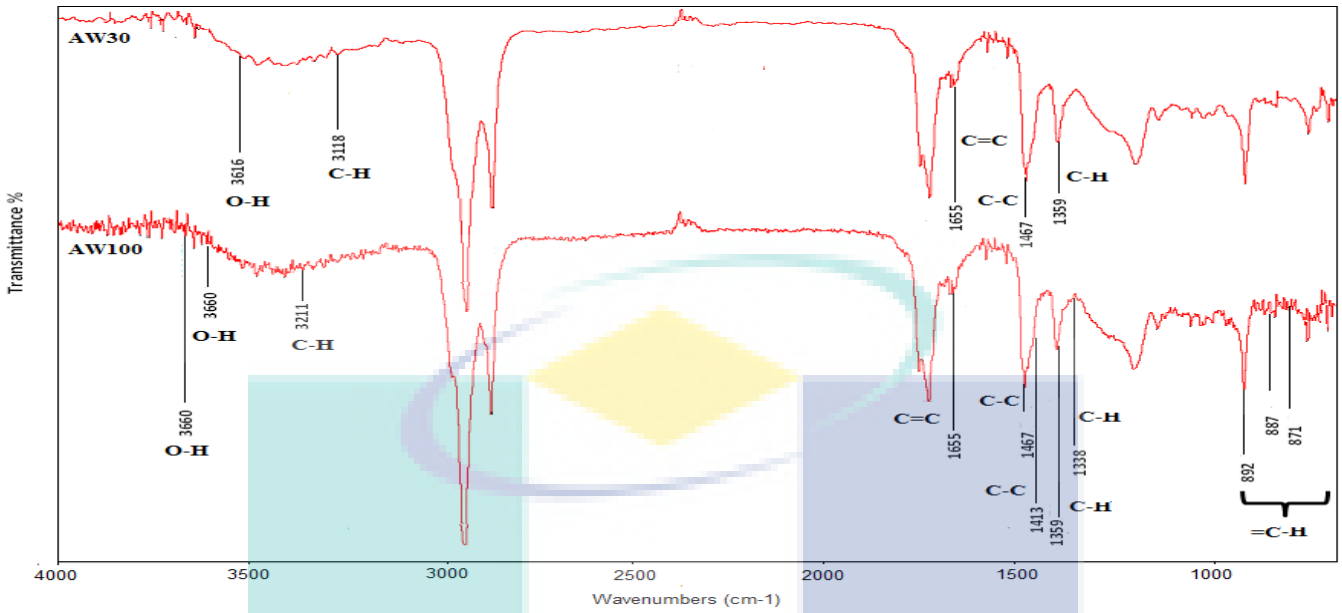


Figure 4.5: FTIR spectrum of agarwood water at 30°C (AW30) and 100°C (AW100)

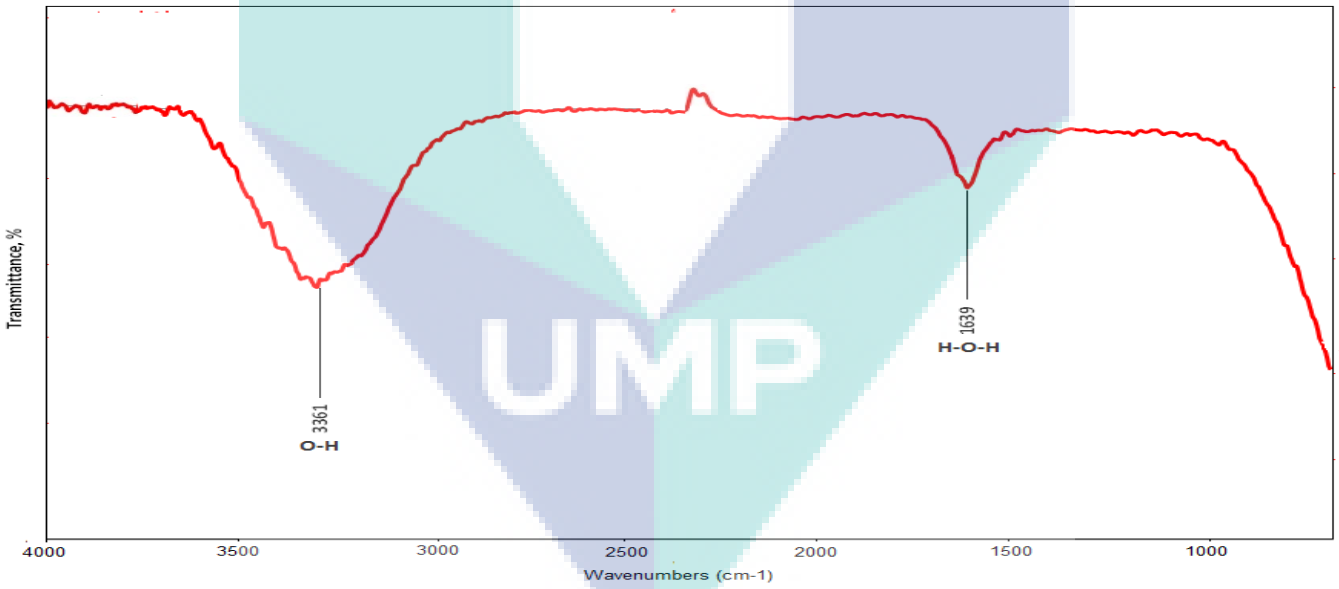


Figure 4.6: FTIR spectrum of pure water

4.2 Application Of Polypiperazine (PPA) Based Membrane For The Recovery of Water Soluble Agarwood (Aquilarria Malaccensis) Marker Molecules

Figure 4.7 illustrates the FTIR plots for feed (industrial agarwood hydrosol), retentate and permeates from membrane filtration process. Based on the generated wavenumbers (Table-2) bands in feed were identified as hydroxyl (O-H), aromatic alkane (C-C, C-H,) and alkene (=C-H) stretches. All of them resemble the functional groups of water soluble agarwood sesquiterpenoids as previously displayed in Table 4.2, thus supportively confirms the presence of these compounds in the hydrosol. H-O-H bond from water was also detected in feed since the agarwood hydrosol itself is a homogenous mixture of water and agarwood sesquiterpenoids. The FTIR characteristic of retentate is almost similar to feed except for the absence of H-O-H stretch in retentate. Permeate on the other hand, shows the presence of only O-H and H-O-H stretch, exhibiting an analogous chromatogram to the FTIR of pure water. From this evidence, it is postulated that, this PPA membrane has been successful in separating majority of agarwood sesquiterpenoids from its water fraction, by retaining them in retentate and allowing only water to pass through into permeate. This prediction is further strengthened by the fact that, MWCO of this PPA membrane is 200 Da. Definitely, agarwood sesquiterpenoids which have MW above 200 Da are not permeable to this membrane, as compared to water with MW of 18 Da. The significance size difference also explain their efficient segregation disregard the fact that, both water and agarwood components actually have the same ability to be pulled towards the hydrophilic vicinity of PPA membrane.

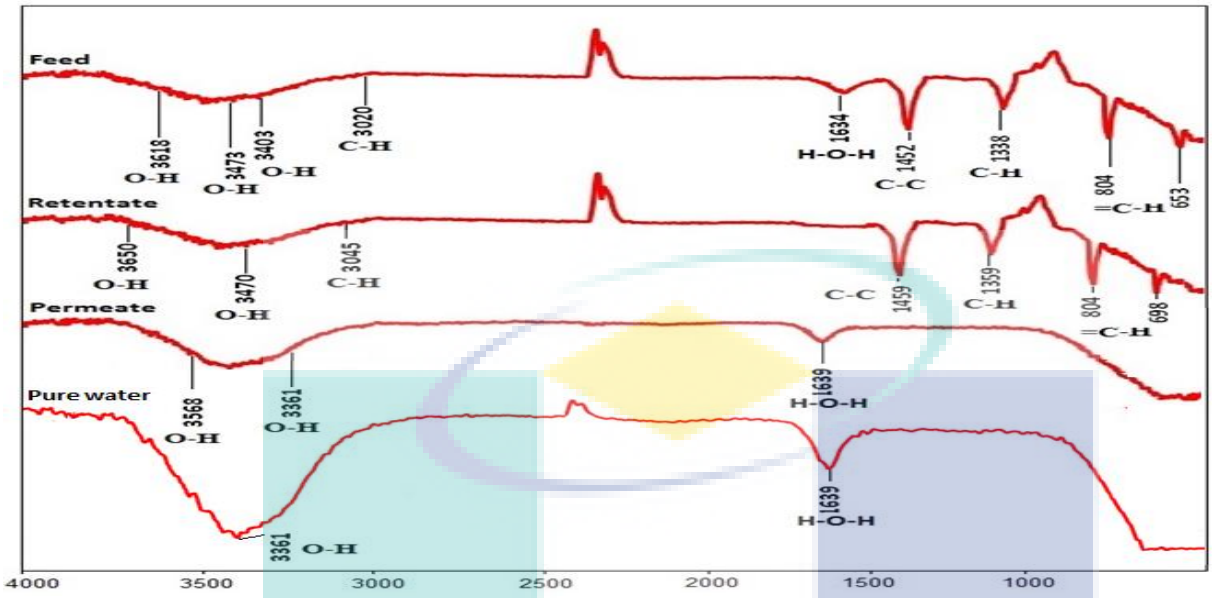


Figure 4.7: FTIR spectroscopy of feed, retentate, permeate and pure water

Table 4.2 : Vibrational bands assignments of feed, retentate, permeate and pure water [(Schulz et al., 2005), (Fang et al., 2011), (Li et al., 2013), (Santosa et al., 2013)]

Wavenumbers (cm^{-1})	**Band assignment**
3400- 3600	O-H (alcohol)
3000-3100	C-H (aromatic alkane)
1400- 1500	In ring C-C stretches (aromatic alkane)
1330-1370	C-H (alkane)
650-800	=C-H bending (alkene)
3300-3400	O-H (water)
1630-1640	H-O-H (water)

4.3 Mass Transfer Flux Study for Gaharu Recovery Using Polypiperazine Amide (PPA) Membrane

The graph of pure water flux vs pressure is presented in Figure 4.8 and summarized results are tabulated in Table 4.3. Regression coefficient, (R^2) is high (0.9975) indicating an outstanding linearity between pressure and flux. However, the flux and permeability (P_m) is quite low, which may be caused by the tight cross sectional structure of this membrane, as portrayed by Scanning Electron Microscopy (SEM) image in Figure 4.9. The average flux is 23.30 L/m^2.h, with 6.76 L/m^2.h.bar of P_m. Data from the graph showed that, the produced pure water fluxes in the pressure of 2-5 bars are approximately 10-35 L/m^2.h, constitutes the lower range of typical NF flux, which is 20-200 L/m^2.h. This is not feasible especially in large scale agarwood industry, as it will restrain the flow rate of water, consequently inhibiting a rapid separation process (Khalil et al., 2013), (Kumar et al., 2011), (Konwar et al., 2011), (Bergo et al., 2012), (Koris et al., 2011).

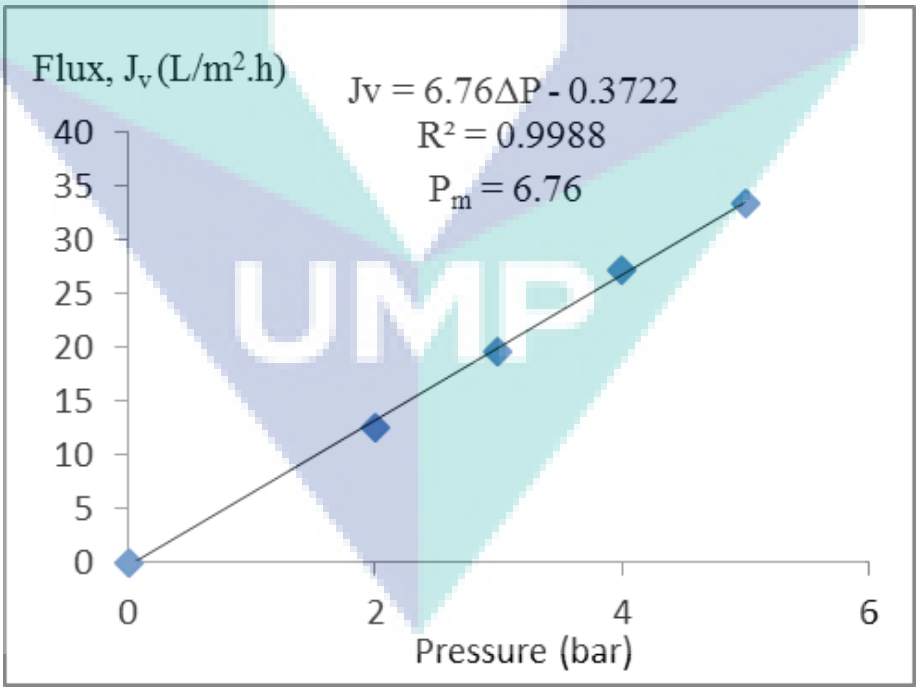
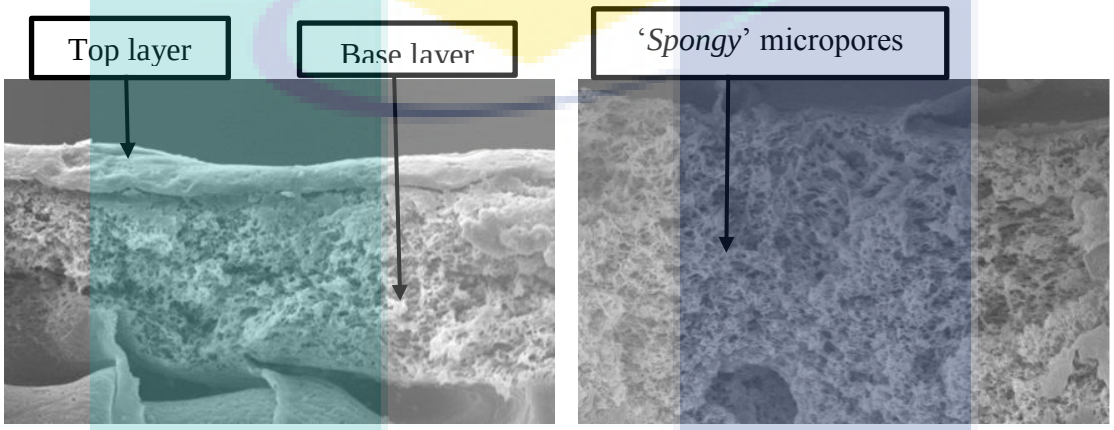


Figure 4.8: Flux of PPA membrane at different operating pressure

Table 4.3: Performance characteristics of PPA membrane

Membrane	Characteristics	
	P_m	Average flux (L.m^2.h)
PPA	6.76	23.20



(a) (b)

Figure 4.9: SEM cross sectional image of PPA membrane (a) Top and base layer at 100x magnification (b) Base support layer at 300x magnification.

4.4 Mass Transfer Flux Study for Gaharu Recovery Using Polyethylene Sulfone (PES) Membrane

4.4.1 Gaharu water nanofiltration

The gaharu water was filtered by using PES membrane in nanofiltration process in order to get the permeate which mainly contained sesquiterpenoids groups.The volume of permeate was taken in 5 minutes with difference pressure and was recorded in Table 4.4.

Based on the Figure 4.10, it shows the increasing of flux with increasing in pressure. The increasing of the applied pressure lead to the increment of the water flux (Nora'aini et al., 2011). This is because the pressure is the driven force for the membrane to filter gaharu water in order to get the permeate which contain sesquiterpenoids groups. The determination of permeability (Pm) is to evaluate the stability and hydrolic properties of the membrane. It can be measure from the slope of the graph below. The permeability coefficient of the gaharu water filtration is 3.5984 (L/m2.h.bar).

Table 4.4: The volume and Flux recorded in 5 minutes for difference pressure

Pressure (bar)	Volume of permeate (mL)	Flux, J_v (L/m^2.h)
2	0.2	0.84
3	1.0	4.18
4	1.8	7.53
5	2.8	11.72

Where,

Flux = V/ (t x A)
V = volume of permeate (collected in 5 minutes)
t = times in hour
A = Effective membrane area, which is 0.00287 m^2.
Flux = $P_m \Delta P$

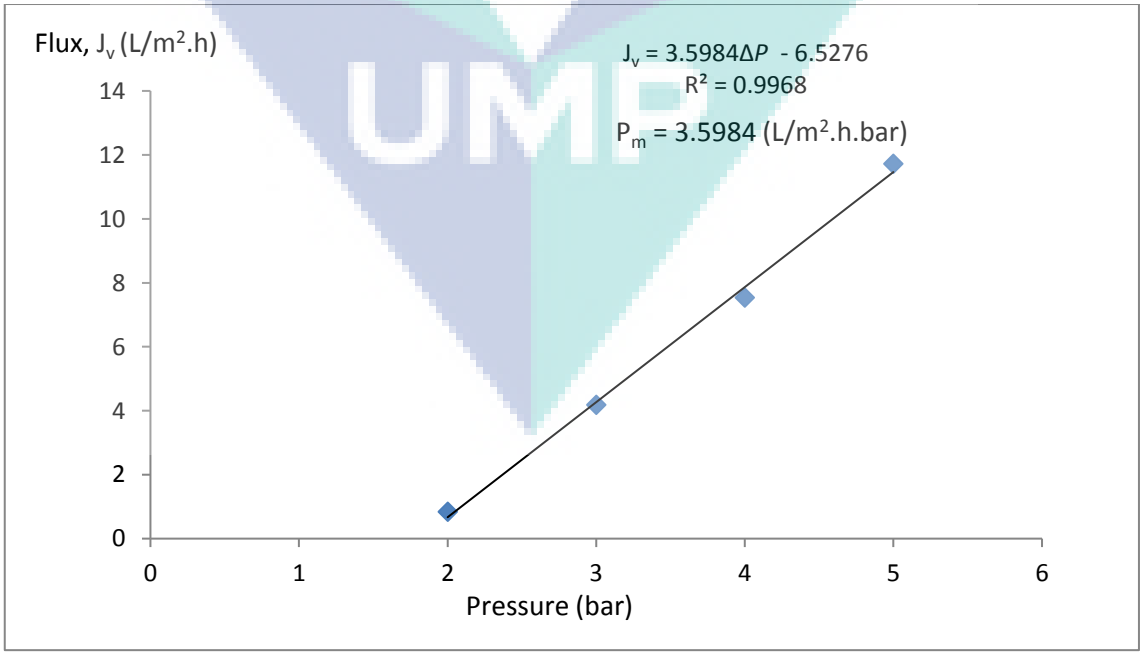


Figure 4.10:The effect of pressure on flux for gaharu water separation process

4.4.2 Pure water nanofiltration

The pure water was also being filtered by PES membrane in nanofiltration process. The function of pure water filtration process was to determine the membrane permeability. The data of pure water permeability is used to explain the morphological attributes of the membrane. It is a fundamental quantity with respect to the membrane and could reflect the porosity of the membrane. The volume of permeate was taken in 5 minutes with difference pressure and was recorded in Table 4.5.

Table 4.5: The volume and Flux recorded in 5 minutes for difference pressure

Pressure (bar)	Volume of permeate (mL)	Flux, J_v ($L/m^2.h$)
2	2.8	11.71
3	6.2	25.92
4	10.0	41.81
5	14.2	59.36

Figure 4.11 show the effect of pressure on flux for pure water separation. The plotted graphs show a linear profile, indicating that the pure water flux is directly proportional to the applied pressure. Thus, by measuring a dependence of the membrane's pure water permeability on pressure, a state of the membrane's active layer porosity can be characterized (Kosutic et al., 2006). At applied pressure of 2 bar, the membrane used gave the lowest flux and increased of flux occurred linearly as the pressure increased up to 5 bar. The permeability (P_m) of pure water is 4.1786 $L/m^2.h.bar$

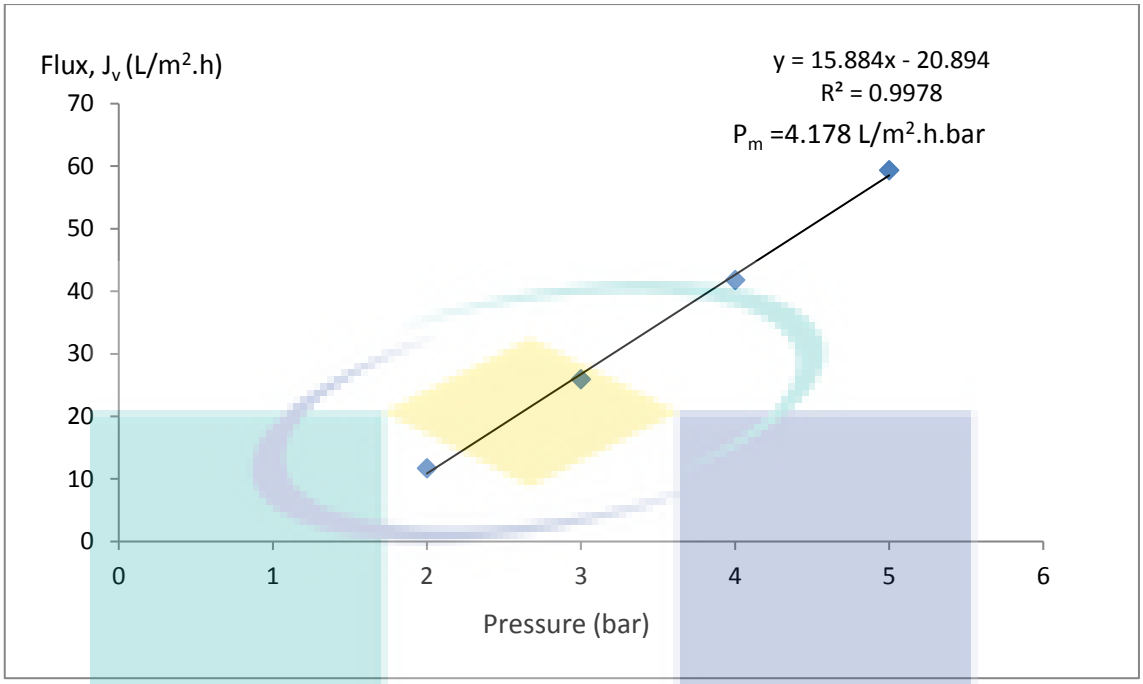


Figure 4.11: The effect of pressure on flux for pure water separation process

Based on the Figure 4.10 and Figure 4.11, it shows that there is only slightly decreasing between permeability of gaharu water separation (3.5984 L/m^2.h.bar) from pure water (4.1786 L/m^2.h.bar).This is due to the more composition in gaharu water compare to pure water which only contained water component that lead to membrane fouling. From the permeability value, it can be conclude that the PES membrane has high permeability of gaharu water separation.

4.4.3 Scanning electron microscopy (SEM)

After the membrane filtration, the surface images of the membrane used in this experiment is captured by using Scanning Electron Microscope (SEM).The cross sectional image of the membrane is shown in Figure 4.1.3.The membrane has asymmetric structure comprising of a dense skin layer, a porous intermediate layer and microvoids at the bottom. At 25 wt.% of PES, membranes have a good permeability but flux show instability in time. Because of this behaviour for industrial application membrane with higher concentration of PES need to be selected (Stefan *et al.*,2009)

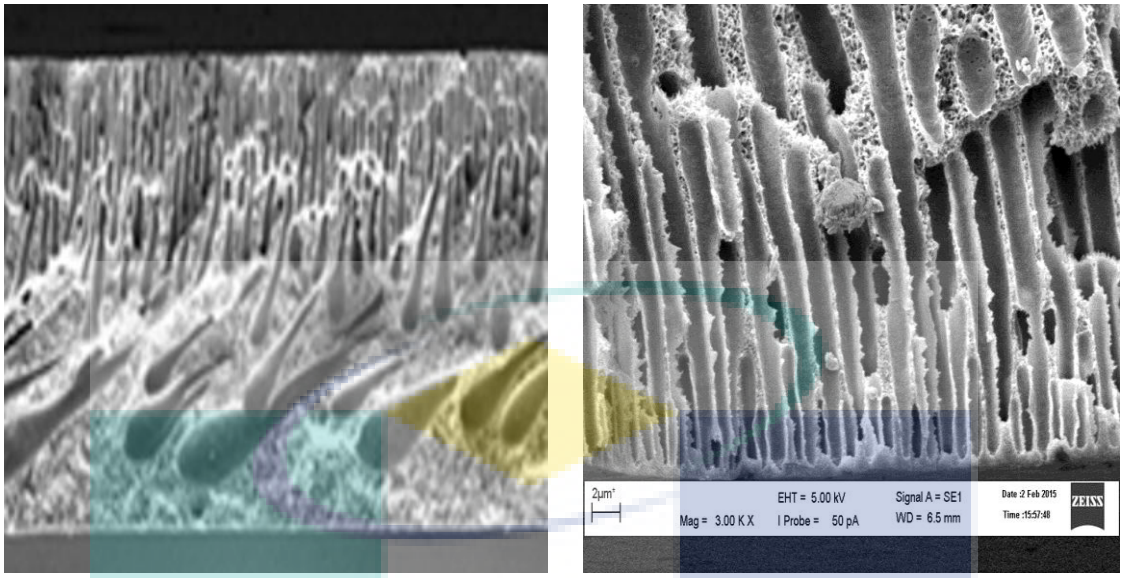


Figure 4.1.3: SEM (500x) cross sectional image of PES membrane.

CHAPTER 5

CONCLUSION

5.1 Conclusion

The recovery of agarwood marker compounds using commercial polypiperazine (PPA) NF membrane yield a positive outcome to recover the highly prized sesquiterpenoid marker compounds. Some property of Gaharu Hydrosol Nanosiever is aimed to be customized towards the achievement of optimum mass transfer flux industrial productivity.

The combined approach of HPLC and FTIR are notably helpful for the predictive assignment of chemical compounds. The spectrum of all samples (AW30, AW100 and pure agarwood oil) was recorded in the range of 4000 to 400 cm^{-1} (mid-infrared spectroscopy) at 4 cm^{-1} resolution.

The recovery of agarwood marker compounds using commercial polypiperazine (PPA) NF membrane yield a positive outcome. FTIR analysis showed that most of hydroxyl bearing components has been extracted into permeate. This might be a fundamental indicator of the successful separation of the agarwood sesquitpeneoids from their hydrosol. Nevertheless, additional studies should be carried out for a more detail clarification, especially on the biological and chemical information of these compounds. The endowed working flux is also low, and this seems to be not efficient, especially for bulk application. Therefore, the operational membrane properties are yet to be improved. Using this PPA membrane as reference prototype, a new formulation of thin film composite (TFC) NF membrane with the combination of 'fine-tuned' flux and high

rejection property is aimed to be customized towards the achievement of optimum industrial productivity.

5.2 Recommendation for Further Study

For big scale membrane operation, it is not recommended to use flat sheet type of membrane. If membrane separation if were to use as a method to extract gaharu essential oils, hollow fiber membranes are recommended. It is because hollow fiber membranes offers more effective membrane area as to compare to flat sheet membrane.

Besides that, a careful and deep consideration also need to be taken when commercializing the membrane technique. This is because membrane separation techniques are very costly. On the other hand, more research project should be done in this field. Other type of membranes such as ultrafiltration, microfiltration and reverse osmosis can also be considered as the technique to separate the gaharu marker compounds.

Further experiment should be conducted to examine how the gaharu marker compounds are attracted to the surface of the membrane. If the diffusion and mass transfer processes are known, ways of improving the rate of diffusion and mass transfer can be implement so that the separation of gaharu marker compound from water mixture can be faster and more efficient.

Another recommendation is to include the amount of gaharu oil collected from the experiment. This is in order to show the effectiveness of the experiments. It is also recommended to include the permeability constant for the membrane separation process.

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ACHIEVEMENTS

- Name of articles/ manuscripts/ books published

  One paper has been published in *ARPN Journal of Engineering and Applied Sciences*, Vol. 11, No. 4, February 2016 Issn 1819-6608

- Title of Paper presentations (international/ local)

  One paper have been presented at the International Conference of Fluids & Chemical Engineering (FLUIDCHE) 2015, November 25-27th, 2015, Langkawi, Malaysia

  Molecular nano-sieve application for the recovery of water soluble agarwood (Aquilarria Malaccensis) marker molecules

- Awards

  One silver medal awarded from the Creation, Innovation, Technology and Research Exposition (CITREX 2015), March 9th, Universiti Malaysia Pahang

- Patent

  Patent application entitiles "GAHARU HYDROSOL NANO SIEVER." has been submitted to Pejabat Penyelidikan dan Inovasi (PNI), Universiti Malaysia Pahang. Patent drafted has been carried out by IPVolusi Sdn Bhd and has been submited to the MyIPO for patent filling with patent no of PI 2016702081.

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APPENDIX A – APRN JOURNAL

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MOLECULAR NANO-SIEVE APPROACH BY THE APPLICATION OF POLYPIPERAZINE (PPA) BASED MEMBRANE FOR THE RECOVERY OF WATER SOLUBLE AGARWOOD (AQUILARRIA MALACCENSIS) MARKER MOLECULES

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ABSTRACT

This study is the groundwork on the ability of nano-sieve technique in the recovery of water soluble agarwood marker compounds from agarwood hydrosol. To achieve this purpose, a polypiperazine (PPA) based nanofiltration (NF) membrane was used. The effectiveness of this membrane on the separation of agarwood marker molecules was analysed and FTIR results showed that most of marker compounds (i.e. agarospirol, jinkohol, jinkoh eremol and khusenol) has been successfully separated from the aqueous agarwood. However, the performance of the membrane in terms of flux and permeability is quite low, which is 23.30 L/m^2.h, with 6.76 L/m^2.h.bar, respectively. These initial findings will be used as the forecast to improve the future development of NF membrane, specifically *'tailor-made'* for the large scale production of agarwood marker compounds.

Keywords: agarwood, aquilarria malaccensis, nanofiltration, agarospirol, jinkohol, jinkoh eremol, khusenol.

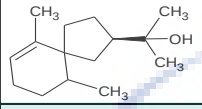
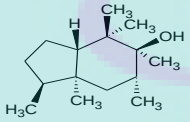
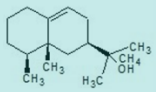
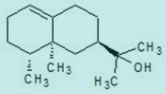
INTRODUCTION

Compound Formula Structure Functional groups MW(Da) Agarospirol C15H26O Aromatic Hydroxyl Alkene Alkane 222.36 Jinkohol C15H26O Aromatic Hydroxyl Alkane 222.36Agarwood, or 'karas/gaharu' in Malay is the resin produced by Aquilarria Malaccensis tree, results from their defence mechanism against wounding (Chong *et al.* 2014), (Li *et al.* 2013). It is often occurs as dark coloured patches or streaks in the tree. The essential oil of agarwood is significantly prized, especially for perfume, incense, religious rituals and medicinal purpose. At present, hydro-distillation technique, utilizing water as solvent, is commercially being used to extract the agarwood oil from its wood (Islam *et al.* 2014), (Azah *et al.* 2013) (Naef *et al.* 2010). Although this method is widely applied in agarwood industry, it is still not yet proficient. This reason is because most main marker compounds of agarwood which are responsible for their aromatic scent as shown in Table-1 are from sesquiterpenoids groups which possess hydroxyl (OH) moieties, have the ability to form hydrogen bonding with water (Subasinghe *et al.* 2012), (Tajuddin *et al.* 2010) (Fadzil *et al.* 2013). This reflects to their possibility to dissolve in solvent during processing, leaving them in the by-product of distillation, known as distillate or hydrosol (Figure-1). As a consequence, they cannot be completely recovered and feasible product yield could not be achieved. Moreover, hydro-distillation is also not very useful for investigating the composition of genuine essential oils and aroma-active compounds because of the tendency in transformation processes due to high temperatures (Richter et al., 2007) (Mohd et al. 2008). Thus, considering the demand and high value of these compounds, a new further alternative separation approach has to be explored. In this research, a commercial NF membrane based on polypiperazine amide (PPA) was applied for the extraction of agarwood marker compounds. The surface of PPA is hydrophilic due to the existence of semi aromatic/aliphatic polyamide as shown in Figure-2. Membrane technology does not require additional energy and also enables a high productivity. Isolation of agarwood marker compounds using membrane technology would bring benefits to the local agarwood industries to control the qualities of agarwood oil, as well as maximizing their profit through the utilization of low cost technology.

MATERIALS

The hydrosol of agarwood was obtained from Kedaik Agarwood Sdn Bhd, a local industry located in Rompin, Pahang, Malaysia. The PPA (TS40) membrane was supplied by Sterlitech Corporation, with theoretical molecular weight cut-off (MWCO) of 200 Da. This membrane is a composite type, consists of thin polypiperazine top layer, supported by polymeric and non- woven bottom layer with 'spongy' micropores. Other chemicals and solvent for analysis (acetone and methanol, both from Fischer Scientific (M) Sdn Bhd) were of reagent grade. The filtration unit was operated in dead end module using Amicon Stirred Filtration Cell (Model 8200). Analyses of both permeate and retentate was accomplished by FTIR.

Table-1. Properties of agarwood sesquiterpenoids.

Chemical compounds	Chemical Formula	Molecular structure	Functional groups
Agarospirol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane
Jinkohol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkane
Jinkoh eremol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane
Kusenol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane

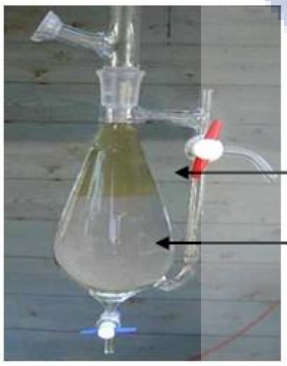


Figure-1. Production of agarwood essential oil by distillation.

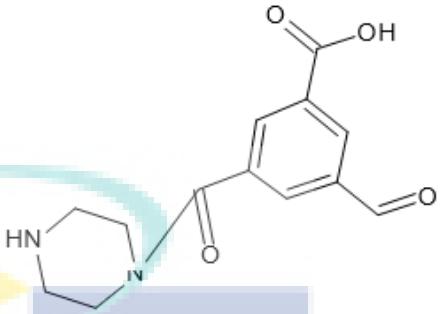


Figure-2. Chemical structure of polyiperazine (PPA).

EXPERIMENTAL PROCEDURE

Permeation process: Optimization of permeation process was performed by applying different transmembrane pressure (TMPs). 20 mL of feed solution (agarwood hydrosol) was poured into permeation apparatus and the volume of permeate collected in five minutes was measured. Permeate flux was calculated according to the following equation:

$$Jv = V/ (t \times A)$$

Where Jv is flux (L/m2.h), V is volume of permeate in liter, t is time in hour and A is the effective area of the membrane, which is 0.00287 m2. The filtration was carried out at different pressure to calculate the permeability coefficient (Pm), which is determined from the slope of the graph. Permeate and retentate stream were both analysed by FTIR.

Fourier Transform Infrared (FTIR): FTIR measures the frequencies at which the sample absorbs, and also the intensities of these absorptions. The frequencies are helpful for the identification of the sample's chemical make-up due to the fact that chemical functional groups are responsible for the absorption of radiation at different frequencies. FTIR is very helpful for the predictive assignment of chemical compounds. The spectrum of all samples (agarwood hydrosol, retentate and permeate) was recorded at range of 4000 – 400 cm-1 (mid infrared spectroscopy) at 4 cm-1 resolution (FTIR model: Nicolet Avatar 370 DTGS).

Scanning Electron Microscopy (SEM): Scanning Electron Microscopy (SEM) is a scientific instrument that uses a principle of a narrow beam of the electron with kinetic energies hits (1 to 25kV) the membrane sample. It allows a clear and concise view of the overall membrane structure. For this work, a scanning electron microscopy (Model: Zeiss Evo 50) was used to probe the morphological features of

membranes. Small pieces of membrane were fractured cryogenically in liquid nitrogen, and sputtered by platinum, prior to viewing under SEM.

RESULTS AND DISCUSSION

Figure-3 illustrates the FTIR plots for feed (industrial agarwood hydrosol), retentate and permeates from membrane filtration process. Based on the generated wavenumbers (Table-2) bands in feed were identified as hydroxyl (O-H), aromatic alkane (C-C, C-H,) and alkene (=C-H) stretches. All of them resemble the functional groups of water soluble agarwood sesquiterpenoids as previously displayed in Table-1, thus supportively confirms the presence of these compounds in the hydrosol. H-O-H bond from water was also detected in feed since the agarwood hydrosol itself is a homogenous mixture of water and agarwood sesquiterpenoids. The FTIR characteristic of retentate is almost similar to feed except for the absence of H-O-H stretch in retentate. Permeate on the other hand, shows the presence of only O-H and H-O- H stretch, exhibiting an analogous chromatogram to the FTIR of pure water. From this evidence, it is postulated that, this PPA membrane has been successful in separating majority of agarwood sesquiterpenoids from its water fraction, by retaining them in retentate and allowing only water to pass through into permeate. This prediction is further strengthened by the fact that, MWCO of this PPA membrane is 200 Da. Definitely, agarwood sesquiterpenoids which have MW above 200 Da are not permeable to this membrane, as compared to water with MW of 18 Da. The significance size difference also explain their efficient segregation disregard the fact that, both water and agarwood components actually have the same ability to be pulled towards the hydrophilic vicinity of PPA membrane.

Table-2. Vibrational bands assignments of feed, retentate, permeate and pure water [(Schulz *et al.* 2005), (Fang *et al.* 2011), (Li *et al.* 2013), (Santosa *et al.* 2013)].

Wavenumbers (cm^{-1})	Band assignment
3400- 3600	O-H (alcohol)
3000-3100	C-H (aromatic alkane)
1400- 1500	In ring C-C stretches (aromatic alkane)
1330-1370	C-H (alkane)
650-800	=C-H bending (alkene)
3300-3400	O-H (water)
1630-1640	H-O-H (water)

The graph of pure water flux vs pressure is presented in Figure-4 and summarized results are tabulated in Table-3. Regression coefficient, (R^2) is high (0.9975) indicating an outstanding linearity between pressure and flux. However, the flux and permeability (P_m) is quite low, which may be caused by the tight cross sectional structure of this membrane, as portrayed by Scanning Electron Microscopy (SEM) image in Figure-5. The average flux is 23.30 L/m^2.h, with 6.76 L/m^2.h.bar of P_m. Data from the graph showed that, the produced pure water fluxes in the pressure of 2-5 bars are approximately 10-35 L/m^2.h, constitutes the lower range of typical NF flux, which is 20-200 L/m^2.h. This is not feasible especially in large scale agarwood industry, as it will restrain the flow rate of water, consequently inhibiting a rapid separation process (Khalil *et al.* 2013), (Kumar *et al.* 2011), (Konwar *et al.* 2011), (Bergo *et al.* 2012), (Koris *et al.* 2011).

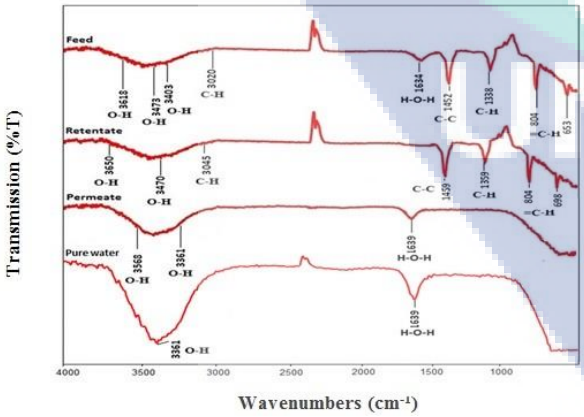


Figure-3. FTIR spectroscopy of feed, retentate, permeate and pure water.

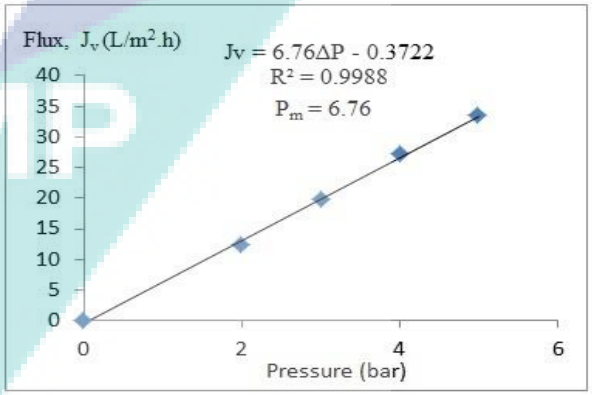


Figure-4. Flux of PPA membrane at different operating pressure.

Table-3. Performance characteristics of PPA membrane.

Membrane	Characteristics	
	P_m	Average flux ($L.m^2.h$)
PPA	6.76	23.20

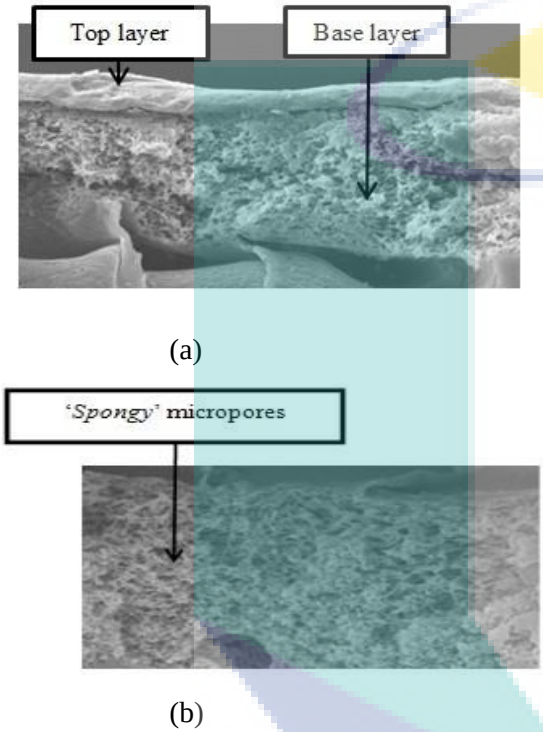


Figure-5. SEM cross sectional image of PPA membrane (a) Top and base layer at 100x magnification (b) Base support layer at 300x magnification.

CONCLUSIONS

The recovery of agarwood marker compounds using commercial polypiperazine (PPA) NF membrane yield a positive outcome. FTIR analysis showed that most of hydroxyl bearing components has been extracted into permeate. This might be a fundamental indicator of the successful separation of the agarwood sesquitpeneoids from their hydrosol. Nevertheless, additional studies should be carried out for a more detail clarification, especially on the biological and chemical information of these compounds. The endowed working flux is also low, and this seems to be not efficient, especially for bulk application. Therefore, the operational membrane properties are yet to be improved. Using this PPA membrane as reference prototype, a new formulation of thin film composite (TFC) NF membrane with the combination of 'fine-tuned' flux and high rejection property is aimed to be customized towards the achievement of optimum industrial productivity.

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APPENDIX B – PATENT

GAHARU HYDROSOL NANO SIEVER

1. INVENTORS

Principal : Fatmawati binti Adam

Associates : Saidatul Syaima binti Mat Tari

Mazrul Nizam bin Abu Seman

2. INVENTION SUMMARY

This disclosed invention relates to the fabrication of Thin Film Composite Nanofiltration (TFC-NF) membrane which is a novel application to separate and recover the water soluble sesquiterpenoids compounds from gaharu hydrosol. The highly prized sesquiterpenoid which is water soluble or hydrophilic is normally during the hydrodistillation process of gaharu essential oil. This is because the sesquiterpenoid components of agarwood are the OH-bearing compounds as listed in Table 1 (please refer to the attachment).When more sesquiterpenoid components soluble in

water, the less quality and price of gaharu essential oil produced from the hydrodistillation process in the market. A poor quality of gaharu essential oil in the market ranges from RM20 000 to 30 000 per kilogram. Meanwhile a high quality essential oil price ranges from RM40 000 to 60 000 per kilogram, Thus, a novel application of gaharu Hydrosol Nanosiever will be synthesised and applied to recover the loss sesquiterpenoid in water solvent.

This Gaharu Hydrosol Nanosiever membrane comprised of two layers: a) a porous polyethersulfone (PES) base and b) a piperazine amide (PPA) thin film. Schematic diagram of the membrane is shown in Figure 1 and the SEM image is shown in Figure 2.

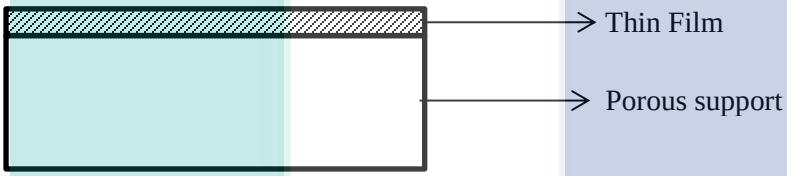
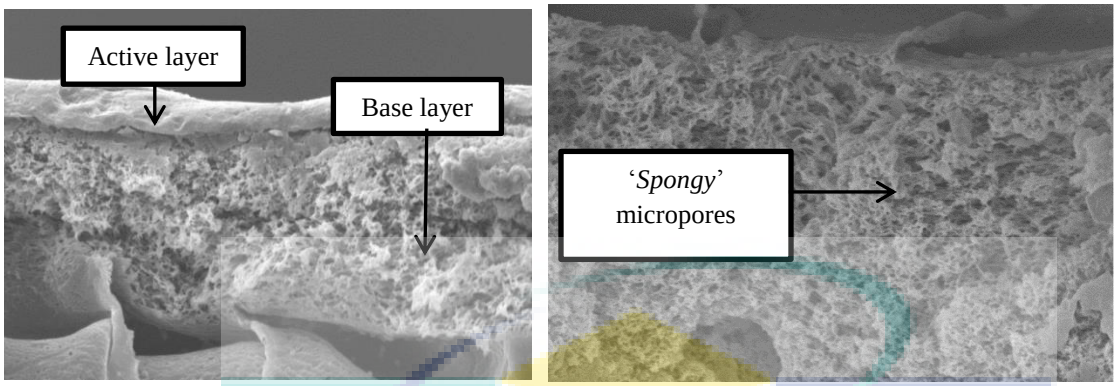


Figure 1: Schematic diagram of Gaharu Hydrosol Nanosiever

The development of this TFC-NF membrane involves two steps. In the first step, the dope solution for porous support is prepared from the mixture of PES and N-methyl-2-pyrollidone (NMP) as solvent. In second step, polymer was dissolved in a suitable organic solvents. NMP which is a highly polar, aprotic organic solvent with a low viscosity, which is easily miscible with water and other organic solvents and which is used as a common solvent in many applications. Once regarded as benign, the solvent N-methylpyrrolidone (NMP) is under scrutiny because of concerns over its potential health effects.



(a) (b)

Figure 2 SEM cross sectional image of PPA membrane (a) Top and base layer at 1000x magnification (b) Base support layer at 3000x magnification

The mixture of polymer and solvent is then casted on glass support by method of dry-wet phase inversion forming a flat sheet polymeric membrane. The membrane may also be formed as hollow fiber or tubulets which do not require a support for practical use; or the support may be of such shape and the membrane is cast internally thereon. The concentration of polymer in dope solution may vary between 5-25 %, as a function of its molecular weight (MW). The temperature for dope preparation may vary from 10°C to 60°C, depending on the particular polymer, and the solvents.

The polyamide thin film is formed onto the surface of fabricated flat sheet membrane by interfacial polymerization method of an amine aqueous solution and amine-reactive compound includes the steps of forming an active layer through interfacial polymerization by contacting a surface of a porous support with an amine aqueous solution (piperazine and sodium hydroxide) containing a polyfunctional aromatic amine monomer and an organic solution containing polyfunctional acyl halide monomer (e.g. trimesoyl chloride) as an amine-reactive compound and

(b) performing post-treatment preceded by the forming of the active layer by contacting the active layer with an aqueous solution containing 0.1 to 100 wt % of polyfunctional tertiary alcohol amine. The polyamide thin film composite reverse osmosis membrane prepared by using the polyfunctional tertiary alcohol amine as a post-treatment compound has improved water permeability and rejection characteristics compared to a case of using various post-treatment agents or methods.

Figure 3 illustrates the FTIR plots for feed (industrial agarwood hydrosol), retentate and permeates from membrane filtration process. All of them resemble the functional groups of water soluble agarwood sesquiterpenoids as listed in Table 1, thus supportively confirms the presence of these compounds in the hydrosol. H-O-H bond from water was also detected in feed since the agarwood hydrosol itself is a homogenous mixture of water and agarwood sesquiterpenoids. The FTIR characteristic of retentate is almost similar to feed except for the absence of H-O-H stretch in retentate. Permeate on the other hand, shows the presence of only O-H and H-O-H stretch, exhibiting an analogous chromatogram to the FTIR of pure water. From this evidence, it is postulated that, this PPA membrane has been successful in separating majority of agarwood sesquiterpenoids from its water fraction, by retaining them in retentate and allowing only water to pass through into permeate. This prediction is further strengthened by the fact that, MWCO of this PPA membrane is 200 Da. Definitely, agarwood sesquiterpenoids which have MW above 200 Da are not permeable to this membrane, as compared to water with MW of 18 Da. The significance size difference also explain their efficient segregation disregard the fact that, both water and agarwood components actually have the same ability to be pulled towards the hydrophilic vicinity of PPA membrane.

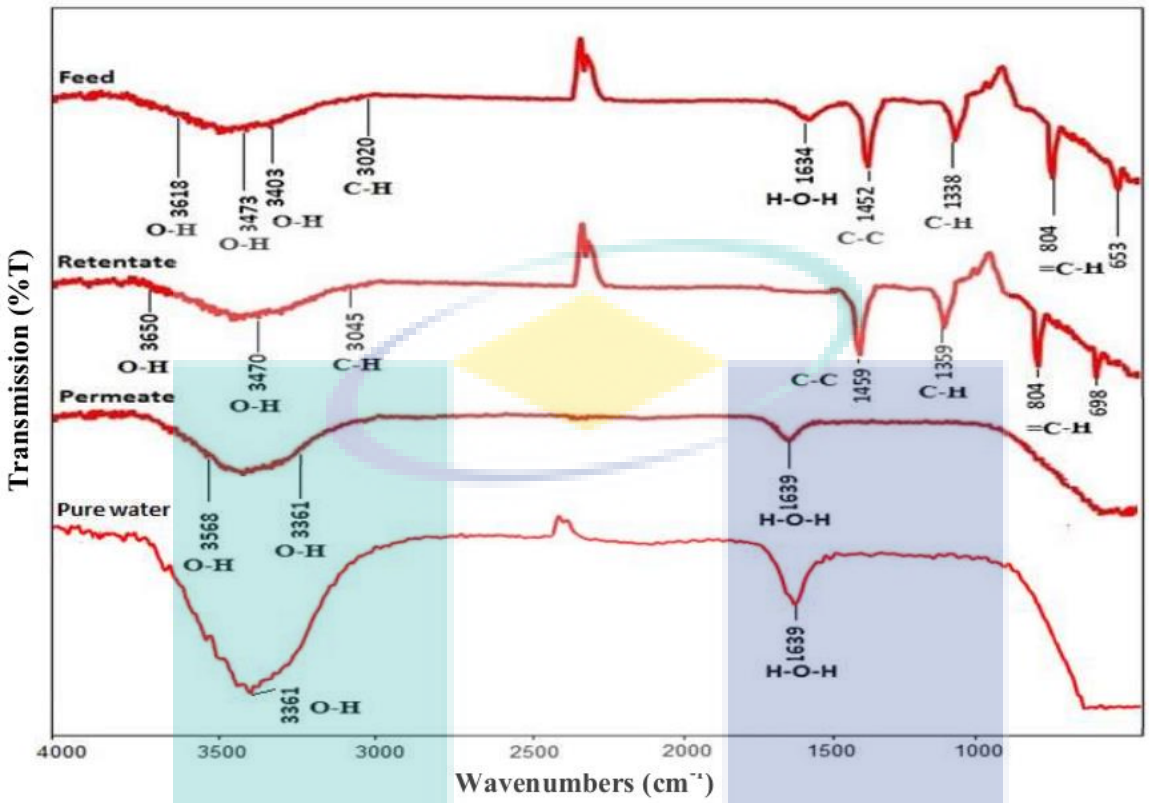


Figure 3: FTIR spectroscopy of feed, retentate, permeate and pure water

The mass transfer flux testing has resulted the average flux of 23.30 L/m^2.h, with 6.76 L/m^2.h.bar of Pm as summarised in Table 2. The produced pure water fluxes in the pressure of 2-5 bars are approximately 10-35 L/m^2.h using the synthesised membrane.

Table 2: Performance characteristics of PPA membrane

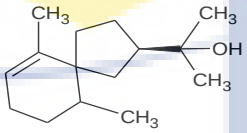
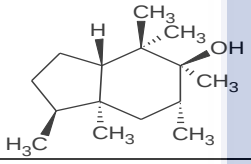
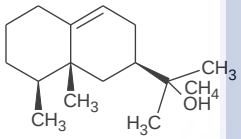
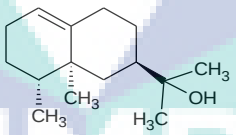
Membrane	Characteristics	
	P_m	Average flux (L.m^2.h)
PPA	6.76	23.20

3. CONCLUSION

The recovery of agarwood marker compounds using commercial polypiperazine (PPA) NF membrane yield a positive outcome to recover the highly prized sesquiterpenoid marker compounds. Some property of Gaharu Hydrosol Nanosiever is aimed to be customized towards the achievement of optimum mass transfer flux industrial productivity.

ATTACHMENT

Table 1: Water-soluble agarwood marker compounds

Agarwood compounds	Chemical Formula	Molecular structure	Functional groups
Agarospirol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane
Jinkohol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkane
Jinkoh eremol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane
Kusenol	$C_{15}H_{26}O$		Aromatic Hydroxyl Alkene Alkane

UMP