MISCIBILITY STUDY OF GAHARU ESSENTIAL OIL IN WATER AND ORGANIC SOLVENTS

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Special Dedication to My…

Beloved father and mother :

Haji Abu Bakar Bin Haji Saad and ***Hajjah Siti Fatimah Bin Haji Hussin***

Loving brothers and sisters :

Abu Yazid, Aishah, Faizah, Salehah, Fathiyyah, Mohd Zaki

Supportive families;

Uncles, Aunties and Cousins

For their support, love and best wishes

ABSTRACT

This research is a study about miscibility of gaharu essential oil in water and organic solvents**.** The scope of this study is to compare polar and non-polar compound which miscible well in gaharu essential oil. Gaharu essential oil is very viscous, smelly, non-sticky and dark-brown oil. This essential oil has a highly market demand because of the value such as it used in incense, perfume and medicine. In this study, the miscibility of gaharu is studied by comparing the chemical components in gaharu essential oil in two different solvent, which is polar and non-polar. Miscibility of gaharu essential oil in water and organic solvents is actually a continuous research work to improve the extraction of gaharu hydro-distillation technique that using water as a solvent and to identify any recovery gaharu maker compound that dissolve in water for each stage of multistage extraction. To perform analysis, FTIR and GCMS are used to determine the chemical compound in gaharu essential oil. The main chemical component in gaharu essential oil is agarospirol, eudesma-4(14),7(11)-diene, α-guaiene, selina-4(15),7(11)-diene, and selinene. But, after gaharu essential oil mixed with water and let for one day, percentage of quality chemical components in gaharu essential oil is reduced. It shows that some of the chemical component tends to dissolve in the water. Furthermore, the smell of water itself just likes gaharu essential oil smell. It coincides with the theory that polar molecule will tend to dissolve anything polar molecule and non-polar molecule will tend to dissolve anything non-polar molecule.

ABSTRAK

Tesis ini membentangkan penyelidikan mengenai kajian tentang kelarutan minyak gaharu dalam air dan pelarut organik. Skop kajian ini ialah untuk membandingkan sebatian berkutub dan tidak berkutub yang boleh bercampur dalam minyak pati gaharu. Minyak pati gaharu sangat likat, berbau, dan berwarna coklat gelap. Minyak pati ini mempunyai permintaan pasaran yang tinggi disebabkan oleh nilainya seperti dalam penggunaan setanggi, minyak wangi dan ubat-ubatan. Dalam kajian ini, kelarutan minyak pati gaharu dapat dikaji dengan membandingkan komponen-komponen kimia yang terdapat dalam minyak gaharu dengan menggunakan dua pelarut yang berbeza, iaitu air dan *hexane*. Minyak pati gaharu dalam air dan pelarut-pelarut organik sebenarnya ialah penyelidikan yang berterusan yang bertujuan untuk meningkatkan kualiti pengekstrakan yang menggunakan teknik penyulingan yang menggunakan air sebagai pelarut.Selain itu, tujuan kajian ini juga ialah untuk mengenal pasti sebarang komponen kimia daripada minyak pati gaharu yang terlarut dalam air untuk setiap peringkat pengekstrakan. FTIR dan GCMS digunakan untuk menentukan komponen kimia dalam minyak pati gaharu. Komponen kimia utama dalam minyak pati gaharu ialah *agarospirol, Eudesma-4(14),7(11)-diene*, *α-Guaiene*, *Selina 4(15),7(11)-diena*, dan *selinene*. Tetapi, selepas minyak gaharu dicampur dengan air dan dibiarkan selama satu hari, peratusan kualiti komponen-komponen kimia dalam minyak pati gaharu berkurang. Ia menunjukkan yang beberapa komponen kimia yang terdapat dalam minyak pati gaharu cenderung untuk dilarutkan dalam air. Tambahan pula, bau air selepas proses pengekstrakan sama sepeti bau minyak gaharu. Ia bertepatan dengan teori yang molekul berkutub akan cenderung untuk melarutkan sesuatu molekul berkutub dan molekul tak berkutub akan cenderung untuk melarutkan sesuatu molekul tak berkutub.

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

Gaharu is the most precious wood in the world. It is even called “Wood of Gods” because of the value. Even in the lyrics, people said about gaharu such as in “The Song of Songs," Solomon says: “What wealth of grace is here…no lack of Spikenard or Saffron, of Calamus or incense tree, of Myrrh…of Aloes or any rarest perfume." “Aloes or any rarest perfume” in the lyrics is gaharu essential oil.

This aromatic resinous wood has different names in different countries such as gaharu in Malaysia, agarwood, eaglewood and aloeswood in europe, jinkoh quaoi in Japan, chen xiang in China, oudh in Arabic, and karas in Indonesia (obmar, 2006). There are many geniuses of gaharu in Malaysia. The details of genus and species gaharu in Malaysia are shown in table 1 below. Gaharu has graded of quality, which grade A, B, C and D but, in peninsular Malaysia, gaharu is the mostly grade C quality.

Table 1.1 are listed the distribution gaharu in Malaysia based on the species and genus.

Table 1.1 : Genus and species gaharu in Malaysia

Genus	Species	Local name	Distribution
Aetoxylon	*A. sympetalum*		Sarawak
Aquilaria	*A. malaccensis*	Karas, kekaras chandan, engkaras	Peninsular Malaysia, Sabah & Sarawak
	A. microcarpa	Engkaras	Sabah & Sarawak
	A. hirta	Chandan buluh	Peninsular Malaysia
	A. rostrata	-	Peninsular Malaysia
	A. beccariana	Gaharu, tanduk	Peninsular Malaysia, Sabah & Sarawak
Gonystylus	*G. bancanus*	Ramin, bidaru, lunak Melitan, melawis	Peninsular Malaysia, Sabah & Sarawak
	G. macrophyllus	Gaharu melintan	Peninsular Malaysia, Sabah & Sarawak
Enkleia	*E. malaccensis*	-	Peninsular Malaysia, Sabah & Sarawak
Wikstroemia	*W. polyantha*	Chandan pelanduk	Peninsular Malaysia, Sabah & Sarawak
	W. tenuiramis	-	Sabah & Sarawak

Source: Chang et.al (2002)

According to Biolande's website, the gaharu tree has the special characteristic of reacting to damage that caused by lightning, insects, fungi, cutting, etc., by forming localized concretions of an extremely fragrant, dense, colour wood. It bears white flowers that are sweetly scented. Gaharu essential oil is a pretty impressive oil to find. It begins to smell the way that it does because of an infection from a mould that has taken hold called *Phialophora Parasitica*. Before the mould infected the sap, it had a light green mild scent, but after it takes hold it transforms into a wonderfully deep, dark, luscious unguent scent.

Gaharu use and trade have played an important historical role in many cultures. The resin is used by Traditional Chinese as medicinally. They believe that gaharu is a good therapy for nervous disorders such as neurosis, obsessive behaviour, and exhaustion. Gaharu is highly psychoactive, and it is used in spiritual rituals. Many religious groups use gaharu as a meditation incense, to calm the mind and spirit. In Ayuravedic medicine, gaharu essential oil is used to treat a wide range of mental illness and to drive evil spirits away. In Japan, it is considered by many to be sacred, and is used to anoint the dead. In Buddhism, it serves as a major ingredient in many incense mixtures, and it is considered to be one of the three integral incenses, together with sandalwood and cloves (obmar, 2006).

1.2 PROBLEM'S STATEMENT

Current extraction of gaharu is using hydro-distillation technique. In hydro-distillation technique, water is used as solvent extraction. Mostly water is miscible with unknown chemical constituents from gaharu essential oil. So, percentage of quality chemical compounds in gaharu essential oil that using hydro-distillation technique is less compared than using solvent extraction method, which is use hexane as a solvent. That's why in this research study, researcher wants to compare the chemical compound in gaharu essential that mixed with water with chemical compound that mixed with hexane. Researcher also wants to identify what kind of gaharu chemical constituents can be miscible well in the water. Furthermore, researcher also will understand further the miscibility of gaharu essential oil in water, which is polar compound and in hexane, which is a non-polar compound.

1.3 RESEARCH OBJECTIVES

The main objectives of this research study are:

I. To compared chemical compound in gaharu essential that mixed with water with chemical compound that mixed with hexane.
II. To understand a mass transfer this involves molecule component in gaharu.
III. To understand the miscibility of gaharu essential oil in polar and non polar compound.
IV. To analyze the chemical compounds present in the essential oil using Gas Chromatography – Mass Spectrometer (GCMS).
V. To analyze chemical constituents from gaharu essential oil that miscible well in the water using FTIR.

1.4 SCOPE OF RESEARCH

The scope of this study is to compare polar and non-polar compound which miscible well in the same raw material which is gaharu essential oil. There are two solvent that researcher will use, which is water and organic solvents. For an organic solvent, researcher focuses on hexane. The GCMS was used to perform analysis to determine the chemical compound constituents in gaharu essential oil and FTIR was used to analyze chemical constituents from gaharu essential oil that miscible well in the water.

1.5 RATIONALE & SIGNIFICANCE

Miscibility of gaharu essential oil in water and organic solvents is a continue research work to improve the current extraction of gaharu hydro-distillation technique that using water as a solvent by studying the miscibility or chemistry of solution in gaharu extraction process. This research can help to control the gaharu extraction process in order to get optimize quality of gaharu essential oil. Furthermore, gaharu essential oil has a highly market value because of the usage such as in medicine, perfume and etc.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

In this chapter, background of gaharu, usage of gaharu, properties of gaharu essential oil, and extraction of gaharu essential oil is discussed. Besides that, the detail of gaharu essential oil, miscibility, and hexane is explained.

2.2 BACKGROUND OF GAHARU

Gaharu is a pathological phenomenon. Gaharu that also known as agarwood, aloeswood, eaglewood, oudh, jinkoh, chenxiang and karas is the most expensive wood in the world. Susanne Fischer–Rice, in her book, 'The Complete Incense Book', explains that agarwood has balsamic, ambergris, woody and deep fragrance. He also said that to experience of this unique substance is like a journey on the road to spiritual perfection.

Gaharu is the occasional product of two tofour genera in the family *Thymelaeaceae* which is species of A*quilaria* (PT. RahayuEkaManuggal, 2009). According to the rain forest project website, the tree grows in natural forests at an altitude of a few meters above sea level to about 1000 meters, and it grows best around 500 meters. Trees grow very fast, and start producing flowers and seeds as early as four years old. At least fifteen species of *Aquilaria* trees are known to produce the much sought-after agarwood. In South Asia, *Aquilariaachalloga* is found particularly in India, *Aquilaria Malaccensis* is mostly

known from Malaysia and Indonesia, and *Aquilaria crassna* principally grows in Indochina.

2.3 USAGE OF GAHARU

Gaharu products are produced in three main forms, namely woodchips, sawdust and oil. (Lim HinFui, 2007). Usually, gaharu grade that has the low grade, which grade C and D were used to process oil through hydro-distillation technique to get the oil. In this distillation process, the final product was the gaharu oil and the gaharu sawdust as a byproduct.

2.3.1 Incense

Gaharu has been used to make high-quality incense since antiquity. Chinese described its smell as a sweet, deep but balanced fragrance. As with Japanese incense, gaharu is the most important ingredients in Chinese's incense. Some other uses of this oil are in cooking. People have used it to add flavor to curries.

2.3.2 Perfume

Oil extracted from gaharu is used in Arabic countries as a perfume as well (PT. RahayuEkaManuggal, 2009). According to RK Sons & Company, the primary usage of gaharu is for incense, perfume and medicine. . In European perfume brands such as Fragonard Zizanie, Jacques Bogart One Man Show, La Prairie Silver Rain, Lacoste, Yves Saint Laurent M7 and Roberto Cavalli, gaharu essential oil is based in the perfume. Unlike many industrial perfumes, it is suitable for hot climates as the longer wear it, the better it smells (PT. RahayuEkaManuggal, 2009).

2.3.3 Medicine

Gaharu is also part of many traditional pharmacopoeias, dating back to medieval times and Chinese doctors still prescribe it for colds and digestion problem. (PT. RahayuEkaManuggal, 2009). Ancients have beliefs that gaharu aroma helps cleanse our body and mind and help us think clearly. Agarwood also has been used for traditional medicine, especially in Japan becaause of its effectiveness as a sedative in detoxifying the body and in maintaining stomach health (Akiko Ishihara, n.d).

2.4 GAHARU ESSENTIAL OIL

Gaharu essential oil is also known as Agarwood oil, Oud oil, Aloeswood oil, Eagleswood, Ud, Kiara, etc., is extremely rare and precious natural oil obtained froms pecies of Agarwood trees. The essential oil from wild agarwood trees is likewise, one of the most expensive oils in the world. Gaharu trees must be infected by *fungi*, prior to producing an oleoresin which saturates the wood. Infected trees secrete a fragrant, protective oil into wounded areas such as roots, branches or sections of the trunk which gradually become harder and darker (PT. RahayuEkaManuggal, 2009). Usually hydro-distillation method is usedin order to extract gaharu essential oils. The gaharu essential oil is a very viscous, dark-brown oil and non-sticky.

2.5 PROPERTIES OF GAHARU ESSENTIAL OIL

Essential oils are multi-component chemicals in one solution. The mixture of oil compounds that constitute the essential oil comprises polar and non-polar compounds (Bohra, 1994).

2.5.1 Chemical Properties of Gaharu Essential Oil

Essential oils is like all organic compounds, are made up of hydrocarbon molecules. It can be classified as alcohols, esters, aldehydes, and phenols etc (Nor Azah et. al, 2008). In peninsular of Malaysia, the gaharu were mostly of grade C quality. The main chemical component in Agarwood grade C quality such as:

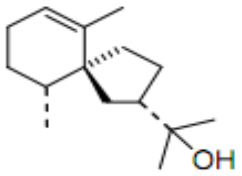

Figure 2.1 : Agarospirol

Source : Jutarut Pornpunyapat (2010)

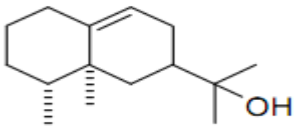

Figure 2.2 : Jinkoh eremol

Source : Nor Azah et al. (2008)

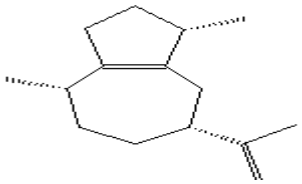

Figure 2.3 : α-Guaiene

Source : Nor Azah et al., (2008)

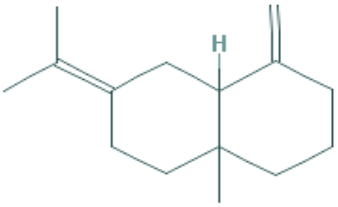

Figure 2.4 : Eudesma-4(14),7(11)-diene

Source : Nor Azah et al., (2008)

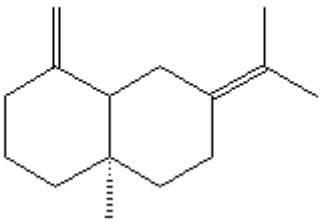

Figure 2.5 : Selina-4(15),7(11)-diene

Source : Nor Azah et al., (2008)

2.5.2 Physical Properties of Gaharu Essential Oil

The gaharu essential oil is very viscous, dark-brown oil and non-sticky. Gaharu essential oil is easily evaporated.

2.6 MISCIBILITY

According to science encyclopaedia, miscibility means how completely two or more liquids dissolve in each other. It is a qualitative rather than quantitative observation such as miscible, partially miscible, and not miscible. Two completely miscible liquids will form a homogeneous (uniform) solution in any amount. Miscibility depends on the forces of attraction between the molecules of the different liquids. The rule of thumb "like dissolves like" means that liquids with similar molecular structures, in particular, similar polarity, will likely dissolve in each other. Miscibility depends on the forces of attraction between the molecules of the different liquids. The rule of thumb "like dissolves like" means that liquids with similar molecular structures, in particular, similar polarity, will likely dissolve in each other. Polarity means the extent to which partial positive and negative charges appear on a molecule, because of the type and arrangement of its component atoms.

2.7 HEXANE

Hexane is a chemical made from crude oil. Pure hexane is a colourless liquid with a slightly disagreeable odour. It evaporates very easily into the air and dissolves only slightly in water. Hexane is a non-polar compound. Hexane is highly flammable, and its vapours can be explosive. The major use for solvents containing hexane is to extract vegetable oils from crops such as soybeans, cleaning agents in the printing, textile, furniture and shoemaking industries.

2.8 EXTRACTION OF GAHARU ESSENTIAL OIL

2.7.1 Hydro-Distillation

Hydro-distillation is the traditional method of extraction. It is fairly rare these days. Instead of having a steam pas s through the powdered wood in hydro distillater the powder is allowed to soak in water. A fire from below the vessel then heats the water & carries off the steam which is allowed to cool. The essential oil molecules and steam are carried along a pipe and channelled through a cooling tank, where they return to the liquid form and are collected in a vat. Essential oils which are lighter than water will float on the surface. Furthermore, since essential oils not water soluble they can be easily separated from the water (Ashwin Charles Benedict, 2009).

2.9 CONCLUSION

This chapter provides a general description of the gaharu essential oil, miscibility, and hexane. Besides that, details about background of gaharu, usage of gaharu, properties of gaharu essential oil, and extraction of gaharu essential oil is explained.

CHAPTER THREE

METHODOLOGY

3.1 INTRODUCTION

In this chapter the detail of techniques of multistage extraction and gaharu essential oil characterization is explained. Multistage extraction involving two different solvent which is water and hexane. Meanwhile, the characteristic of essential oil and recovered water is characterized using GCMS and FTIR.

3.2 PREPARATION OF SAMPLE GAHARU ESSENTIAL OIL IN HEXANE

Firstly, 20 ml of gaharu essential oil is filled up into a test tube. Then, 80 ml of hexane is measured and fill it inside the cone cylinder. Next, the essential oil is poured into the cone cylinder that containing hexane. The solution is shaking for 15 minutes using an orbital shaker. After 15 minutes, visually observe the solution if has any layer formation. Then, the solution is transferred into the separating funnel and let it for a day. After a day, visually observe the solution again if it has any layer formation. The solution is analyzed using GC-MS to analyze the chemical constituents in gaharu essential oil.

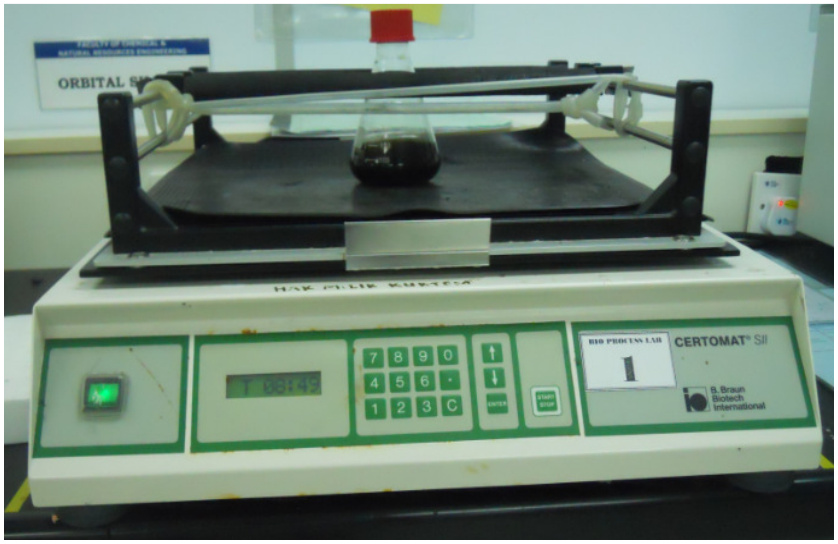

Figure 3.1: Gaharu essential oil is shaking using an orbital shaker.

3.3 PREPARATION OF SAMPLE GAHARU ESSENTIAL OIL IN WATER

Firstly, 20 ml of gaharu essential oil is filled up into a test tube. Second, 80 ml of hot water is measured and fill it inside the cone cylinder. Next, the gaharu essential oil is poured into the cone cylinder that containing hot water. The solution is shaken for 15 minutes using an orbital shaker. After 15 minutes, visually observe the layer formation of the solution. Then, the solution is transferred into the separating funnel and let it for a day. After a day, visually observe the thickness of layer formation. Then, the layer formation of water in separating funnel is transferred into a beaker as a sample first stage of multistage extraction. Gaharu essential oil that remained in the separating funnel is added with 80 ml of hot water. The solution is shaken for 15 minutes using the orbital shaker. The other step is same and repeated for four times in order to get the fives samples of water. Lastly, each sample of water is analyzed using FTIR to identify what kind of chemical constituents from gaharu essential oil was miscible in the water. Gaharu essential oil that remained is analyzed by GC-MS to analyze the chemical constituents in gaharu essential oil after mixed with water.

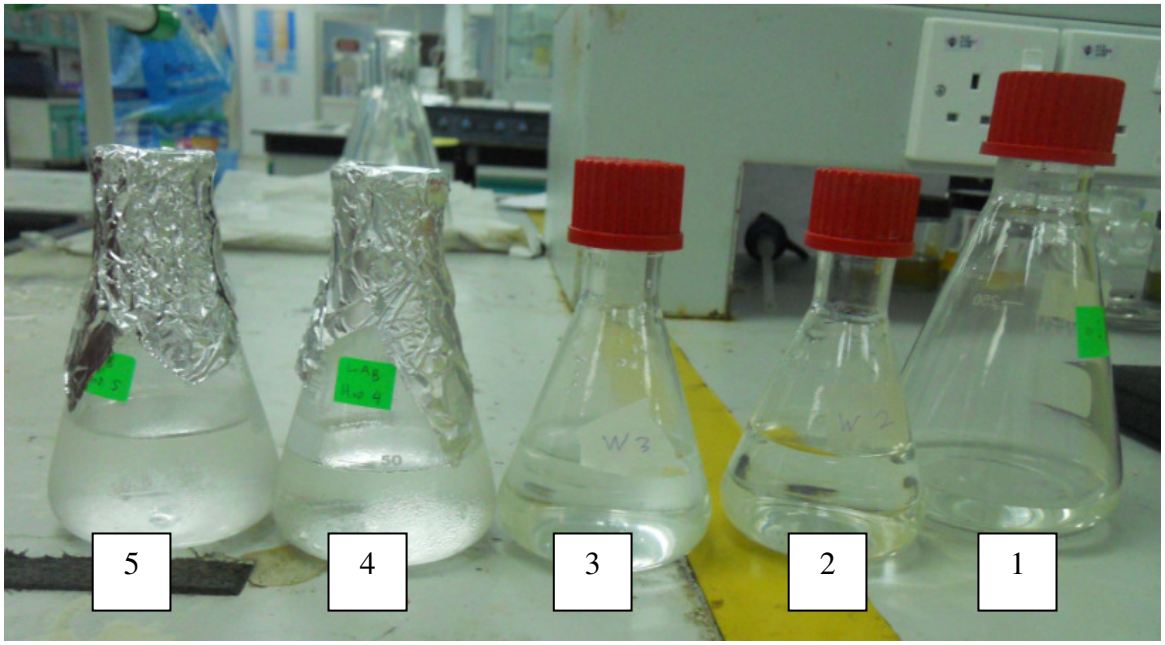

Figure 3.2: Fives samples of water after mixed in gaharu essential oil after 5 stages of multistage extraction.

3.4 METHOD OF ANALYSIS

3.4.1 GAS Chromatography-Mass Spectrometry (GC-MS)

GC-MS is one of the so-called hyphenated analytical techniques. The purposed of used GC-MS is to separate mixtures of chemicals into individual components. Once isolated, the components can be evaluated individually. In GCMS, inert gas or also called carrier gas such as helium is used as mobile phase. The mixture of compounds in the mobile phase interacts with the stationary phase at a different rate. Those that interact with the fastest will exit (elute from) the column first. Those that interact slowest will exit the column last. By changing characteristics of the mobile phase and the stationary phase, different mixtures of chemicals can be separated.

Each gaharu essential oil is injected in 1µl volume in the split mode. Before inject the sample, each sample is diluted in hexane. The capillary column used is DB-1ms 30m x 0.25 µm mm I.D; 0.25 µm film thickness. The oven temperature was programmed for 60

°c for 1 min. The capillary column is in the oven was programmed to increase the temperature gradually at 3°c/min to 250 °c and held for 10 min. Injector inlet and detector temperature were set at 250°c (Dr. Saiful Nizam Tajudin).

Figure 3.3: GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

3.4.2 Fourier Transform Infrared Spectroscopy (FTIR)

According to Handbook of analytical method for material, Fourier Transform Infrared Spectroscopy (FTIR) is describing as a powerful tool for identifying types of chemical bonds in a molecule by presenting an infrared absorption spectrum." This technique measures the absorption of infrared radiation by the sample material versus wavelength.

20	1H-Cycloprop [e] azulene, decahydro-1,1,7-trimethyl-,4-methylene [1aR- (1α,4aα,7α, ,7aα ,7aβ)]		✓	
21	1(10)-Aristolene	✓	✓	✓
22	Cyclolongifolene oxide, dehydro-		✓	
23	6-isopropenyl-4, 8a dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphtalene-2-ol		✓	
24	(+)-γ-Gurjunene	✓	✓	✓
25	Agarospirol	✓		
26	Tetradecanoic acid	✓	✓	✓
27	Pentadecanoic acid	✓	✓	
28	n-hexadecanoic acid	✓	✓	✓
29	Heptadecanoic acid	✓	✓	✓
30	Tridecanoic acid			✓
31	(-)-isoaromadendrene-(v)			✓
32	dinene			✓
33	Cycloheptane,4-methylene-1-methyl-2-(2-methyl-1-propen-1-yl)-1-viny-1			✓
34	hexadecanoic acid, methyl ester			✓
35	pentadecanoic acid, 14- methyl-, methyl ester			✓
36	Octadecanoic acid	✓	✓	✓
37	9,12-octadecanoic acid			✓
38	6-tetradecyne			✓
39	Eudesma-4(14),7(11)-diene		✓	✓
40	(R)-(-)-14-methyl-8-hexadecyn-l-ol		✓	
41	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-			✓