CHEMISTRY SOLUTION STUDY OF GAHARU WATER-MIXTURE

NURUL SALWA AZLIYANA BINTI HAMZAH

BACHELOR OF CHEMICAL ENGINEERING
UNIVERSITI MALAYSIA PAHANG

NURUL SALWA AZLIYANA BINTI HAMZAH

BACHELOR OF CHEMICAL ENGINEERING 2013

UMP

CHEMISTRY SOLUTION STUDY OF GAHARU WATER-MIXTURE

NURUL SALWA AZLIYANA BINTI HAMZAH

Report submitted in partial fulfilment of the requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering

UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

SUPERVISOR'S DECLARATION

I hereby declare that I have checked this project and in my opinion, this project is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering.

Signature :

Name of Supervisor : DR. FATMAWATI BINTI ADAM

Position :

Date : 25th January 2012

STUDENT'S DECLARATION

I hereby declare that the work in this project is my own except for quotations and summaries which have been duly acknowledged. The project has not been accepted for any degree and is not concurrently submitted for award of other degree.

Signature :

Name : NURUL SALWA AZLIYANA BINTI HAMZAH

ID Number : KA09107

Date : 25th January 2013

DEDICATION

Special dedication to my supervisor, Dr. Fatmawati binti Adam your Time, Guidance, and Support.

And,

To my beloved parents (Hamzah bin Embong &Ainun binti Othman) and friends, that encouraged and fully supports me throughout completing this thesis.

ACKNOWLEDGEMENT

During the completion of this project, I met a number of people, namely lecturers and professional. All of them have assisted me in many ways towards completing this research. I would like to thank several people who have contributed either directly or indirectly to the completion of this final year project 1 and 2. I would like to express my sincere appreciation to my supervisor, DR. Fatmawati binti Adam for her valuable guidance, advices, constant support and her valuable time towards finishing my final year project 1. Following her guidance, I am able to understand more about this project, where to collect the information that is needed and how to use the information properly to produce successful report. I am really thankful and indebted with her for all her cooperation, tolerance and commitment in assisting me to make this project success.

I also would like to thanks the personnel of Faculty of Chemical Engineering and Natural Resources (FKKSA), especially lecturer, for their assistance and corporation. Not to be forgotten, my biggest thanks to PM.Nordin , DR. Mazrul, DR Syed Mohd Syaufi and DR Sabri as my panels for their comments. Lastly, I am also obliged to express my appreciation towards my beloved family members for their moral supports due to complete this project. Special thanks to all my friends that also entitled for an appreciation, as they have been all very supportive and always assist me in various occasions. Thanks to all of you for everything.

CHEMISTRY SOLUTION STUDY OF GAHARU WATER-MIXTURE

ABSTRACT

This research is about chemistry solution study of Gaharu-water mixture. The fundamental of chemistry properties of Gaharu-water mixture will be investigated by using Preparative High Performance Liquid Chromatography (PREP-HPLC) and Fourier Transform Infrared Spectroscopy *(*FTIR). This will give benefits for the development of economics and help the engineers provide the accurate properties of Gaharu compound solubility in water. Then, the gaharu marker compound characterized from gaharu water mixture may provide the useful information to engineers and scientists to give an insight to control the gaharu extraction process in a better way. The first objective of the study is to develop the method (protocol) in identify and recognize the Gaharu marker compound in water mixture. The second objective if this research is to identify the compound and molecular chemistry of Gaharu oil via Fourier Transform Infrared Spectroscopy (FTIR) and Preparative High Performance Liquid Chromatography (PREP-HPLC). As the conclusion, Gaharu marker compound that dissolve in the water mixture can be successfully identified by using FTIR and PREP-HPLC. Main marker compound dissolved in water are agarospirol, jinkohol-eremol, jinkohol and kusenol.

KAJIAN KIMIA PENYELESAIAN CAMPURAN GAHARU-AIR

ABSTRAK

Kajian ini adalah mengenai kajian penyelesaian kimia campuran Gaharu-air. Asas sifat kimia campuran Gaharu air akan disiasat dengan menggunakan persiapan Cecair Prestasi Tinggi Chromatography (PREP-HPLC) dan Spektroskopi inframerah transformasi Fourier (FTIR). Ini akan memberi manfaat untuk pembangunan ekonomi dan membantu jurutera untuk mendapatkan sifat kompaun Gaharu yang larut di dalam air dengan tepat. Kemudian, sebatian gaharu penanda yang dicirikan dari campuran air gaharu boleh memberikan maklumat yang berguna kepada jurutera dan saintis untuk mengawal proses pengekstrakan gaharu dengan cara yang lebih baik. Objektif pertama kajian ini adalah untuk membangunkan kaedah (protokol) dalam mengenal pasti sebatian penanda Gaharu dalam campuran air. Objektif kedua kajian ini adalah untuk mengenal pasti kompaun dan kimia molekul minyak Gaharu melalui Spektroskopi inframerah transformasi Fourier (FTIR) dan persiapan Kromatografi Cecair Prestasi Tinggi (PREP-HPLC). Sebagai kesimpulan, Gaharu sebatian penanda yang larut dalam campuran air telah berjaya dikenal pasti dengan menggunakan FTIR dan PREP-HPLC. Sebatian penanda Utama larut dalam air adalah agarospirol, jinkohol-eremol, jinkohol dan kusenol.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Introduction

This research aims to gain a better understanding of chemistry solution study of Gaharu-water mixture. Gaharu is the resinous heartwood from Aquilaria trees, which is the native to Southeast Asia and Asia (Frank & James, 2012). In this study, the fundamental of chemistry properties of Gaharu-water mixture will be investigate by using Preparative High Performance Liquid Chromatography (PREP-HPLC) and Fourier Transform Infrared Spectroscopy (FTIR). The analytical method to analyse the gaharu marker compound that dissolved in water mixture will be identified. Thus, this will give benefits for the development of country economics and help the chemical or process engineers provide the accurate properties of gaharu compound solubility in water.

1.2 Problem Statement

This research aims to identify the Gaharu marker compound in water mixture. Basically, water and essential oil will never mix together. When the gaharu oil contacts the surface of the water, it spreads out to form a thin layer. This is because the density of gaharu oil and water are differences. Nevertheless, there are some gaharu marker compounds able to dissolve in the water and their presence can be identified because there is an odour of gaharu in the water after conducting the distillation process. Thus, this research will develop and identify a new analytical method by using Preparative High Liquid Chromatography (PREP-HPLC) and Fourier Transform Infrared Spectroscopy (FTIR) to recognize the marker compound in gaharu-water mixture. Once the marker compound able to be recognized, engineers are able to optimize and purify the gaharu oil from the extraction process.

1.3 Research Objective

At present study, there is a scarcity of chemistry properties of gaharu water mixture from extraction process. Therefore, the study is aimed to achieve several objectives which are as follow:

I. To develop the method (protocol) in identify & recognize the gaharu marker compound in water mixture.

II. To identify the compound and molecular chemistry of Gaharu oil via Fourier Transform Infrared Spectroscopy (FTIR) and Preparative High Liquid Chromatography (PREP-HPLC)

1.4 Scope of the Proposed Study

The scope of this study is to identify the important chemical constituent in Gaharu oil- water mixture from the local Malaysian gaharu essential oil. In addition, this research will investigate the chemistry structure of gaharu water mixture using Fourier Transform Infrared Spectroscopy (FTIR) and Preparative High Performance Liquid Chromatography (PREP-HPLC). The marker compound characterized from gaharu water mixture may provide the useful information to engineers and scientists to control the gaharu extraction process in better and efficient way.

1.5 Expected Outcome

In this research, it is expected that Gaharu marker compound that dissolve in the water mixture can be successfully identified by using FTIR and PREP-HPLC. Main marker compound which possibly dissolve in water are agarospirol, jinkohol-eremol, jinkohol and kusenol. This is because, the sesquiterpene alcohol compound able to form the intermolecular interaction between water molecules. The stronger the intermediate interaction between marker compound-water, the higher the solubility of marker compound in water solvent.

1.6 Significant of Propose Study

This study wills focus on developing and identifying the gaharu marker compound in water mixture. This research will give the benefit for the development of economics and improve the analytical method to analyse the gaharu marker compound that dissolve in the water mixture. In addition, the data can provide an engineer the accurate properties of “gaharu marker compound” solubility in water as solvent during the extraction process. The result would help the local gaharu extractor improve their conventional extraction process to much more efficient techniques.

1.7 Conclusion

In conclusion, this study is about Chemistry Solution Study of Gaharu-Water Mixture. Thus, this research will develop and identify gaharu essential oils that dissolve in the water mixture by using Fourier Transform Infrared Spectroscopy (FTIR) and Preparative High Liquid Chromatography (PREP-HPLC).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

In the world of development, people nowadays are realized the existence of Gaharu in their daily life. Gaharu is the resinous heartwood from Aquilaria trees, which is the native to Southeast Asia and Asia. The trees occasionally become infected with mold and begin to produce an aromatic resin in response to this attack. As the infection grows, it results in a very rich, dark resin within the heartwood. It is this precious resinous wood that is treasured around the world. The resin is commonly called Gaharu, Agarwoods, Oud, Jinko, or Aloeswood and is valued in many cultures for its distinctive fragrance, thus it is used for incense or perfumes.(Frank and James, 2012).

The picture of Gaharu is show in Figure 2.1 in order to get clear understanding about Gaharu.

Figure 2.1 Gaharu Wood (Source: Burhan, 2012)

There are five species of *Aquilaria* (gaharu) were recorded in Peninsular Malaysia,. *Aquilaria malaccensis* is the most popular species among the *Aquilaria* family. There are some chemical components that contribute to the characteristic aroma of Gaharu. Those components are Agarospirol, a-agarofuran, Jinkoh-eremol, 10-epi-g-eudesmol, b-agarofuran, Nor-ketoagarofuran, Kusunol, Jinkohol and Jinkohol II (Surita, 2008).

2.2 Gaharu/ *Malaccensis*

Gaharu or Aquilaria is known under many names based on different cultures. In Malaysia, *Aquilaria malaccensis was* the first agarwood-producing species in 1995. (Lim. & Nooraine, 2010,). Besides, in Chinese is known as Chen-xiang, Jin-Koh in Japanese whereas in Europe it was referred to as Lignum Aquila (eagle-wood) because of the similarity in sound of agila to gaharu. Gaharu can be categorized in many species.

There are five species of *Aquilaria* in Malaysia namely *Aquilaria malaccensis, A.microcarpa, A. hirta,A.rostata and A.beccariana.* Normally, *Aquilaria malaccensis* is the most popular species among the *Aquilaria* family. Gaharu consist of two principles uses namely medicine and perfume.

Gaharu is widely used in traditional medicine as sedative, analgesic and digestive (Alimon et.al, 2011). In China, it is widely used to treat gastralgia, gastric ulcers, gastroptosis, kidney, liver and respiratory problems (Commercial Gaharu Cultivation in Sarawak, 2010). In addition, Gaharu is valued in many types of cultures in all country for its distinctive fragrance and has been widely used as a perfume. Usually, the perfume will come out with resin. The resin looks like a dark to black or a chunk with a fragrant smell if it is burns. Basically, it is found in the heartwood or roots of gaharu producing trees undergoing a chemical and physical change due to the existence of fungus infection. Unfortunately, not all plants can produce resin. (http://www2.thejakartapost.com, 2012).

2.3 Gaharu Extraction

Extraction is a process of obtaining something by chemical or physical or mechanical means from a mixture of compounds (die.net, n.d). There are many types of extraction such as liquid-liquid extraction, and solid phase extraction. Knowing that, liquid-liquid extraction is a separating technology that is based on the distribution of one or more components between two immiscible or almost immiscible liquids. This type of extraction has many advantages such as very large capacities are possible with minimum energy consumption, heat sensitive products are processed at ambient or moderate temperature and also separation of small contents of high-boiling impurities. Whereas, for solid phase extraction, Solid-phase extraction is an extraction method that uses a solid phase and a liquid phase to isolate one, or one type, of analyte from a solution. It is usually used to clean up a sample before using a chromatographic or other analytical method to quantitate the amount of analyte in the sample (Aldrich, 1998).

2.3.1 Gaharu Essential Oil

Essential oils can be defined as a volatile oil that is usually having the characteristic odor or flavor of the plant from which it is obtained, and used to make perfumes and flavorings (essential oil, 2000). Knowing that, Generally, gaharu oils are mixture of sesquiterpenes,sesquiterpene alcohols, oxygenated compounds, chromone derivatives and resins. Some of the more important compounds are agarospirol, jinkohol-eremol, jinkohol and kusenol that may contribute to the characteristic aroma of gaharu. There are many different of comparisons of chemical at different origin of the Gaharu. The comparison is stated in the Table 2.1.

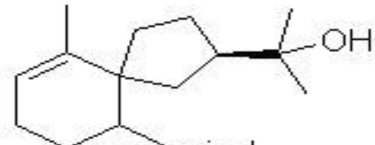
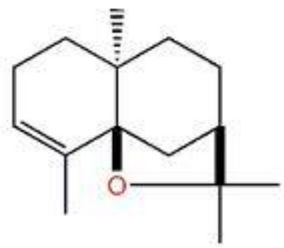
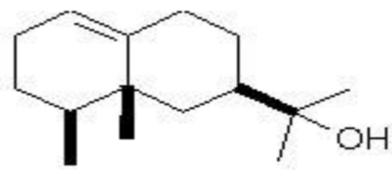
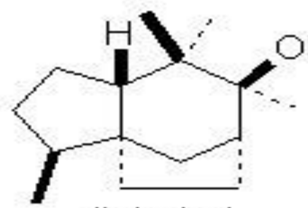
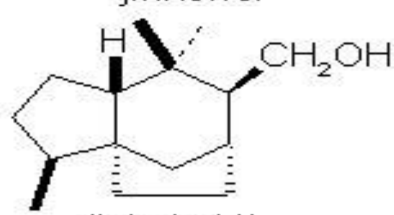
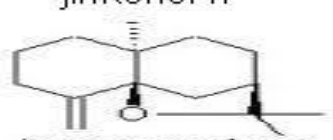
Table 2.1 Comparisons of Chemical at Different Origin of the Gaharu

Gaharu	Chemical components
Type A (A. agallocha)	Agarospirol Jinkoh-eremol Oxo-agarospirol α- and β-agarofuran Dihydroagarofuran Kesunol
Type B (Aquilaria spp.)	Agarospirol Kusunol Jinkoh-eremol Oxo-agarospirol α-agarofuran (-)-10epi-γ-eudesmol Jinkohol Different

(Source: Surita, 2008)

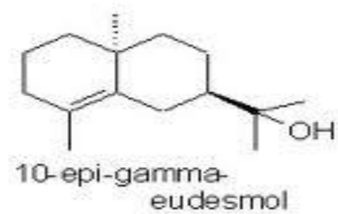
Knowing that, usually different kind of thing will gives different types of its characteristics. Same goes to the Gaharu, Different chemical component in gaharu oil will determine the different characteristic or quality of the gaharu. Thus, for the better and clear understanding, Table 2.2 is tabulated to show some chemical component structure in gaharu essential oil.

Table 2.2 Chemical Component Structures in Gaharu Essential Oil.

Chemical components	Chemical structure
Agarospirol	 agarospirol
a-agarofuran	
Jinkohol-eremol	 jinkoh-eremol
Jinkohol	 jinkohol
Jinkohol II	 jinkohol II
Beta-agarofuran	 beta-agarofuran

(Source:Surita,2008)

Table 2.2 (Continue)

Chemical Components	Chemical Structure
10-epi-gamma-eudesmol	

People nowadays are realized the importance of using essential oils in their daily life. In the world of development, essential oils are more widely used in modern products than one might expect. Usually the essential oils are extracted by using distillation. They are used to fragrance bathing products, incenses, perfumes and cosmetics. But, in terms of alternative medicine, essential oils are most frequently used in aromatherapy. Same to Gaharu essential oils also, it also used to fragrances, incenses, perfumes and so on.

2.3.2 Gaharu Marker Compound

Gaharu marker compound is the compound that will be used flame photometry to detect certain substances. The main component gaharu marker compounds are agarospirol, jinkohol-eremol, jinkohol and kusenol that may contribute to the characteristic aroma of gaharu. (Adam et.al, 2005). Table 2.3.1 shows the physical and chemical properties of gaharu maker compound.

Table 2.3 Physical and Chemical Properties of Agarospirol

Name of component	Agarospirol
Chemical Formula	$C_{15}H_{26}O$
Molecular Weight	222.366g/mol
Hydrogen Bond Acceptors/donors	Donor
Polarity	Polar

Table 2.4 Physical and Chemical Properties of Jinkohol-Eremol

Name of component	Jinkohol-Eremol
Chemical Formula	$C_{15}H_{26}O$
Molecular Weight	222.366g/mol
Hydrogen Bond Acceptors/donors	donor
Polarity	polar

Table 2.5 Physical and Chemical Properties of Jinkohol

Name of component	Jinkohol
Chemical Formula	$C_{17}H_{14}O_4$
Molecular Weight	250g/mol
Hydrogen Bond Acceptors/donors	donor
Polarity	polar

Table 2.6 Physical and Chemical Properties of Khusenol

Name of component	Khusenol
Chemical Formula	$C_{26}H_{32}O_8$
Molecular Weight	472.527 g/mol
Hydrogen Bond Acceptors/donors	donor
Polarity	polar

2.4 Intermolecular Interaction in Organic Solution

Intermolecular forces are forces of attraction or repulsion which act between neighboring particles such as atoms, molecules or ions. Intermolecular forces are particularly important in terms how molecules interact and form biological organisms or even life. In general, there are four classifications of intermolecular forces such as dipole-dipole forces, van der Waal's forces, Hydrogen bond and Covalent bonding.

2.4.1 Dipole-Dipole Forces

Dipole-dipole interaction is the attraction between a partially negative portion of one molecule and a partially positive portion of a nearby molecule. Dipole-dipole interaction occurs in any polar molecule as determined by molecular geometry. The dipole-dipole force exists in all molecules that are polar. Polar molecules have permanent dipoles that interact with the permanent dipoles of neighboring molecules.

The positive end permanent dipole is attracted to the negative end to another (Figure 2.2).

The dipole-dipole forces much weaker than ionic or covalent bonds and have a significant effect only when the molecules involved are close together (touching or almost touching).

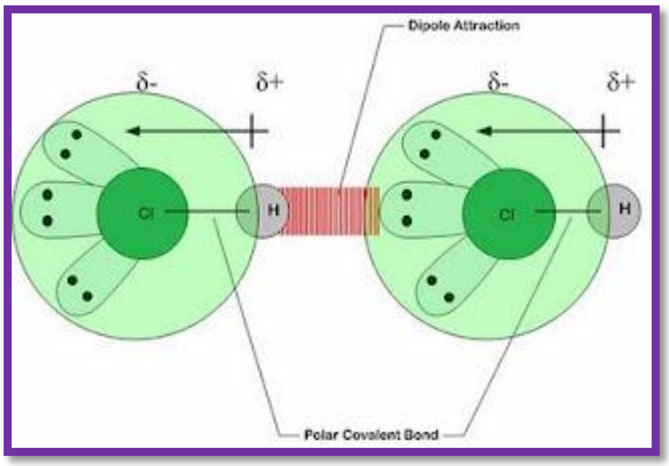


Figure 2.2 Dipole-Dipole Forces (Source: Burhan, 2012)

2.4.2 Van Der Waal's Force

The force arisen from induced dipole and the interaction is weaker than the dipole-dipole interaction. In general, the heavier the molecules, the stronger the van der Waal's force of interaction. The Figure 2.3 shows the van der Wall’s force between the molecules. Van der Waals' forces are much weaker than all other types of bonding. They are only significant in atoms and molecules which have no other types of intermolecular forces of attraction.

For example, discrete non-polar molecules and the Group 0 elements.Van der Waals' forces are a result of electrostatic attraction between temporary dipoles and induced dipoles caused by movement of electrons in atoms and molecules.

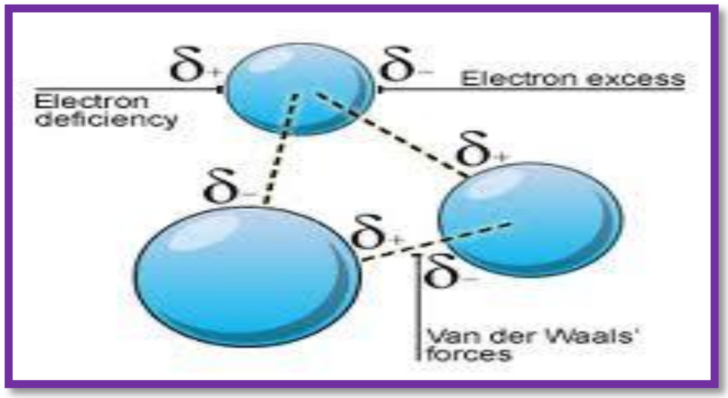


Figure 2.3 Van Der Wall's Force (Source: Scottich, 2012)

2.4.3 Hydrogen Bond

Hydrogen bond is a type of attractive intermolecular force that exists between two partial electric charges of opposite polarity. Although stronger than most other intermolecular forces, the hydrogen bond is much weaker than both the ionic bond and the covalent bond. Usually, Hydrogen bonding is stronger than normal dipole forces between molecules. Basically, to recognize the possibility of hydrogen bonding, the Lewis structure of the molecule is examined first. The electronegative atom must have one or more unshared electron pairs as in the case of oxygen and nitrogen and also has a negative partial charge.

The hydrogen, which has a partial positive charge tries to find another atom of oxygen or nitrogen with excess electrons to share and is attracted the partial negative charge as present in Figure 2.4(Charles, 2003)

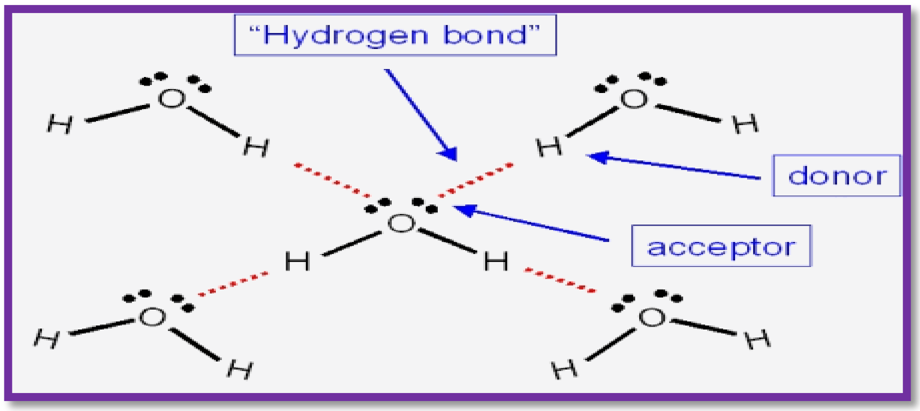


Figure 2.4 Hydrogen Bonding (Source: John, 2011)

2.4.4 Covalent Bonding

A covalent bond is a form of chemical bonding that is characterized by the sharing of pairs of electrons between atoms. Using the Wave Theory, the covalent bond involves an overlap of the electron clouds from each atom. The electrons are concentrated in the region between the two atoms.

In covalent bonding, the two electrons shared by the atoms are attracted to the nucleus of both atoms. Neither atom completely loses or gains electrons as in ionic bonding.

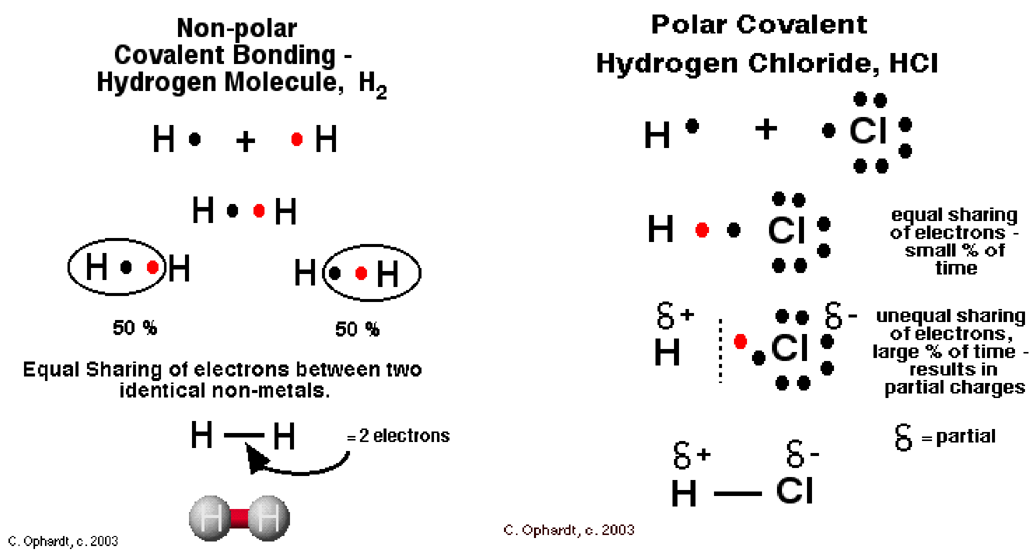


Figure 2.5 Covalent Bonding (Source: C.Ophardit, 2003)

2.5 Analytical Determination of Organic Water Mixture

An organic compound is any member of a large class of gaseous, liquid, or solid chemical compounds whose molecules contain carbon. Organic compound can be classified in two types which are natural compound and synthetic compound. Natural compounds refer to those that are produced by plants or animals whereas synthetic compound refers to Compounds that are prepared by reaction of other compounds are referred to as "synthetic".

Organic compounds having one or more double bonds,usually shows π to π* transition. Then, this organic compound can be observed by far ultraviolet (UV) region. Basically, U.V spectrum is not used to direct identification of functional groups. It mainly used in identification of double bond, conjugation system, aromatic ring and location of substituent (Mallick, 2008).

Besides, functional groups are quickly responding to the Infrared Spectrometry (IR) spectra. Absorption in IR region is highly selective to each functional group. All the functional groups absorb light energy within 1000cm^{-1} to 3000 cm^{-1}. Each and every functional group has a characteristic of IR frequency. Any noticeable change in characteristic IR frequency indicates the structural change of functional group. For example, presence of hydrogen bonding in atomic vibration and it is reflected in IR absorption (Mallic, 2008).

2.6 Analytical Determination of Gaharu Essential Oil

There are two types of analytical determination in order to indentify the gaharu maker compound that are dissolve in water such as liquid Chromatography–Mass Spectrometry (LCMS) and Fourier Transform Infrared Spectroscopy (FTIR). The explanations for both instruments are stated Section 2.6.1 and 2.6.2.

2.6.1 Preparative High Liquid Chromatography (PREP-HPLC)

High Liquid Chromatography is a chemistry based tool for quantifying and analyzing mixtures of chemical compounds. It is used to find the amount of a chemical compound within a mixture of other chemicals. Unlike gas chromatography, which is unsuitable for nonvolatile and thermally fragile molecules, liquid chromatography can safely separate a very wide range of organic compounds, from small-molecule drug metabolites to peptides and protein (Agilent, 2001).

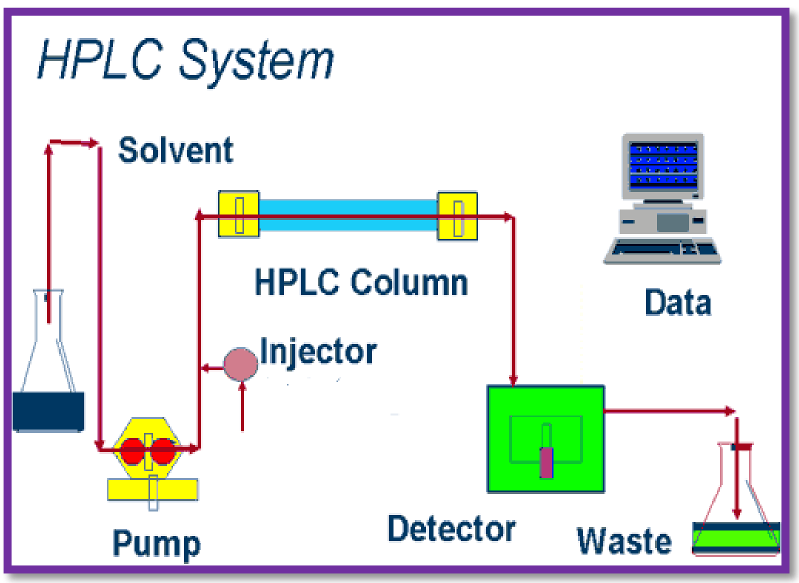


Figure 2.6 System of HPLC (Source: NMSU, 2012)

The system of PREP-HPLC can be explained based on the figure above. The sample is dissolve in a solvent (like water or alcohol), thus the term LIQUID chromatography and then a detector measures response changes between the solvent itself, and the solvent plus the sample when passing through. The electrical response is digitized and sent to a data system. Figure 2.7 below shows the spectra of some gaharu marker compound.

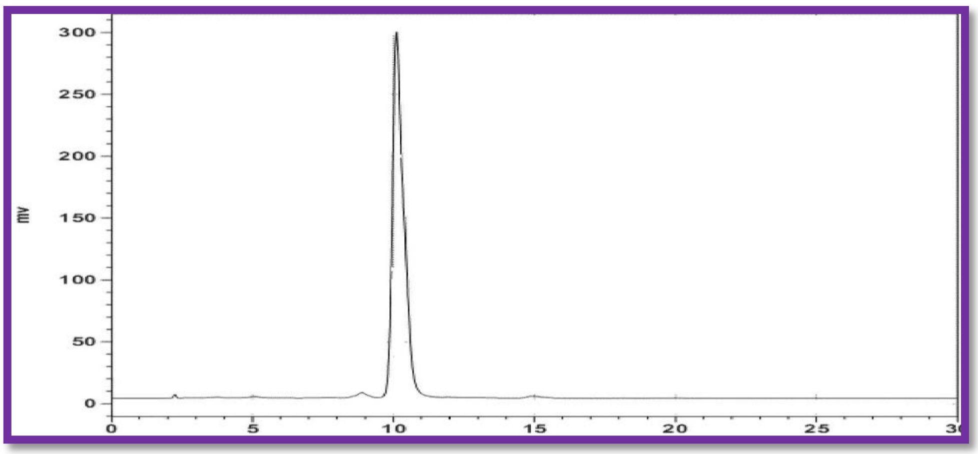


Figure 2.7 Spectra Gaharu Marker Compound (Source: Ting-Ling 2013)

2.6.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR is a technique used to obtain an infrared spectrum of absorption,emission, photoconductivity or Raman scattering of liquid,solid or gas. Normally, FTIR can identify unknown materials, determine the quality or consistency of a sample and also determine the amount of components in a mixture. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. Figure 2.8 shows the spectra of some gaharu marker compound.

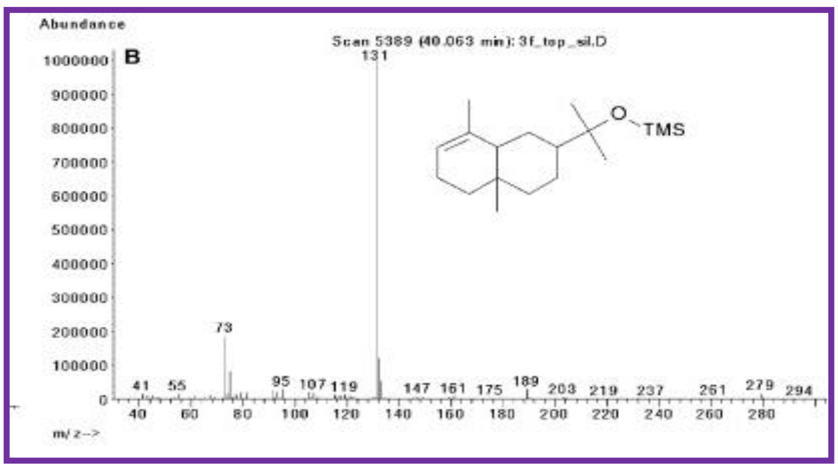


Figure 2.8 Isomeric $C_{15}H_{26}O$ Sesquiterpene Alcohols (Source: Valery, 2011)

Infrared spectroscopy deals with the interaction of infrared light with matter. The energy of an infrared photon can be calculated using the Planck energy relation. The Plank energy relation is as follow:

$$E = hn \qquad (2.1)$$

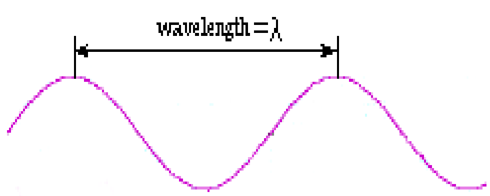
Where h = 6.6 x 10^{-34} joule second

n = frequency of the photon.

The frequency, n, and speed of light, c, are related through the relation

$$c = ln \qquad (2.2)$$

Where c = 3.0 x 10^{8} meter/second and l = wavelength for the light



These two equations can be used to identify a common spectroscopic unit called wavenumber, $\bar{\nu}$, which is the reciprocal of the wavelength.

$$E = hn = h\,c\left(\frac{1}{\lambda}\right);\ E = hn = h\,c\,\bar{\nu}; \qquad (2.3)$$

$$\bar{\nu} = \text{wavenumber} = \left(\frac{1}{\lambda}\right) \text{ has units of } (cm^{-1}) \qquad (2.4)$$

2.7 Conclusion

Gaharu or Aquilaria is known under many names based on different cultures. Gaharu is valued in many types of cultures in all country for its distinctive fragrance and has been widely used as a perfume. Some of the more important compounds are agarospirol, jinkohol-eremol, jinkohol and kusenol that may contribute to the characteristic aroma of gaharu. PREP-HPLC and FTIR is the instrument used in order to detect gaharu maker compound that dissolved in the water.

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Gaharu-Water Equilibrium

This study intends to develop and identify the components of Gaharu maker compound in water mixture and also to identify the compound of Gaharu oil that dissolve in water via Fourier transform infrared spectroscopy (FTIR) and Preparative High performance Liquid Chromatography (PREP-HPLC). In this research, a new method will be developing in order to analyse the component of Gaharu marker compound that dissolve in water mixture by using FTIR and PREP-HPLC. This method will help the engineers and scientists to get the accurate properties of gaharu marker compound in extraction process.

3.2 Material

There are several of materials used in these studies. The materials play an importance role to identify gaharu maker compound that dissolve in water mixture. In order to identify gaharu maker compound that dissolve in water mixture, the chemical material used are gaharu essential oils distilled water and also acetone. Acetone will be used when conducting the FTIR instrument. Figure 3.1 show the picture of gaharu essential oil.

Figure 3.1 Sample from Load Extractor

3.3 Instrumentation

The instrument used for this study is Fourier transform infrared spectroscopy (FTIR) and Preparative High Performance Liquid Chromatography (PREP-HPLC). Knowing that, FTIR is an important technique in organic chemistry. It is an easy way to identify the presence of certain functional groups in a molecule. In addition, it also can use the unique collection of absorption bands to confirm the identity of a pure compound or to detect the presence of specific impurities. Figure 3.2 show the picture of FTIR and figure 3.3 show the picture of PREP-HPLC.

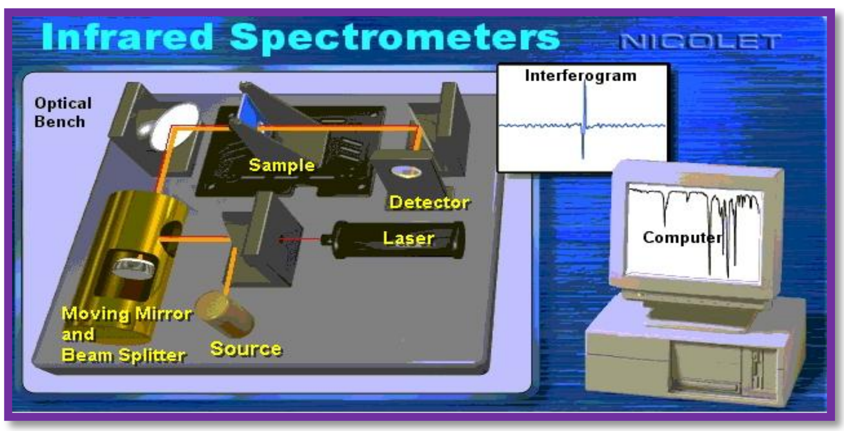


Figure 3.2 FTIR (Source: Nicolet, 2012)

Figure 3.3 PREP-HPLC (Source: Waters Alliance, 2012)

3.4 Procedure

In order to identify the Gaharu maker compound that dissolves in the water mixture, there are several steps that must be following. The major procedure in this research for using FTIR is as section 3.4.1, 3.4.2, 3.4.3 and 3.4.4.

3.4.1 General Set Up for Using FTIR and PREP-HPLC

FTIR is a very sensitive to contaminants. Persons that want to run the experiment should wear rubber gloves at all times. Also, do not use any lubricant on the pellet press. A hydrocarbon, such as steric acid, will seriously degrade any spectrum that is obtained, and the press (die assembly) has to be replaced. Then, Liquid chromatography is a fundamental separation technique in the life sciences and related fields of chemistry.

Figure 3.4 showed the schematic of FTIR and Figure 3.5 showed the schematic for PREP-HPLC.

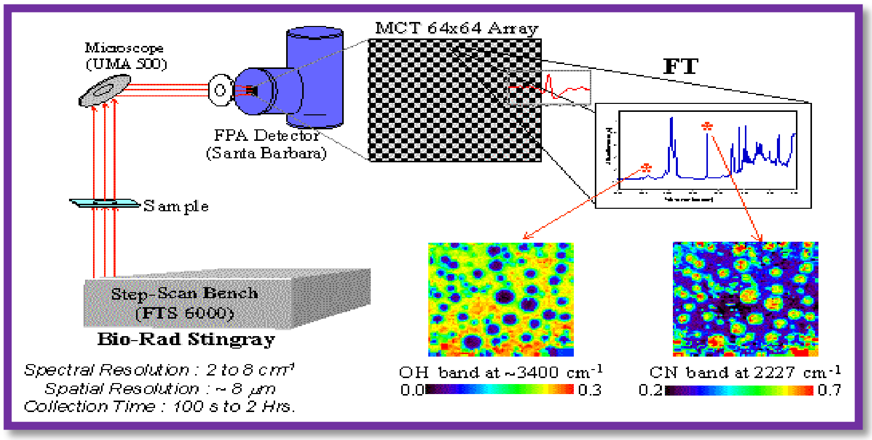


Figure 3.4 A schematic of the FTIR (Source: Nicolet, 2012)

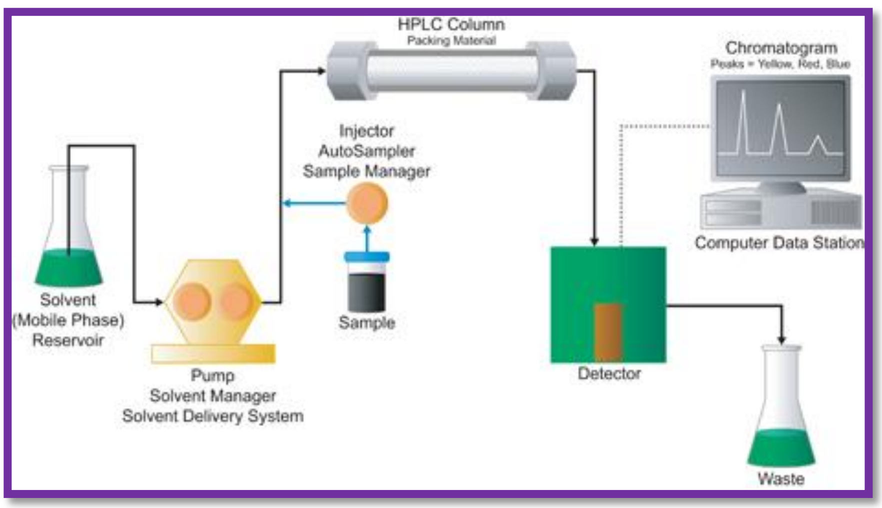


Figure 3.5 A schematic of the PREP-HPLC (Source: Janet, 2012)

3.4.2 The Sample Preparation Process

In order to run the experiment, the parameters for this research are as follow:

I. Temperature (room temperature and boiling point temperature)

II. Time (24hours)

III. Ratio of water and Gaharu essential oil(1:10)

The experiment will be conducted with difference of temperature which is at room temperature (30^{o}C) and boiling point temperature (100^{o}C). 10mL of distilled water and 1mL of gaharu essential oil is filling up into the test tube by using measuring cylinder. The picture of distilled water and gaharu essential oil is shown in Figure 3.6.

Figure 3.6 Gaharu -Water Mixture

Then, the sample will be putting into the thermo mixer in order to maintain the temperature, to mimic the extraction process and also to stir the mixture as shown in Figure 3.7 to achieve an equilibrium point.

Figure 3.7 Gaharu-Water Mixture in Thermo Mixer

After the sample is kept into the thermo mixer, the temperature is set to be 30^{o}C for the first sample and 100^{o}C for the second sample, the stirrer speed is 300rpm and the time is sixteen hours. The samples were stir up until 16hours to mix the solution and the temperature was maintained at 30^{o}C. The mixing process was stop after sixteen hours and leaves it for 8hours and observes the formation of layer. Then, the process is stopped and the sample is leaved for 10 minutes to allow it to cool. Figure 3.8 and 3.9 showed the picture of sample after a day.

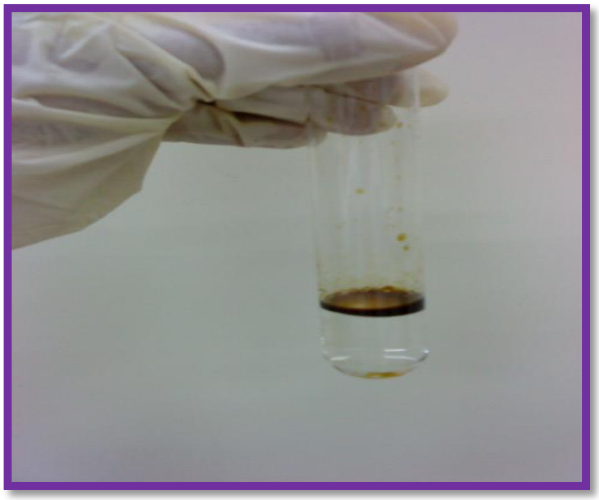


Figure 3.8 Gaharu-Water Mixtures at $30^{O}C$

Figure 3.9 Gaharu-Water Mixtures at $100^{O}C$

After that, the sample (gaharu-water mixture) will be separate by using syringe as shown in figure 3.10.

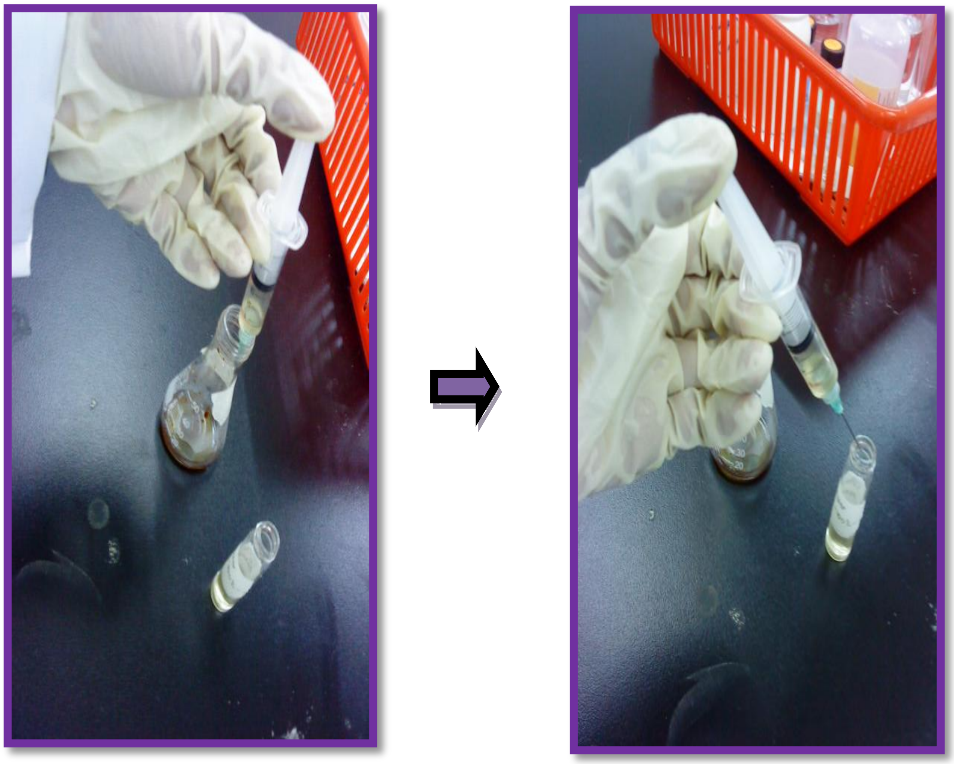


Figure 3.10 Separating Gaharu Essential Oil Using Syringe

Then, the sample is filled into the glass test tube as shown in Figure 3.11.

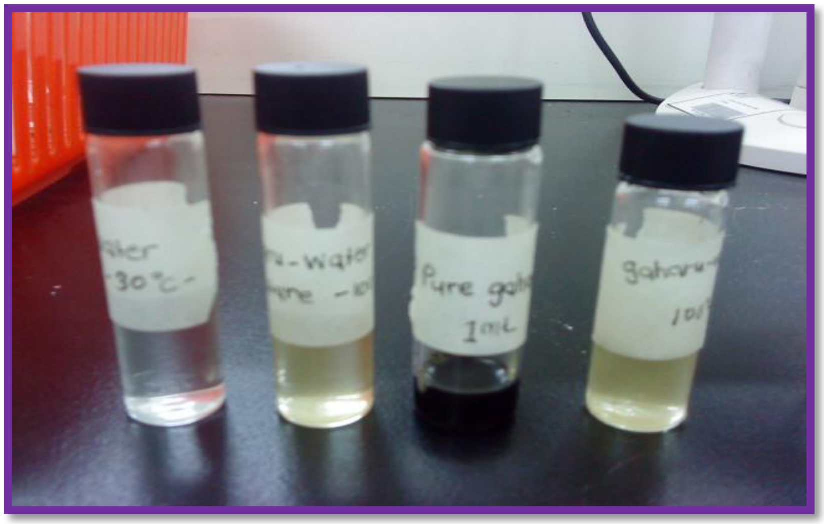


Figure 3.11 Samples after Separation Process

Water and gaharu essential oil will be analysed using FTIR and PREP-HPLC to identify gaharu marker compound that dissolve in water. The procedure of the experiment can be simplified as in flow chart in Figure 3.12.

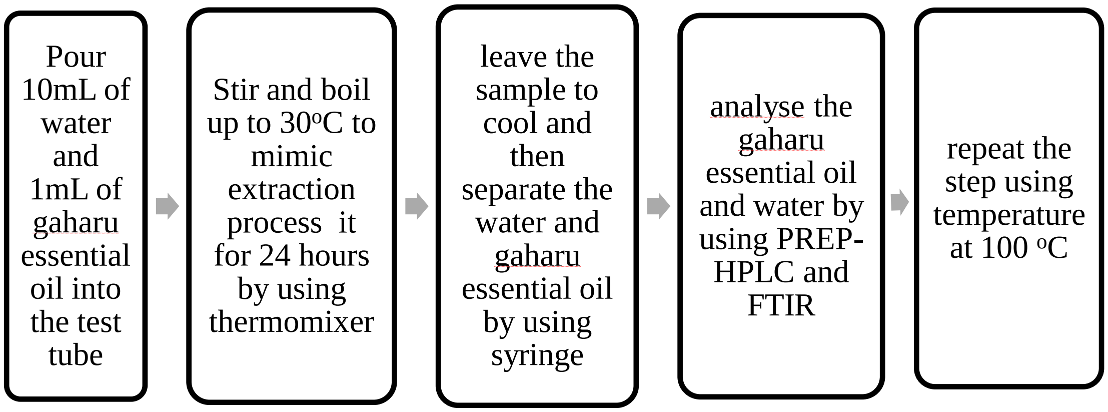


Figure 3.12 Sample Preparation Process

3.4.3 Method of Analysis (FTIR)

Sample of FTIR can be prepared in a number of ways. In this study, the liquid sample will be analyzed. At the end of the experiment, the sample with different temperature and constant stirrer speed for each experiment will be analyzed using FTIR. The sample will be placed into FTIR sample holder in FTIR instrument as shown in the Figure 3.13. In FTIR, the beam enters the sample compartment where it is transmitted through and the radiation or light is passed into the sample. On the other side of the sample is a detector which detects the light that is transmitted through the sample for the final measurement.

The results of FTIR sample is a spectrum that is similar to a molecular fingerprint.

Figure 3.13 Sample placed on the FTIR Sample Holder

Before run the analysis process, the pH value of the sample should be measure first because the plate for FTIR only can be used if the pH of the sample in the range of 1 until 14. The pH of pure gaharu essential oil is 3.55, sample at 30 ^{O}C is 4.23 and sample at 100 ^{O}C is 3.44. Then, the analysis process can be run gently. When conducting the analysis process, the plate should be rinse by using acetone.

The acetone is drop into the wet tissue and rinse the plate. The plate is inserting as shown in Figure 3.14.

Figure 3.14 Plate of FTIR

Firstly, Omn’s software is clicked at the computer programmer. Secondly, smp test mart assence is clicked and the system is calibrated by clicking col bkg button. The system was finishing the calibration process after 32 seconds.

When the confirmation appears, no button is clicked to proceed the analysis process as shown in Figure 3.15.

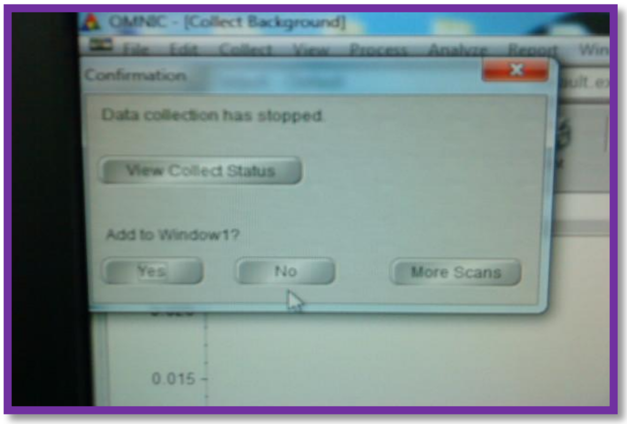


Figure 3.15 Confirmation Button

Next, the experiment set up button is clicked and make sure the gain is 2.0 as shown in Figure 3.16.

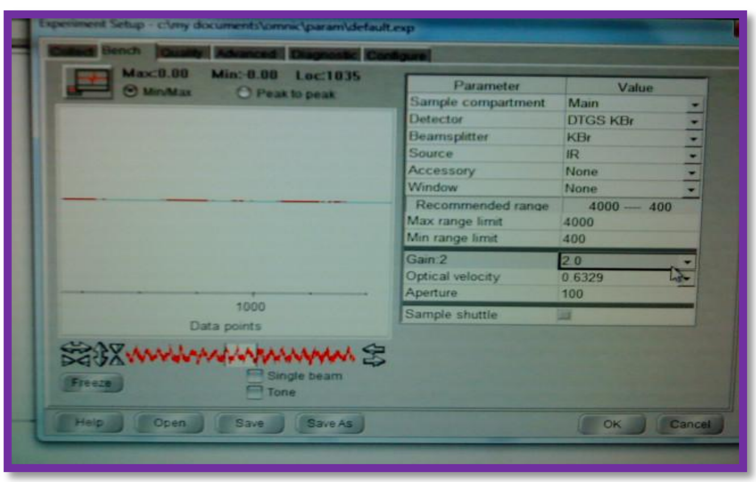


Figure 3.16 Experiment Set Up

After that, the sample is dropped into the plate by using dropper as shown in Figure 3.17.

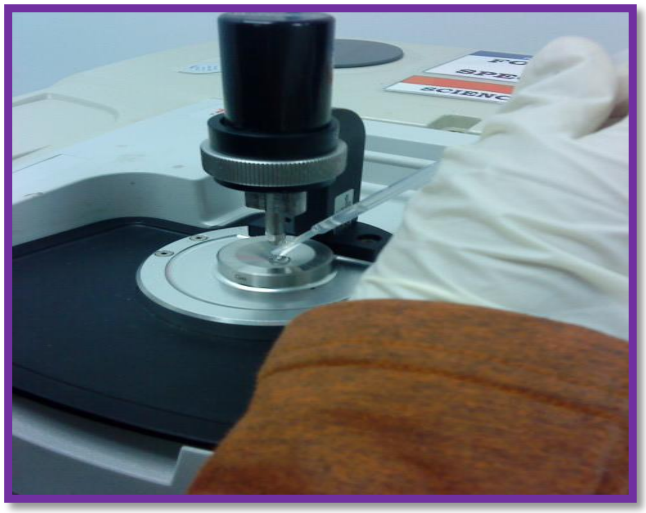


Figure 3.17 Sample Dropped Into the Plate

Then, col smp is clicked and the sample is renamed by typing the name of sample as shown in figure 3.18 below. Next, ok is clicked to save the name of the sample. Besides, the result will be appearing in 32 seconds. Finally, the result is saved and takes the plate and rinsed it using acetone.

The step is repeated for gaharu-water mixture at 100^{o}C, pure gaharu essential oil and distilled water.

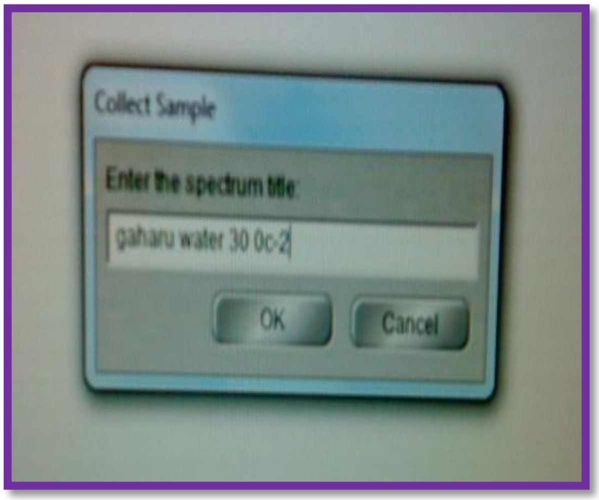


Figure 3.18 Rename the Sample

3.4.4 Method of Analysis PREP-HPLC

In this research, the liquid sample will be analyzed by using PREP-HPLC. HPLC is a chemistry based tool for quantifying and analyzing mixtures of chemical compounds. It is used to find the amount of a chemical compound within a mixture of other chemicals.

The sample will be placed into PREP-HPLC sample holder in PREP-HPLC instrument as shown in the Figure 3.19.

Figure 3.19 Sample Placed on the PREP-HPLC Sample Holder.

Firstly on the PREP-HPLC, the sample is dissolved in a solvent, thus the detector measures response changes between the solvent itself, and the solvent plus the sample when passing through. The electrical response is digitized and sent to a data system. The amount of sample injection is 40 µL. for detail instrument method, the Creation of a gradient is done by a specified table where the percentage of each mobile phase component is determined and the time segments are detailed as shown in Figure 3.20.

The spectrum of PREP-HPLC will appear based on the time that already set. Thus, the compound of Gaharu were identified.

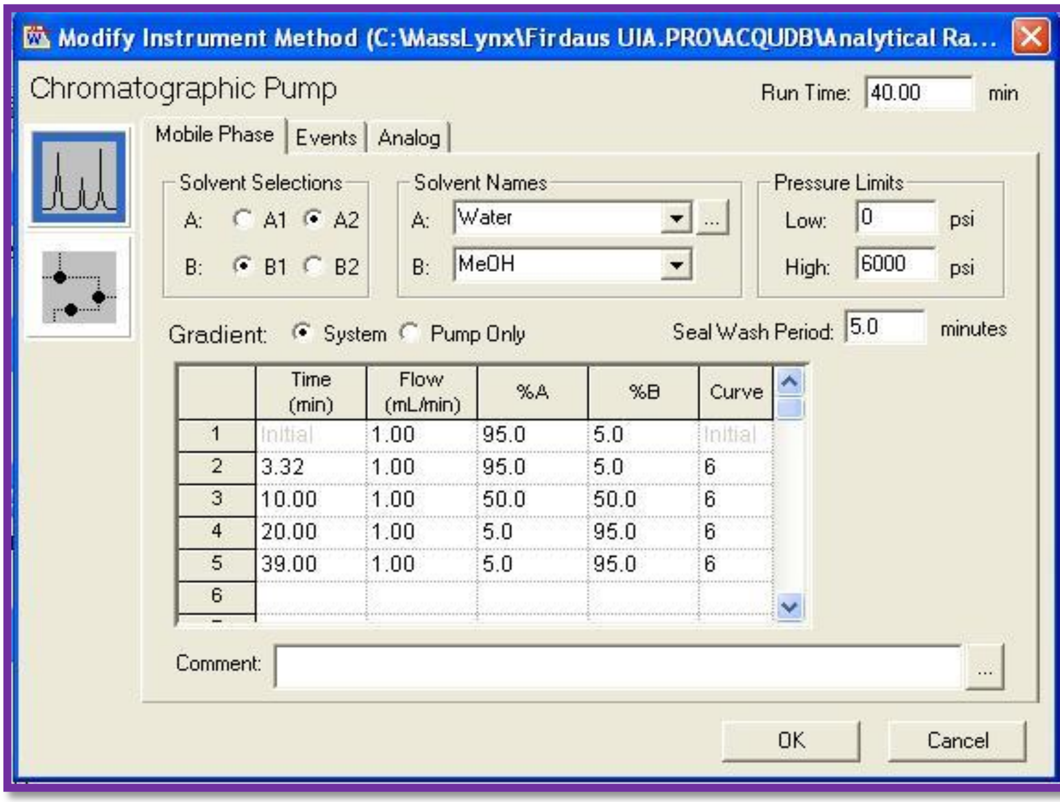


Figure 3.20 Instrumentation Method

Based on the Figure 3.20, the composition of mobile phase (A) is 95% and composition B is 5%. It is stayed in the initial composition for 1 minute. The composition is fixed for about 2 minutes to wash all the strongly eluting composition. The composition is starting to change within 10 minutes and gradually reach a composition of 5:95. Then, get ready for the next injection. The volume of injection if 40µL for each sample.

3.5 Conclusion

As a conclusion, a new analytical method was develop in order to identify the gaharu marker compound that dissolve in the water mixture by using Fourier transform infrared spectroscopy (FTIR) and Preparative High Performance Liquid Chromatography (PREP-HPLC). FTIR analysis will compliment the result obtained from PREP-HPLC to show that there is certain Gaharu marker compounds dissolved in the water mixture.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Introduction

The research of chemistry solution study of Gaharu-water mixture is completed. In this research, the gaharu marker compound that dissolved in the water can be studied by using water as a solvent. FTIR and PREP-HPLC are used to perform the analysis to identify chemical compound of gaharu that dissolve in the water. There are three samples that a researcher can manipulate in this research. The first sample is pure gaharu essential oil that was not adding with water. Second sample is pure gaharu essential oil added into the water and let it for 24 hours at temperature $30^{o}C$ with 300rpm stirrer speed and last sample is pure gaharu essential oil mixture with water at temperature $100^{o}C$ at the same stirrer speed and time.

In this study, each sample is analysed to compare it wavelength, functional group and the color of mixture at different temperatures. The Figure 4.1 and 4.2 were shown the different color of gaharu-water mixture at different temperature.

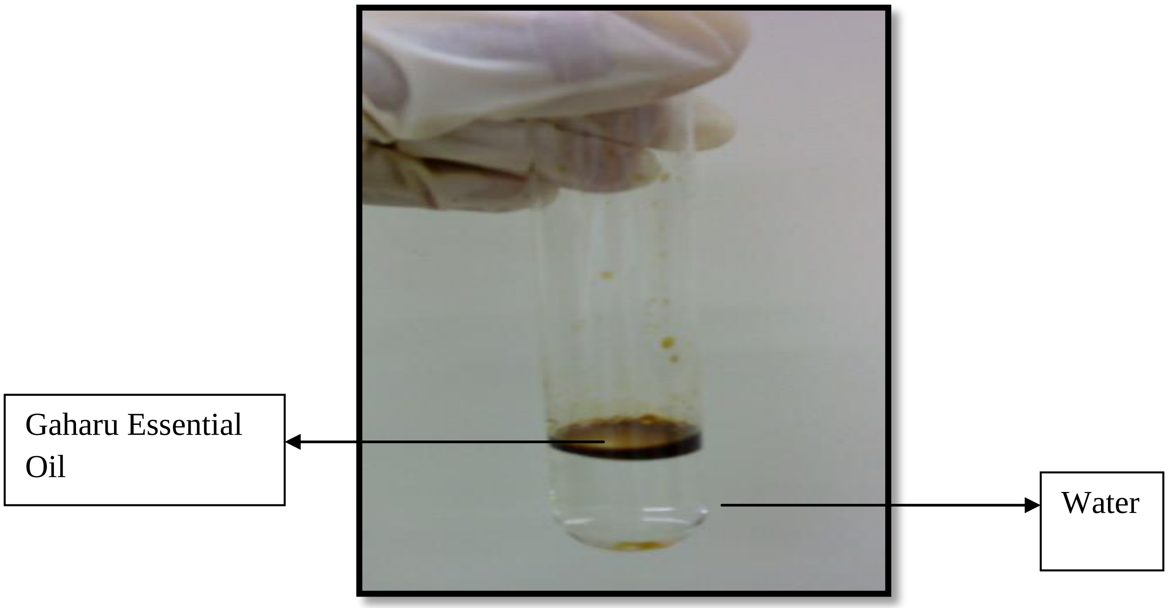


Figure 4.1 Gaharu-Water Mixture at Temperature 30^{o}C

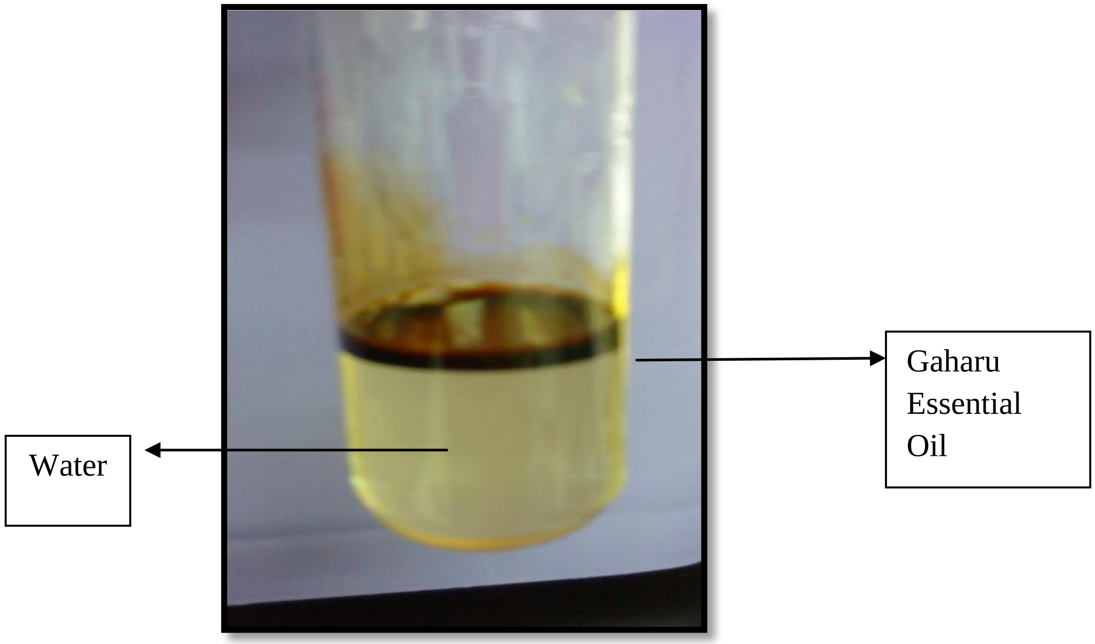


Figure 4.2 Gaharu-Water Mixture at Temperature 100^oC

4.2 Results from Preparative High Performance Liquid Chromatography (PREP-HPLC)

The compound of Gaharu will be compare based on their wavelength, peak height and retention time. At the same wavelength and retention time, it can be concluded that the compound of Gaharu is similar. Figure below showed the results that already get from PREP-HPLC analysis.

The significant peaks were marked as show in Figure 4.3, 4.4 and 4.5.

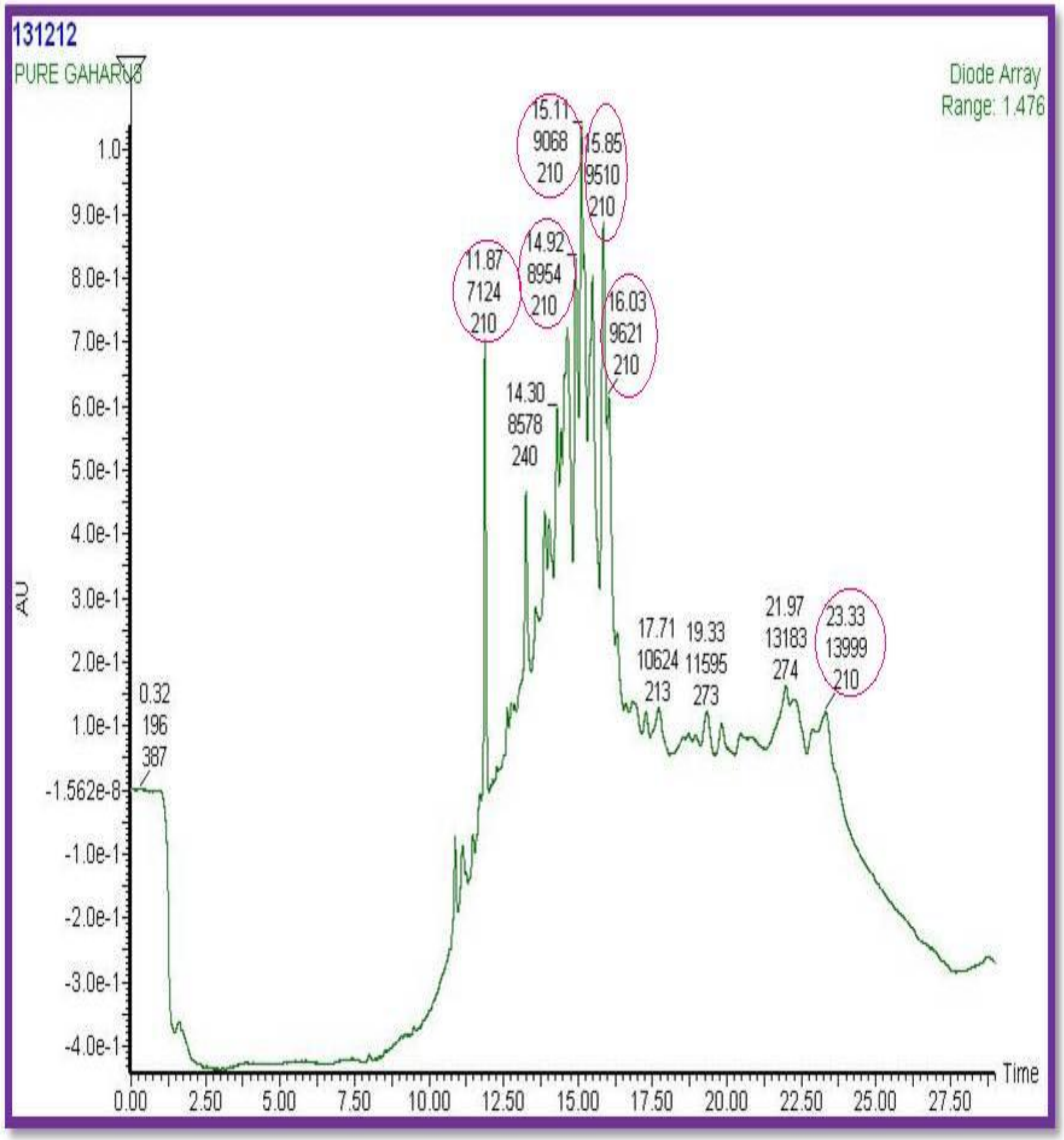


Figure 4.3 Preparative High Performance Liquid Chromatography (PREP-HPLC) Analysis Pure Gaharu Components

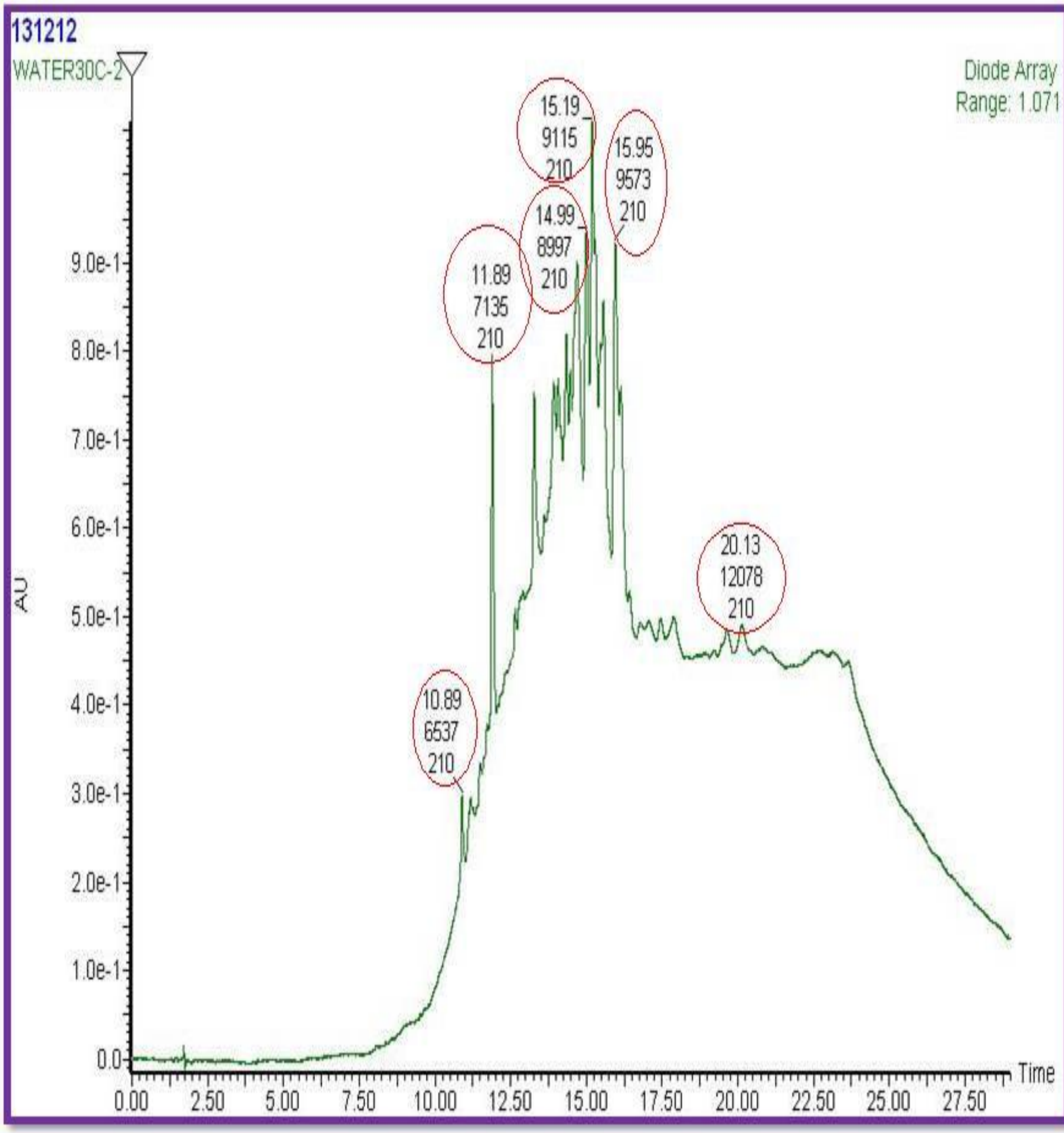


Figure 4.4 Preparative High Performance Liquid Chromatography (PREP-HPLC) Analysis Gaharu-Water Mixture at Temperature 30^{o}C

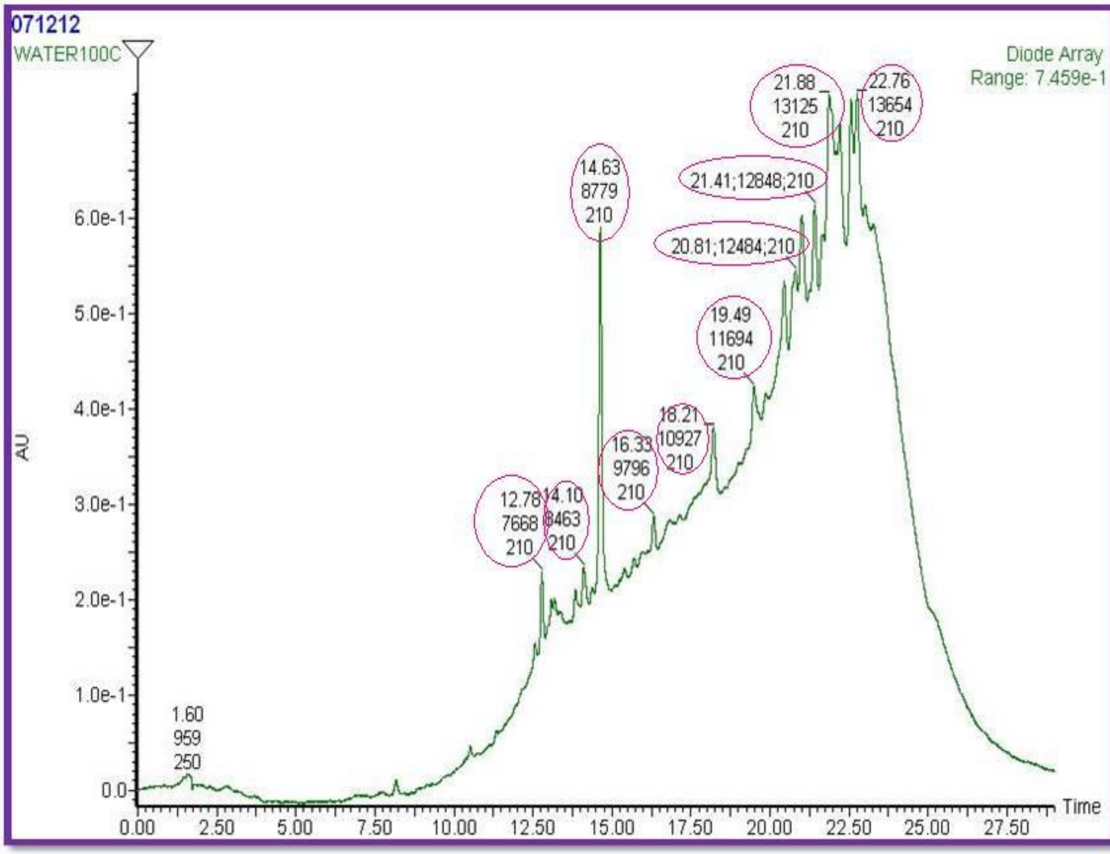


Figure 4.5 Preparative High Performance Liquid Chromatography (PREP-HPLC) Analysis Gaharu-Water Mixture at Temperature 100^{o}C

The bottom value which is 250cm^{-1} wave number, the middle value which is 959 is the height of peak and the top value which is 1.60 is the mass.

4.3 Discussion (PREP-HPLC)

From the graph of pure Gaharu, the wavelengths of gaharu compounds are 210 cm^{-1}, 213 cm^{-1}, 240 cm^{-1},273 cm^{-1},274 cm^{-1} and 387 cm^{-1}. The graph of pure gaharu will be set as reference. So, the graph for gaharu-water mixture at temperature 30^oC and 100^oC will be compared with graph of pure Gaharu. From that, the same wavelength in the graph pure gaharu will be concluded that there are gaharu compound dissolved in water mixture.

Based on both spectrum for Gaharu-water mixture at temperature 30^oC and 100^oC, the wavelength at 210 cm^{-1} is the gaharu marker compound that dissolved in water at different retention time. If the retention time is approximately similar, the spectrum is the same compound of gaharu. The higher the intensity of peak, more gaharu marker compound dissolved in the water. Unfortunately, the compound of gaharu can not be identified due to the limitation of technology on PREP-HPLC. PREP-HPLC can just show that there are gaharu marker compound dissolved in the water but cannot identify the functional group or molecular weight of that compound. Probably, the Gaharu compound dissolved in the water is the compound that has –OH group such as Agarospirol, Jinkohol-eremol and Khusenol.

This is because alcohols group consists of carbon chain which is non polar and –OH group which is polar compound. Water is polar compound, thus it will attract the –OH group. Thus, to support this result, FTIR analysis will be run to proved that there are gaharu marker compound dissolved in water mixture.

4.4 Results from Fourier Transform Infrared Spectroscopy (FTIR)

All samples of Gaharu-water mixture were analyzed by using FTIR in this section. The compound of Gaharu will be identified based on it wave numbers. From the wave numbers, the functional group for compound of gaharu will be identified respectively. Figure below showed the results that already get from FTIR analysis.

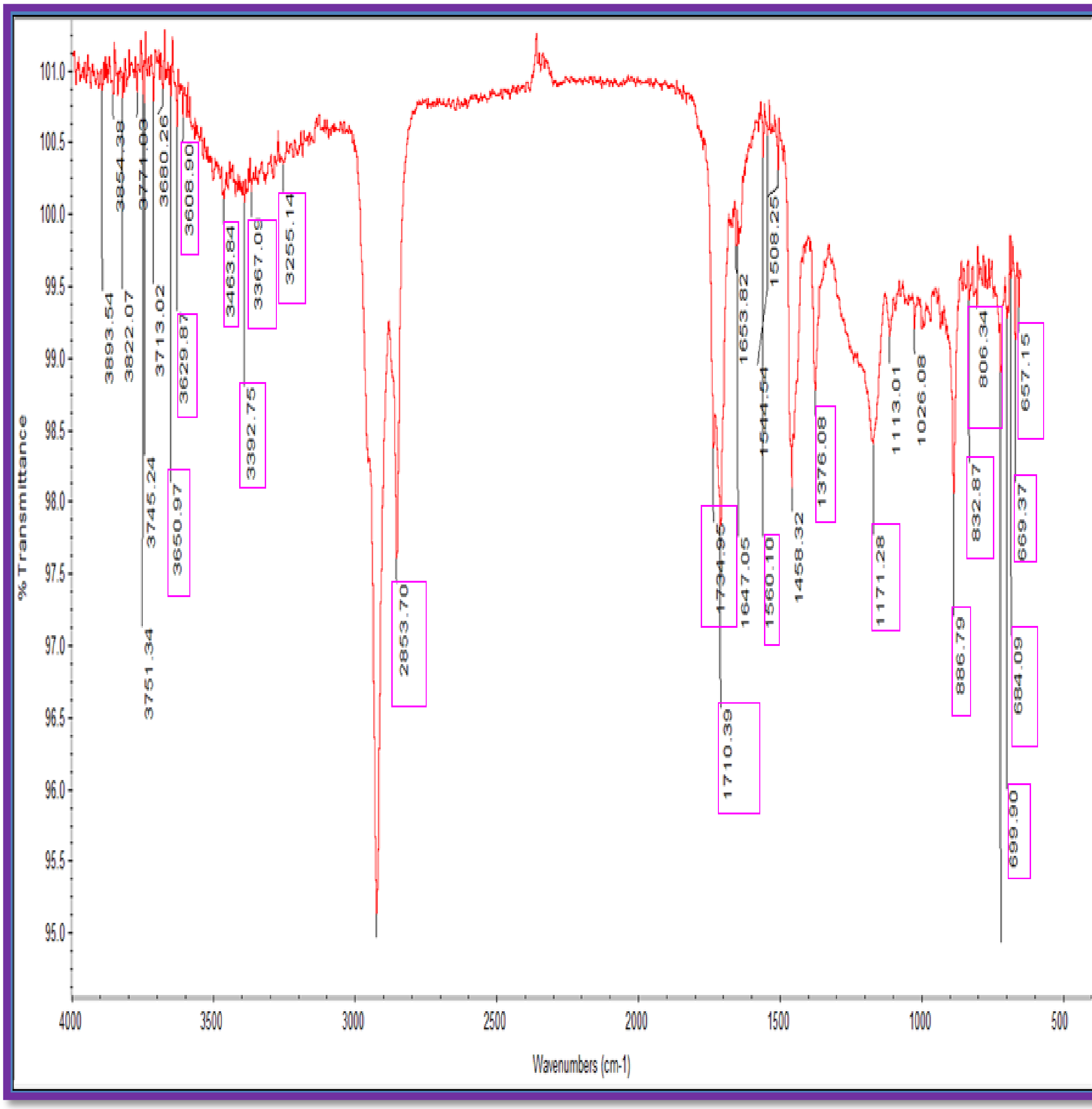


Figure 4.6 IR Spectrum for Pure Gaharu

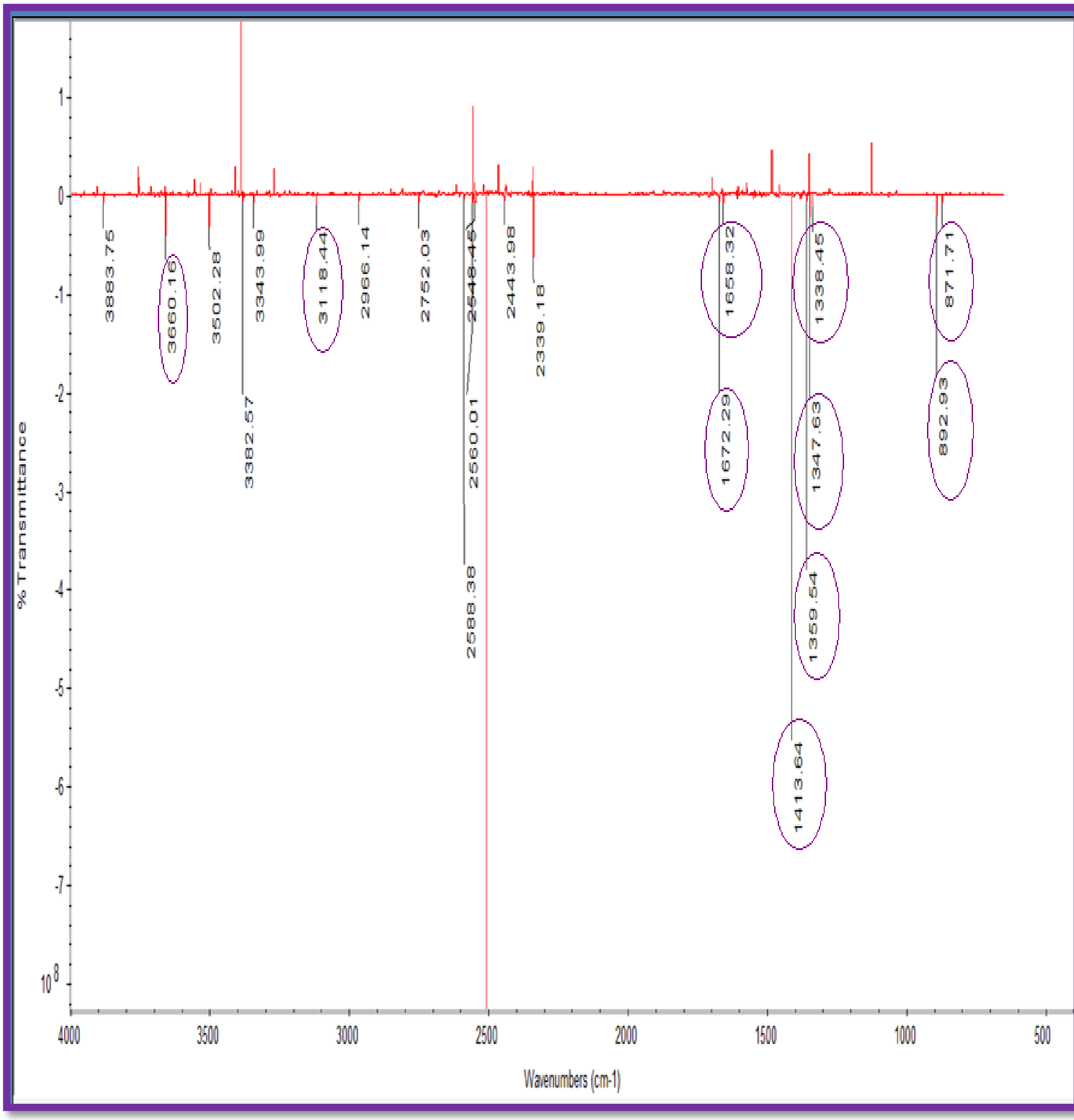


Figure 4.7 IR Spectrum for Gaharu-Water Mixture at Temperature 30°C

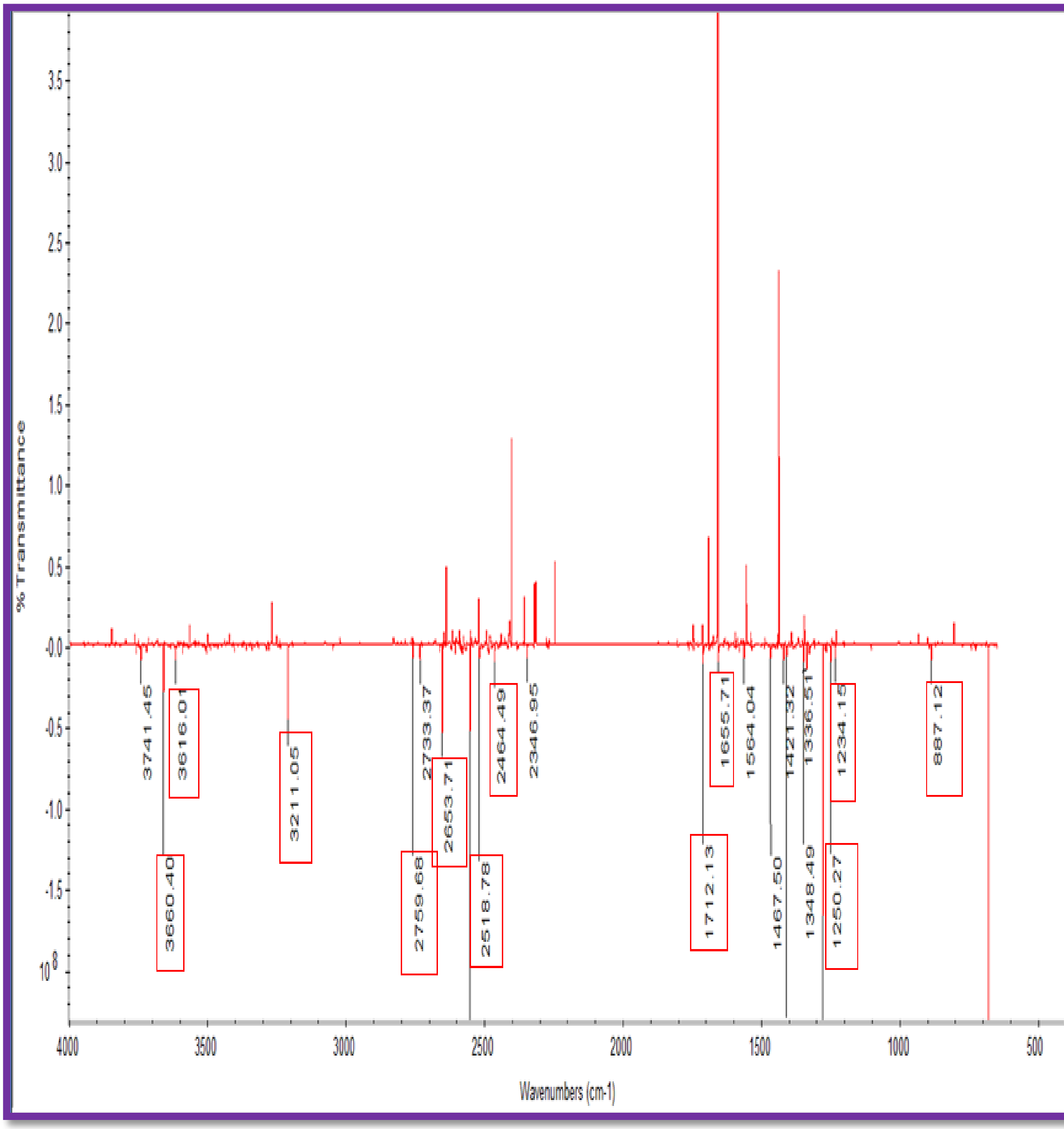


Figure 4.8 IR Spectrum for Gaharu-Water Mixture at Temperature 100°C

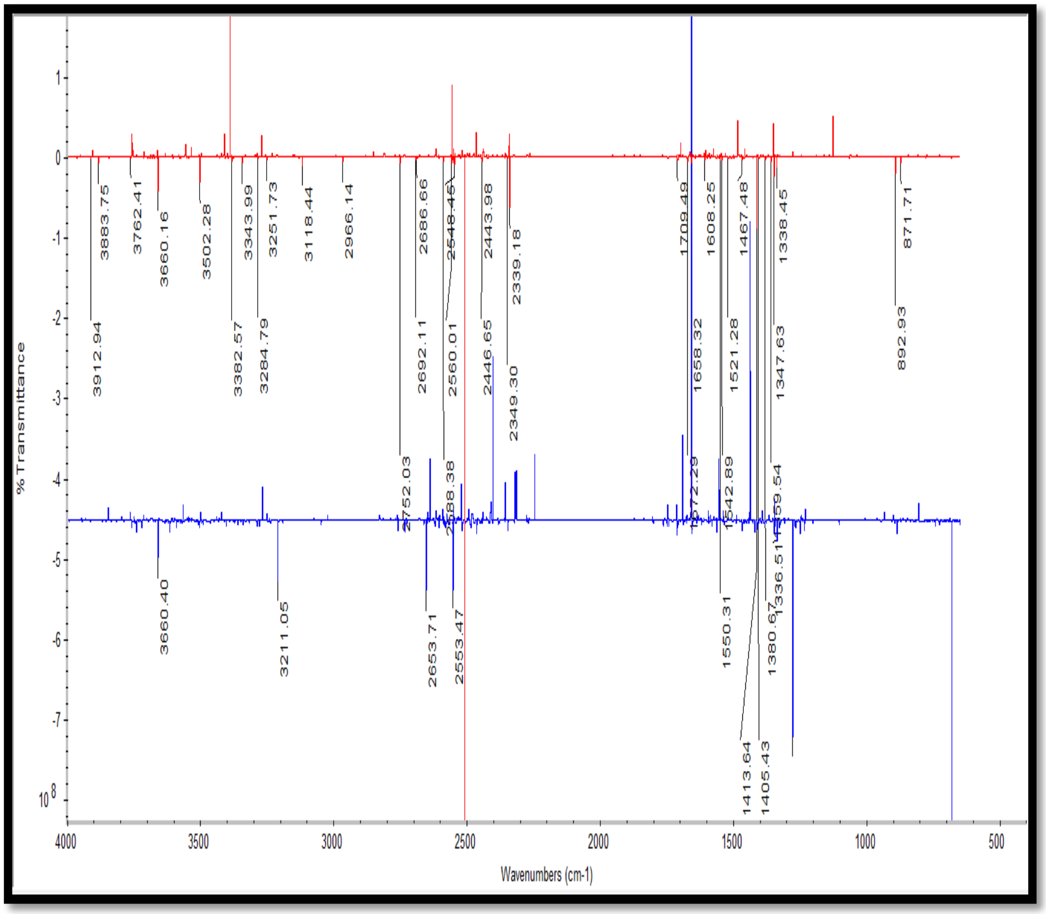


Figure 4.9 Combination of Spectum Gaharu-Water Mixture at Temperature 30^{o}C and 100^{o}C

Temperature 30^{o}C

Temperature 100^{o}C

Table 4.1 FTIR Analysis of Possible Gaharu Component at Temperature 30^{o}C

Wavenumber (cm^{-1})	Functional group	Molecular motion	Possible component
3660.16	Alcohols	O-H Stretch	Agorospirol, 10-epi-gamma-eudesmol & Khusenol
3118.44	Aromatics	C-H Stretch	Agorospirol, 10-epi-gamma-eudesmol & Khusenol
1672.29 1658.32	Ketones	C=O Stretch	agarofuran
1413.64	Carboxylic Acids	O-H Bend	Agarospirol, Jinkohol-eremol & Khusenol
1359.54 1347.63 1338.45	Alkenes	C-H in plane Bend	Agarospirol, Jinkohol-eremol & Khusenol

Table 4.1 (Continue)

Wavenumber (cm^{-1})	Functional group	Molecular motion	Possible component
892.93	Alkenes	C-H Bend (distributed -1,1)	Agarospirol, Jinkohol-eremol & Khusenol
871.71	Aromatics	C-H Bend (meta)	Agorospirol & Khusenol

Table 4.2 FTIR Analysis of Possible Gaharu Component at Temperature 100^{o}C

Wavenumber (cm^{-1})	Functional group	Molecular motion	Possible component
3660.40 3616.01	Alcohols	O-H Stretch	Agarospirol, Jinkohol-eremol, 10-epi-gamma-eudesmol & Khusenol
3211.05 2759.68 2653.71 2518.78 2464.49	Carboxylic Acids	O-H Stretch	Agarospirol, Jinkohol-eremol, 10-epi-gamma-eudesmol & Khusenol
1712.13	Ketones	C=O Stretch	Agarofuran
1655.71 1467.50	Aromatics	C=C Stretch	Agorospirol, 10-epi-gamma-eudesmol & Khusenol
1250.27	Ethers	C-O-C Stretch (diaryl)	Khusenol
1234.15	Ethers	C-O-C Stretch (dialkyl)	Khusenol
887.12	Alkenes	C-H Bend (disubstituted -1,1)	Agarospirol, Jinkohol-eremol & Khusenol

4.5 Discussion (FTIR)

From the graph 4.6, 4.7 and 4.8 and Table 4.1 and 4.2, it is clearly show that Agarospirol, Jinkohol- eremol, khusenol and agafuron are gaharu marker compound that dissolve in the water. Thus, from that, it will support the analysis from PREP-HPLC. Based on the Table 4.1 and Table 4.2, it is clearly showed that gaharu-water mixture at temperature 100^{o}C gives better results. Meaning that, the wave numbers for that sample is quickly identified, more functional group from –OH were identified and more gaharu marker compounds were found from that temperature. Thus, the temperature at 100^{o}C is very suitable for extraction process and help engineer to get the accurate properties of "gaharu marker compound" solubility in water as solvent during the extraction process.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Introduction

This chapter will explain in detail about the conclusion of this study, recommendation to improve the result of this research and what should do in the future works.

5.2 Conclusion

From the result obtained, it is showed that there are certain gaharu compounds dissolved in the water. The Gaharu compound dissolved in the water is the compound that has –OH group such as Agarospirol, Jinkohol-eremol and Khusenol. This is because alcohols group consists of carbon chain which is non polar and –OH group which is polar compound. Water is polar compound, thus it will attract the –OH group.

The objectives of this research were achieved. The aims of this study are to develop the method (protocol) in identify and recognize the gaharu marker compound in water mixture and also to identify the compound and molecular chemistry of Gaharu oil via Fourier Transform Infrared Spectroscopy (FTIR) and Preparative High Liquid Chromatography (PREP-HPLC).

Based on data presented in the figure Preparative High Performance Liquid Chromatography (PREP-HPLC) Analysis Gaharu component at temperature 30^oC, 100^oC and pure Gaharu, it can be seen that there are certain Gaharu marker compound that possibly dissolve in the water. Unfortunately, it is still cannot be proved which compound it is. Thus, Analysis from FTIR was proved that which compound dissolved in the water. From the table FTIR Analysis of possible Gaharu component at temperature 30^oC and 100^oC, it clearly showed that Agarospirol, Jinkohol-eremol and Khusenol are the gaharu marker compounds that dissolve in the water. Even though the chemical compound at temperature 30^oC and 100^oC are similar, but at temperature 100^oC, the gaharu marker compound can be identified faster. Thus, at this temperature, it is very suitable for extraction process and help engineer to get the accurate properties of “gaharu marker compound” solubility in water as solvent during the extraction process.

The table FTIR Analysis of possible Gaharu component at temperature 30^{o}C and 100^{o}C showed the wavenumbers, functional group, molecular motion and possible component for each two sample of gaharu-water mixture. Based on data presented, each sample has different wavenumbers, functional group and molecular motion. The functional group is identified according to its wavenumbers. The highest functional group in each sample is alcohol group. This is because alcohols group consists of carbon chain which is non polar and –OH group which is polar compound. Water is polar compound, thus it will attract the –OH group. So, it can be concluded that, polar molecule will tends to dissolve component from polar molecule and non-polar molecule will dissolved anything non-polar molecules.

5.3 Recommendations

There are recommendations need to be applied for this research in order to improve the quality and better result for this study. One of them is the limitation of handling the equipment. Student does not allow running the equipment by itself.

In addition, there are some equipment already cannot functioning. Students feel too disappointed because unable to use the equipment and had to use another equipment and not get a good result because of different equipment gives a different result. So, the authorities should improve technology or change the new equipments available in the chemistry laboratory.

The recommendation for this research to get better result, researcher should use Liquid Chromatography–Mass Spectrometry (LC-MS). This is because liquid chromatography can safely separate a very wide range of organic compounds, from small-molecule drug metabolites to peptides and protein (Agilent, 2001). Whereas, Mass Spectrometry (MS) identifies substances by electrically charging the specimen molecules, accelerating through a magnetic field, breaking the molecules into charged fragments and detecting the different charges (Frederic, 2012). This means that, molecular weight for each component will be identified by using LCMS. So, the gaharu marker compound will be clearly identified.

5.4 Future Works

All researchers should think what they should do in the future works based on their research. There are some future works of this study. For example, researcher must think what is the most powerful equipment should be used for this study in order to get good result and this result will help the engineer in the extraction process. This is because it is quite difficult to collect the gaharu essential oil due to the small amount of oil extraction. PREP-HPLC was not suitable equipment to identify the compound of gaharu because PREP-HPLC cannot identify the molecular weight or functional group of certain component.

Thus, researcher should find powerful equipment in order to get a better result. The gaharu marker compound will be clearly identified by using Nuclear magnetic resonance (NMR). This is because nuclear magnetic resonance spectroscopy has been used to analyze the microscopic physical and chemical structures of molecules. NMR has become the finest technique for looking closely at the composition of organic compounds.

REFERENCES

Abidi, S.L. (2001). Chromatographic analysis of plant sterols in foods and vegetable oils. Chromatography Journal.[online].935{3}.[Accessed15th April 2012], p.173- 201.Available from World Wide Web: http://naldc.nal.usda.gov/

Bevis, J. (1996). Fourier Transform Infrared Spectroscopy (FTIR)-Pirelli Cables Case Study.[online].[Accessed15th April 2012]. Available from World Wide Web: http://www.azom.com/article.aspx?ArticleID=1562

David. H (2000). Modern Analytical Chemistry.[online].[Accessed15th April 2012]. Available from World Wide Web: http://www.chemmsu.ru/

E.AG Evans Analytical Group (2009*). TN105 EAGLABS* Fourier Transform Infrared Spectroscopy (FTIR) Services. .[online].[Accessed15th April 2012]. Available from World Wide Web: http://www.eaglabs.com/

Fairul Idzwan (2008). Comparison of Agarwood Essential Oil & Water Soluble (Hydrosol). Bachelor thesis. University Malaysia Pahang, Malaysia.

Mallick. A (2008). Engineering Chemistry.[online].[Accessed10th April 2012]. Available from World Wide Web: http://www.che.iitb.ac.in/

Nor Azah et.al. (2008). Comparison of Chemical Profiles of Selected Gaharu Oils from Peninsular Malaysia. Journal of Analytical Sciences in Malaysia.[online]. 12{2}. [Accessed15th April 2012],p. 338-34 Available from World Wide Web:http://pkukmweb.ukm.my/

Nor Ilia Anisa (2008). Study On Pretreatment Of Gaharu Oil Extraction Process. Bachelor thesis. University Malaysia Pahang, Malaysia.

Norsuzieana (2009). Analysis Volatile Compound Of Gaharu Oil Composition Via Solid Phase Micro Extraction (SPME).Bachelor thesis. University Malaysia Pahang, Malaysia.

Nurdiyana (2008). Comparison Of Gaharu (Aquilaria Malaccensis) Essential Oil Composition Between Each Country.Bachelor thesis. University Malaysia Pahang, Malaysia.

Sawant, S., Baravkar, A., and Kale. R. (2011). FT-IR Spectroscopy : Principle, Technique and Mathemathic, 2. International Journal of Pharma and Bio Sciences. [online].6{9}.[Accessed10th April 2012],p. 513-519. Available from World Wide Web: http://www.academicjournals.org/

S. Chand (2009). A Textbook of Engineering Chemistry.[online].[Accessed11th April 2012]. Available from World Wide Web: http://www.vedamsbooks.com/

Shahrizal (2006). Analysis Of Gaharu Essential Oil Using GC/GCMS**.**Bachelor thesis University Malaysia Pahang, Malaysia.

Surita (2008). Comparison between Chemical Compounds in Gaharu Smoke (Burning)And Gaharu Oil (Hidrodistillation). Bachelor thesis. University Malaysia Pahang, Malaysia.

Kanokkantapong V. et al. (2006). FTIR Evaluation of Functional Groups Involved In The Formation Of Haloacetic Acids During The Chlorination Of Raw Water. Journal of Hazardous Materials in Thailand.[online].136{1}.[Accessed12th April 2012],p. 188-196. Available from World Wide Web: http://www .amazon .com/

Walt.V (1999). Organic Compound Identification Using Infrared. Spectroscopy. [online] [Accessed11th April 2012].]. Available from World Wide Web:http://www.800mainstreet.com/

William, W. (2012). Analysis of Fatty Acids By High-Performance Liquid Chromatography. [online].[Accessed11th April 2012]. Available from World Wide Web:http://lipidlibrary.aocs.org/

Zhen, G., Zheng, H., Yanzhen, L. and Yun , W. (2012). Isolation and purification of of heroin from heroin street samples by preparative high performance Liquid chromatography. Forensic Science International Journal. [online].221{1}.[Accessed13th April 2012],p. 120 -124. Available from World Wide Web: http://www.ncbi.nlm.nih.gov/

Zubair (2008). Extraction Of Gaharu Essential Oil Using Ultrasonic Assisted Hydrodistillation. Bachelor thesis. University Malaysia Pahang, Malaysia.

APPENDICES

APPENDIX A

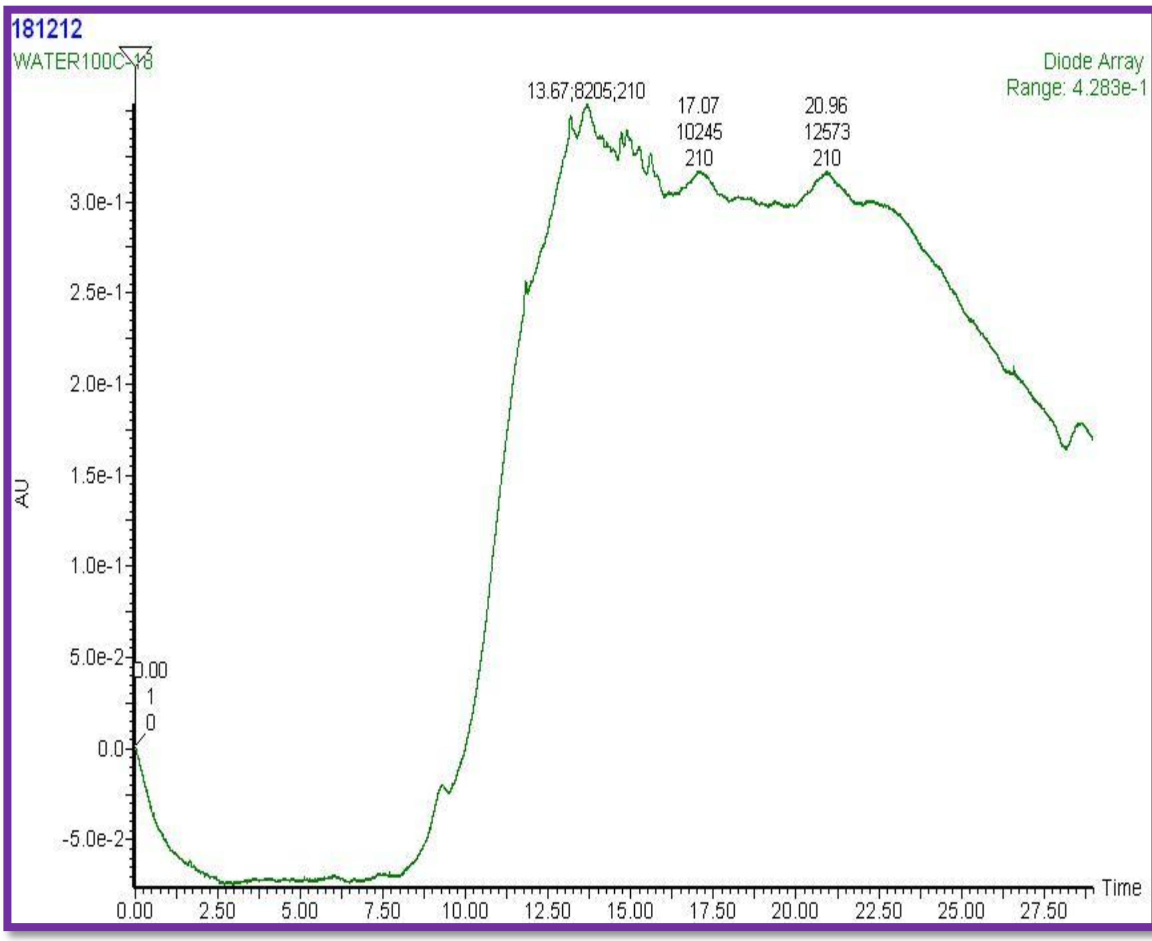


Figure A.1 Gaharu-Water Mixture at 100^{o}C(PREP-HPLC)

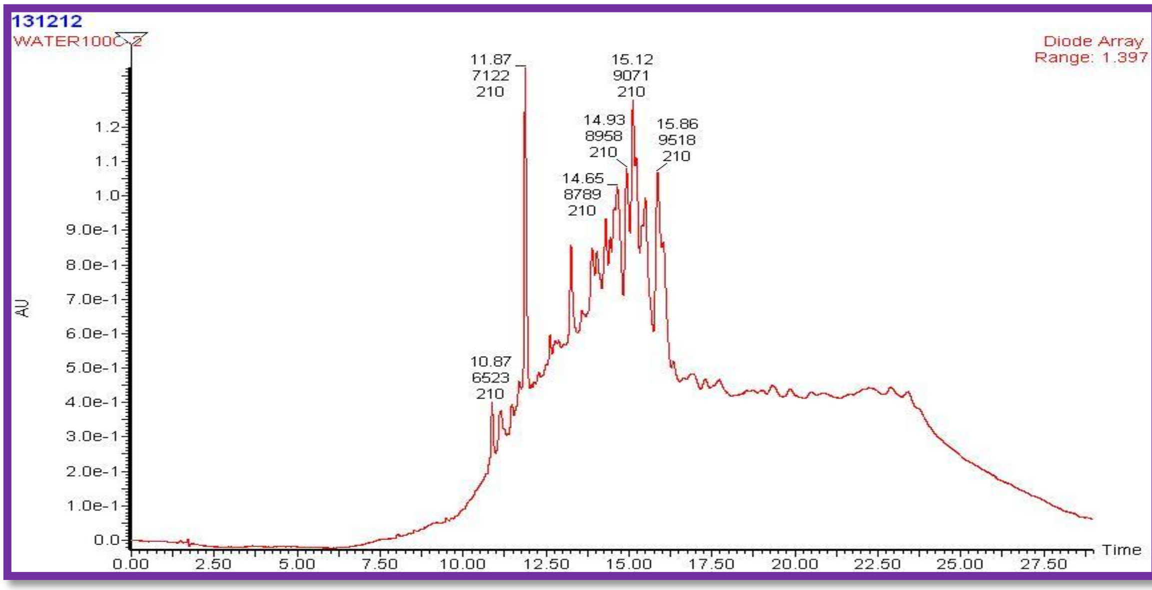


Figure A.2 Gaharu-Water Mixture at 100^{o}C(PREP-HPLC)

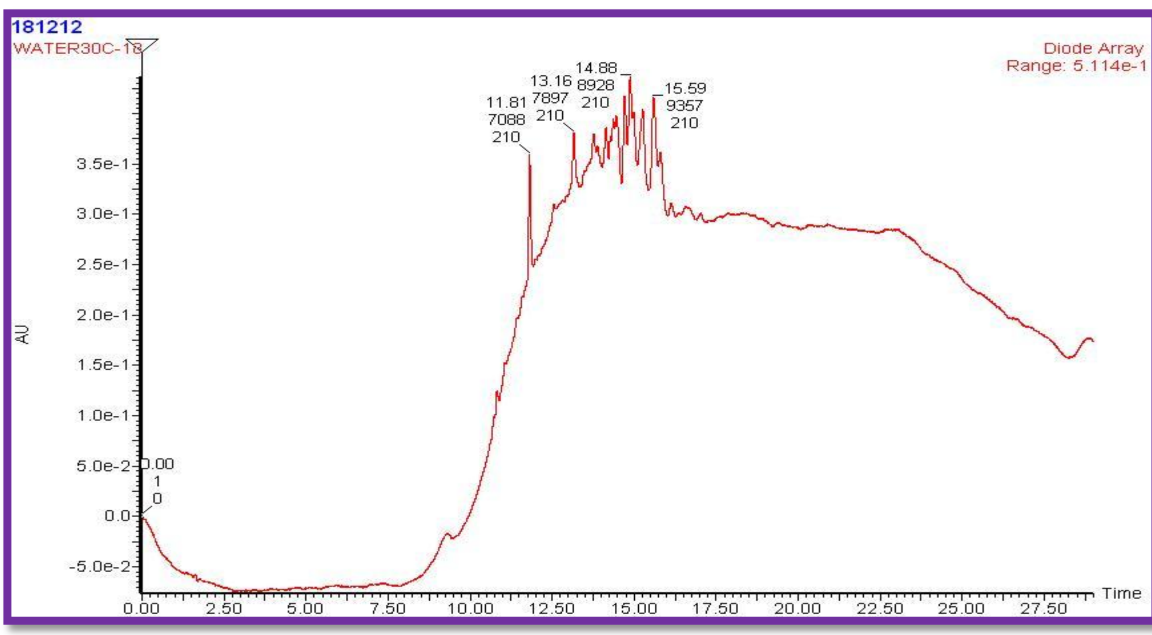


Figure A.3 Gaharu-Water Mixture at 30^{o}C(PREP-HPLC)

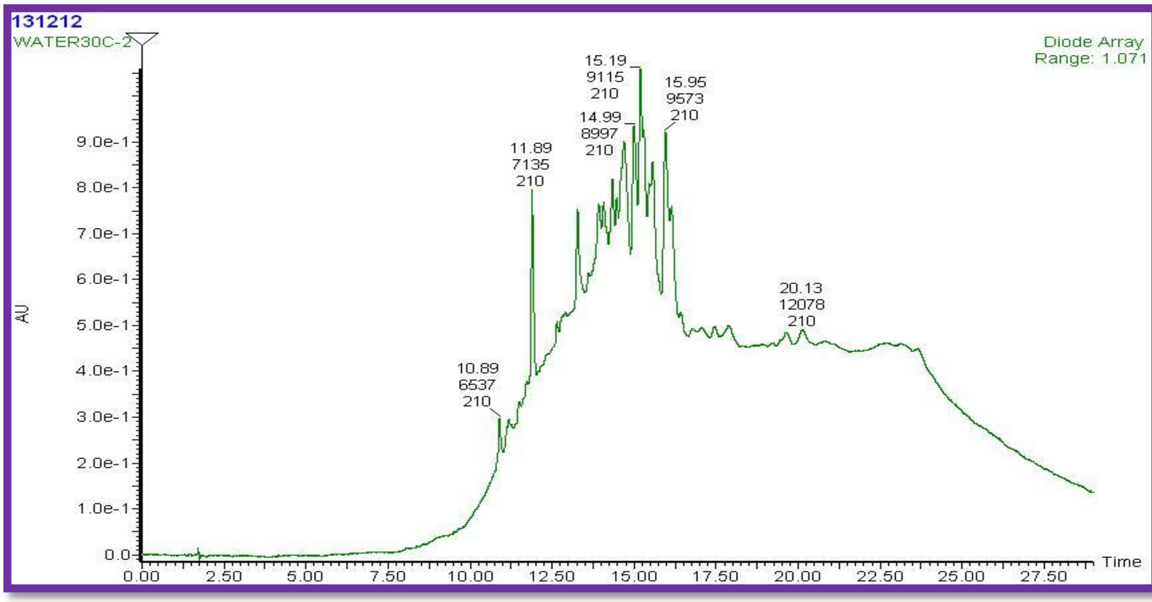


Figure A.4 Gaharu-Water Mixture at 30°C(PREP-HPLC)

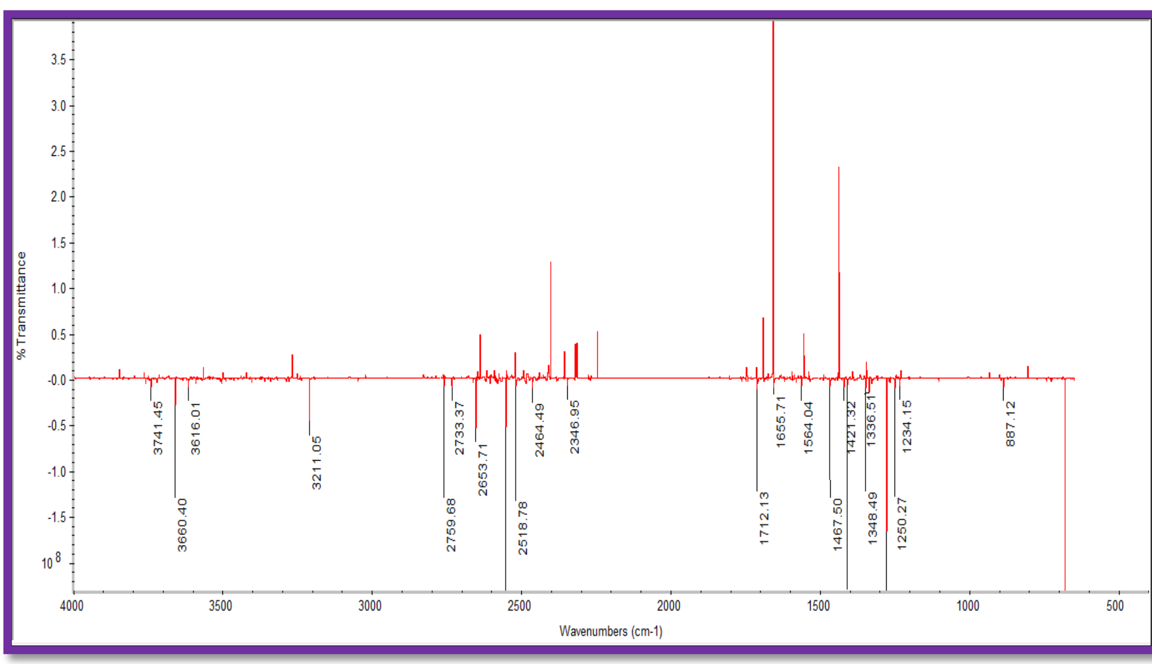


Figure A.5 Gaharu-Water Mixture at 100°C(FTIR)

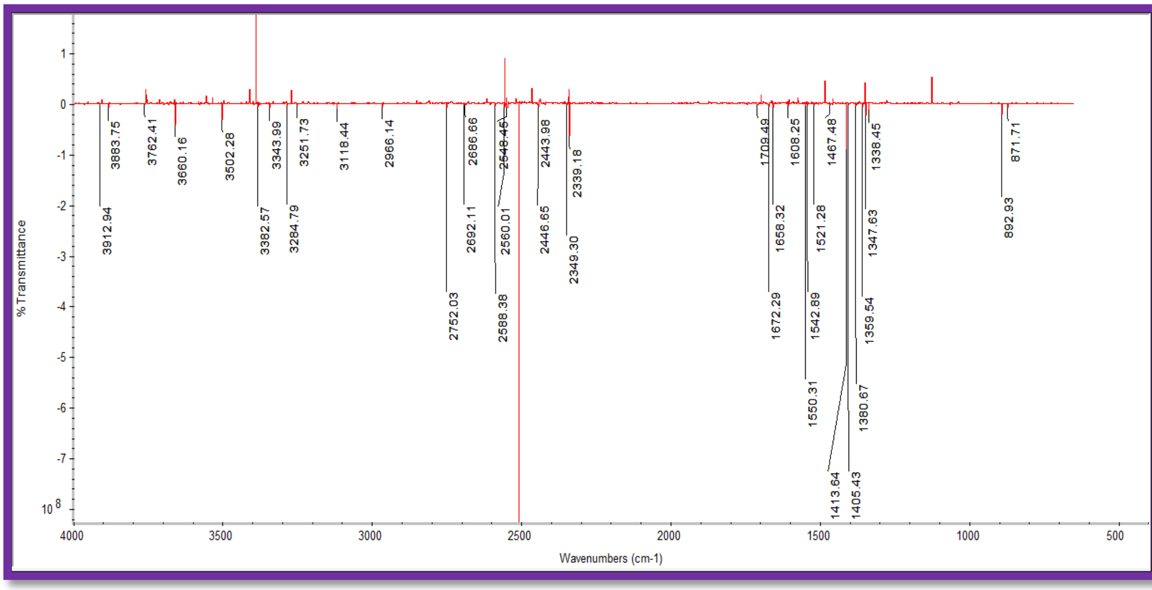


Figure A.6 Gaharu-Water Mixture at 30°C(FTIR)