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Project title Qualitative analysis of essential oil using TLC stationary phase modification

Student names	Mr. Pongrapee Ieosuwan	ID	6033824023
	Mr. Ekkapob Imkool	ID	6033848023

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Qualitative Analysis of Essential Oils using TLC stationary phase modification

by
Mr. Pongrapee Ieosuwan
Mr. Ekkapob Imkool

In Partial Fulfillment for the Degree of Bachelor of Science
Program in Applied Chemistry (International Program)
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Examination committees

1. Associate Professor Somsak Pianwanit, Ph.D. — Chairman
2. Assistant Professor Prompong Pienpinijtham, PhD. — Committee
3. Assistant Professor Luxsana Dubas, PhD. — Advisor
4. Chadin Kulsing, PhD. — Co-advisor

Endorsed and approved by the Head of Department of Chemistry

.....

(Assistant Professor Luxsana Dubas, PhD.)
Advisor

.....

(Chadin Kulsing, PhD.)
Co-advisor

.....

(Associate Professor Voravee Hoven, PhD.)
Head of Department of Chemistry

Date 28 December 2020

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Student Name Mr. Pongrapee Ieosuwan Student ID 6033824023
Mr. Ekkapob Imkool Student ID 6033848023
Advisor Name Assistant Professor Luxsana Dubas, PhD.
Co-advisor Name Chadin Kulsing, PhD.
Department of Chemistry, Faculty of Science, Chulalongkorn University, Academic Year 2020

Abstract

Agarwood essential oil has a unique aroma and is well known in the Arab countries due to its value and price. Due to its high price, it has been developed into various grades. Basically, in order to investigate the adulterants and characteristics of authentic agarwood oil, gas chromatography or gas chromatography-mass spectrometry are used to classify its volatile compounds which is slightly complicated and time consuming. However, thin-layer chromatography (TLC) was modified in stationary phase with silver nitrate to increase the polarity of the stationary phase to successfully classify the unsaturated complex compounds of agarwood oils. This report aims to find the most optimum procedure under suitable conditions which are 0.5% $AgNO_3$ (w/v) with 80:20 ratio (methanol:milli-Q), then immersed the TLC plate in solution for 10 minutes and took in the oven at 110℃ for 15 minutes. To separate the same genus from different procedures. Gas chromatography-mass spectrometry was shown that silver nitrate gave a difference in the compound selectivity. Lastly, Color intensity was to identify as a potential application as can identify a fingerprint of further essential oils.

Keywords: Agarwood, Essential oils, Authentication, Thin Layer Chromatography, Stationary Phase Modification, Metal Ions Impregnated.

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Please note that if you have any corrections and/or additions in this report please feel free to inform us. Your feedback will help us identify the areas where the further improvement may be needed.

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Chapter 1
Introduction

1.1 Introduction to the research problem

Agarwood essential oil is one of the most popular essential oils worldwide especially in the Arab culture.[1] Due to their unique aroma fragrance, it is well-known to be used in hinduism culture and the perfume industry. Currently, the demand is very high which causes authentic essential oil price tags to be very expensive.[2] Since authentic essential oils were expensive and currently there is an outbreak of adulterants and fake synthetic essential oil in the market. Furthermore, Agarwood essential oils that come from different origins or it is an altered species will result in distinct properties which are hard to distinguish and verify.[3] Likewise, Agarwood essential oils have various ways to be produced by both local villagers and corporations which have different equipment and standard. Normally, Agarwood is sold as raw material in a form of solid wood. The lower quality woodchip, unqualified grades, and wood dust were refined into essential oils through the distillation process both by local producers and industrial corporations.[4,5] According to various sources and production methods without globalized standards, the agarwood quality is subjective depending on their sourcing countries and production lines.[6] On the other hand, local farmers usually use their axe, hammer, saw, and other equipment to chop, nail or hold into an agarwood tree which intends to create damage and start the process of healing to collect the resin which shows that the quality can be various. Nonetheless, all of these basic methods are more time consuming and receive insufficient yield.[7] In conclusion, although the essential oil came from the same origin and it is the same species but, its properties might be modified in the production process which affects the way each species or origins to be characterized from others.[8]

To establish essential oil effective inspection methods one of most common techniques used to analyze the volatile organic compounds and properties are gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS).[9] However, GC technique still required a lot of steps to perform and needed time to operate.[10] On the other hand, thin layer chromatography (TLC) technique which is convenient and easier to perform. It is an alternative to use as a separation technique.[11,12] Mostly, the sorbents which used in thin layer chromatography technique include particle size of 12-μm silica gel, impregnated silica gel, chemical-bonded silica gel, alumina, mineral oxides, polyamide, polymeric ion exchange, and chiral phase.[13] However, using a normal silica gel in the thin layer chromatography technique still contains some of the limitations of the separation due to the polarity of silica gel. On the surface of hydrate silica gel could consist of three kinds of silanol groups (free silanol, geminal, and associated silanol). The non hydrogen-bonded free silanol contains highly acidic nature and can be destructive binding of basic solutes or can be said to be silica gel that contains a higher concentrate free and highly acidic silanols generally shows increase in retention and broadening peak tailing for basic compounds.[13] To improve the selectivity of silica gel using thin layer chromatography technique, the silica gel were impregnated with metals ions to change the polarity and tune the selectivity toward components in the agarwood oil. In this experiment, there are three metals that were examined including silver nitrate, nickel sulfate, and copper sulfate to determine their abilities to tune the separation of sesquiterpenes which is the main component of agarwood oil.

1.2 Research objectives

This research aims to develop and perform qualitative analysis by modification stationary phase of thin layer chromatography (TLC) silica gel plate impregnated with silver nitrate.

1.3 Literature review

1.3.1 Agarwood Genus

There are two well-known families of plants which are Aquilaria and Gyrinops which belong to the subfamily Aquilarioideae.[14] Both of them have similar properties but the number of stamens are different, while Gyrinops has five stamens and Aquilaria has about eight to twelve stamens.[15] The Aquilaria genus consist of four main species including Sinensis, Malaccensis, Crassna, and Subintegra.[16] Four of its stamens were known to be used for agarwood production.[17] In addition, Malaccensis and Subintega were mostly found in Thailand.[16]

1.3.2 Volatile compounds identified in Agarwood essential oils and extracts

Agarwood essential oils and extracts are very complex compounds. Major components in Agarwood essential oils are:

(1) Sesquiterpenes

Sesquiterpenes are a class of terpene that consists of three isoprene units. There are various types of sesquiterpenes which can be divided depending on its molecular skeletons such as agarofuran, agarospirane and candinane (**Figure 1.1- Figure 1.3**).[18]

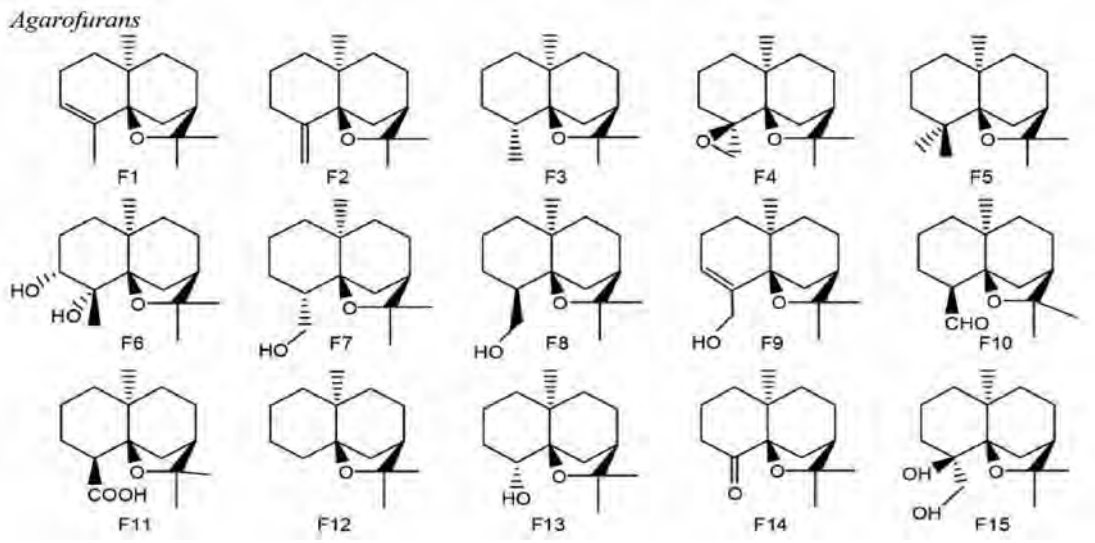

Figure 1.1: The structures of Agarofuran[18]

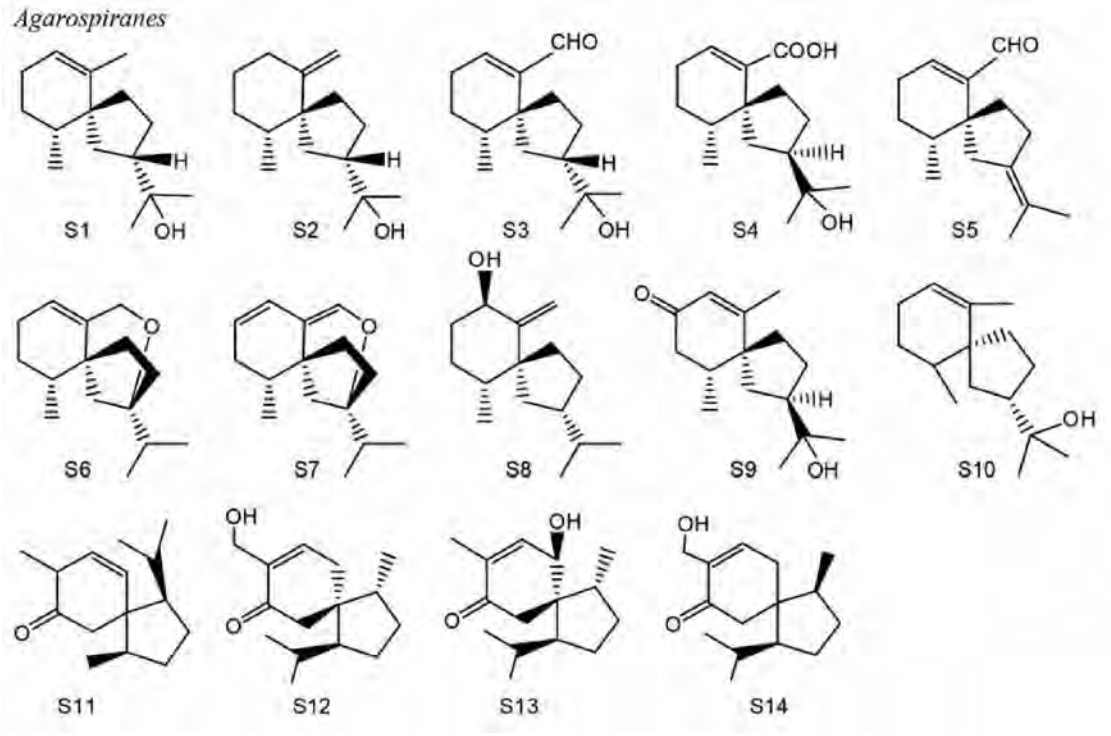

Figure 1.2: The structures of Agarospirane[18]

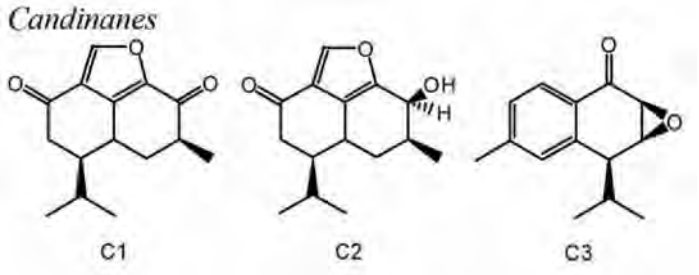

Figure 1.3: The structures of Candinane[18]

(2) Chromones

Chromones is a derivative of benzopyran with a substituted keto group on the pyran ring which is only found in a few plant species including in agarwoods. The main characteristic component of agarwood is 2-(2-phenylethyl)chromone derivative. In addition, chromones can be divided by its molecular skeleton into[18]:

(1) 2-(2-phenylethyl)chromones (**Figure 1.4**)
(2) 5,6,7,8- tetrahydro-2-(2-phenylethyl)chromones
(3) Diepoxy-tetrahydro-2- (2-phenylethyl)chromones
(4) Associated chromones

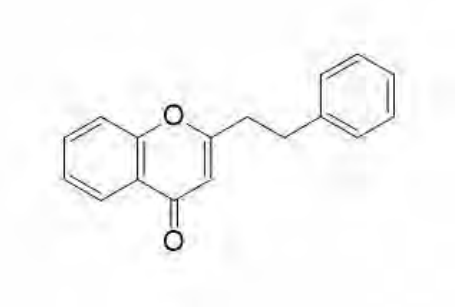

Figure 1.4: The structure of 2-(2-phenylethyl)chromone

1.3.3 Agarwood oil formation

1.3.3.1 Natural method

Oleoresin components in agarwood wood resins are very hard to produce and consume a lot of time. In nature, the wood does not contain oleoresin. The production process of oleoresin would start from harming or wounding a part of the aquilaria tree then letting the tree heal itself. However, this method cannot support the market demand.[19]

1.3.3.2 Fungi injection method

The fungi injection method (FI) was started by drilling some holes around the Agarwood tree then slowly injecting fungi solution (FI) into the hole using a wash bottle. However, this method result was unsatisfactory since the performance is lower than the AIMA method and the resin yield is barely recovered.[4]

1.3.4 Agarwood oil extraction

1.3.4.1 Hydro-distillation method

One of the traditional methods used to extract essential oils is hydro-distillation. Agarwood was grinded into powder and stored at room temperature, and immersed in distilled water followed by assembling the all-glass Clevenger-type distillation apparatus and performing hydrodistillation between 30-35℃ at flow rate of 3-10 mL per minutes. Then the volatile essential oils were retrieved before weighted and stored inside the refrigerator. However, this method required complex scientific equipment to perform and have negative effect such as heat-induced degradation.[5,20]

1.3.4.2 Steam-distillation method

An alternative method is by using steam from a boiler to distill the essential oils. In this method, Agarwood chips were placed in a boiler unit on the overhead inlet. Then, the steam passed through the chips and carried out the essential oil. Nonetheless, steam distillation still has some downside such as vapor pressure issues and when flow rates are high the degradation could occur.[21]

1.3.4.3 Vacuum distillation

While the pressure decreases the boiling point also decreases. Some material with high boiling point properties might cause a problem while researchers intend

to perform an extraction. It would be more effective to reduce the atmospheric pressure rather than increase the temperature to prevent deterioration.[20]

Figure 1.5: Collecting the extracted agarwood oil (Lao agar, 2017)

1.3.5 Grading of Agarwood

1.3.5.1 Appearance

Agarwood can be classified into three main categories depending on the raw material color. Three main categories consist of "Grade A", "Grade B", and "Black Magic Wood" (BMW). The darker Agarwood is regarded to contain more oleoresin and bespeak from older trees and vise versa.[7] The BMW is impregnated wood immersed with petroleum-based oil which generates the darker color on the wood [1] Nowadays, BMW has become an alternative choice for ordinary buyers in the society. However, agarwood could be difficult to assess with only its physical appearance.[6]

Figure 1.6: Different type of agarwood pieces (a) Grade A; (b) Grade B; and (c) Black Magic Wood (BMW) [6]

1.3.5.2 Parts of wood

Each segment of wood contains different properties and can be used to distinguish the agarwood grade and value. The piece that contains a higher amount of resin such as the root will be considered more valuable than the part that has less resin.[8] There is a technique called a sinking test by which wood pieces will be dropped into the water and observed whether the wood sinks or not. If the pieces sink, it can be predicted that the wood contains high resin and assume that it is the finest product. Nonetheless, if the wood were to float, it will be considered as the underlying product and the value will be lower, although this method is easier to exploit using heavier metal inside the wood.[6]

1.3.5.3 Aroma Scent

Their unique aroma is another property which is favored to determine the value and quality of the Agarwood. The piece of Agarwood can be burned and the aroma fume could be inhaled. However, this method is not consistent because different people have their own perception towards the scent.[6] The elite grade of Agarwood oils hold distinct odor and some essential oils can be kept for some time before delivering into the market to increase the intensity of their aroma scent.[1]

1.3.5.4 Sourcing Countries

Each country may have its own grading of agarwood due to there being no international system grading in agarwood. In Southeast Asia, there are two countries that have their own grading system: (1) Malaysia and (2) Indonesia

(1) Malaysia

Malaysia market had grading agarwood depending on their physical appearance based on the ABC system as **Table 1**. In addition, The Malaysian Timber Industry Board (MTIB) is offering a classification system by classifying agarwood products in relation to their end usage as **Table 2**.[6]

Table 1: Agarwood grades in Malaysia[6]

Grade	Resin coverage on the surface	Resin color	Wood shape
Super king	Entire	Total black and shiny	Solid wood chunks (500 g to 3 kg)
Triple super	Entire	Total black and shiny	Solid wood chunks (200–500 g)
Double super	90 %	Less black and shiny	Solid wood chunks (50–200 g)
Super	80 %	Resin is black and grayish	Solid wood chunks of mixed sizes
A (A1-A10)	Entire	Black turning into gray	Solid wood chunks of mixed sizes
AB	Entire	Black turning into brown	Solid wood chunks of mixed sizes
B (B1-B10)	Entire	Black turning into brown	Solid wood chunks of mixed sizes
C	50 %	Gray	Varies in shapes and sizes
D	Entire	Gray and whitish	Varies in shapes and sizes

Table 2: Agarwood classification based on end products in Malaysia[6]

Category	End use	Grade
Aroma	Wood chips and blocks containing fragrant resin for direct burning	Super, A, and B
Block	Wood blocks of various shapes and sizes, containing moderate to high density of fragrant resin, for use in making end products such as sculptures, beads, and bracelets	Tiger stripes and color of the sculpture
Classic	Wood blocks with fragrant resin of unique natural shapes for sale as an aesthetic product	Classic
Dust	Dust and debris, by-product of washing and oil extraction, but has remaining fragrant resin	Black, gray, yellow, dust, incense powder, debris
Extractable wood	Wood blocks and pieces of various sizes with low fragrant resin Suitable for oil distillation	C
Fragrance	Resin covers uniformly on one side of the wood pieces. Low to moderate fragrance	A1, A, and B

(2) Indonesia

The National Indonesian Standard (SNI) proposed a grading system based on physical appearances such as color, size, contamination of wood, density, and burned aroma of agarwood (**Table 3**).[6]

Table 3: Agarwood grades in Indonesia[6]

Type	Grade	Color	Weight	Aroma (burned)
Gubal	Double super	Equally black and shiny	Sink	Soft aroma (*wangi halus*)
	Super A	Unequal black and shiny	Sink	Soft aroma (*wangi lembut*)
	Super B	Black and not shiny	*Melayang* (float)	Aromatic
	Super middle A (under water)	Black	*Melayang* (float)	Aromatic
	Super middle A (up water)	Black	Float	Aromatic
Kemedangan	*Sabah*	Brownish black	*Melayang* (float)	Aromatic
	Kemedang A	Brown with black line	*Melayang* (float)	Aromatic
	Tanggung C	Brown with white narrow line	Float	Aromatic
	Kemedangan hijau	Brown with green line	*Melayang* (float)	Aromatic
	Kemedangan putih	Gray with black narrow line	Float	Pungent aroma (*wangi pedas*)
Agarwood powder	*Gubal* powder	Black	–	Aromatic
	Kemedangan powder	Whitish brown	–	Quite aromatic

1.3.6 Silver-ion

Silver ion chromatography was used in two techniques which are thin-layer chromatography (TLC) and column chromatography. Silver ion chromatography is based on the ability of Ag^+ to form the weak and reversible charge-transfer complex with unsaturated organic compounds.[22] in this instance terpene. Impregnated silver nitrate on silica gel was reported by Lindsay Morris. Moreover, double bonds are one of the common functional groups in organic compounds and silver ions are highly selective in double bond retention.[23] Therefore, silver-ion is widely used for stationary phase modification.

Chapter 2
Experimental

2.1 List of equipment and instrument

Equipment and instrument used: 0.5-10µl micropipette (Eppendoft, Germany); Agilent 7890A Gas Chromatograph (Agilent Technologies, USA); Agilent 7693 Automatic Liquid Sampler (Agilent Technologies, USA); Agilent 7000 Series Triple Quad GC/MS (Agilent Technologies, USA); Memmert UM 400 Drying Oven (Memmert GmbH + Co. KG., Germany); Milli-Q® Reference Water Purification System; Clifton Ceramic Hotplate Stirrer by Nickel-Electro Ltd, Magnetic Stirrers C-MAG HS 7 by IKA; Stainless Steel Box FAI No.333 (Size 14x20x2 cm); SevenCompact Advanced pH Meter. (Mettler Toledo GmbH., Germany); Combination Ion-Selective Electrode, Silver/Sulfide by Cole-Parmer.

2.2 List of chemicals and materials

Chemicals and material used:

(1) Silver nitrate, Analytical reagent by Thomas Baker Chemicals.
(2) Nickel(II) sulfate hexahydrate for analysis by Merck, Germany.
(3) Copper(II) sulfate pentahydrate for analysis by Merck, Germany.
(4) TLC Silica gel 60 F254 aluminium sheet by Merck, Germany.
(5) Methanol for analysis by Merck, Germany.
(6) Dichloromethane for analysis reagent by Carlo Erba Reagents, France.
(7) Vanillin for analysis by Merck, Germany.

2.3 Experimental

2.3.1 Sample preparation

Agarwood essential oils were obtained from Almas Oudh Al manufacturer which were extracted from Aquilaria in Trat, Thailand which is processed by a hydro-distillation method. The agarwood essential oils were sampled into four which are:

Table 4: Agarwood oils sample

	Code	Manufactured Date	Fermentation	Baked oil
Sample A	SD	25 May 2018	38 Days	40-50 ℃ for 10 months
Sample B	S61	29 Nov 2018	10-18 Days	-
Sample C	SW	Aug 2016	19 Days	-
Sample D	S63	9 Aug 2019	13 Days	-

Agarwood essential oils were prepared separately into 1.5mL sterile glass vials with 75µl Agarwood oils and 1,425µl Acetonitrile.

2.3.2 Preparation of metal-ion solution and TLC modification

Silver nitrate ($AgNO_3$), copper sulfate($CuSO_4$), and nickel sulfate($NiSO_4$) were prepared in 50-mL beakers by weighing 1.0000 grams (± 0.01 grams). Next, Each metal solid was dissolved in 20mL of milli-Q water. The solution was transferred into a 100mL volumetric flask and filled with methanol until the solution in each volumetric flask reached 100 mL. and 80mL of methanol was added to make the final volume to 100 mL. 100mL-metal-ion solutions were transferred into Stainless steel boxes No.333 separately. Three TLC plates in each condition were used. 5x10 cm^2 TLC silica gel plates prepared, were immersed in a stainless steel box with metal-ion solution for 10 minutes. Then the impregnated-TLC plates were dried out in the oven at 110℃ for 15 minutes and developed the plates to observe the difference between three metals and a bare silica gel plate regarding separation ability.

2.3.3 Study of impregnated-$AgNO_3$ conditions

2.3.3.1 Ratio of the solvent (methanol and milli-Q)

To test the suitable ratio of the solvent (methanol:mili-Q), the 1% $AgNO_3$ (w/v) in different solvent compositions were prepared in different solvent compositions. Firstly, four silver nitrate solutions weighing from 1.0010 to 1.0023 grams were prepared in 50-mL beakers. The solutions were prepared as described in section 2.3.2 using different solvent composition, 50:50, 60:40, 70:30, and 80:20 (MeOH:milli-Q) respectively. A retention factor was considered due to the difference of solvent ratio.

2.3.3.2 $AgNO_3$ concentration

$AgNO_3$ concentration can play a major role in the tuning of the selectivity. Therefore five different concentrations, 0.3%, 0.5%, 0.7%, 1.0%, and 2.0% $AgNO_3$ (w/v), of silver nitrate were prepared using the suitable solvent composition found in section 2.3.3.1. These are used to compare due to a different concentration, a retention factor and ability of separation were considered.

2.3.3.3 Impregnated time

Silver nitrate solutions were prepared in a 500-mL beaker by weighing 1.5081 grams before adding 60 mL of milli-Q water to dissolve silver nitrate and gently stirred until all solids were dissolved. Afterwards, 240 mL of methanol was added to the solution, giving a total volume of 300 mL 1.5% $AgNO_3$ (w/v). The $AgNO_3$ solution was transferred into three stainless steel boxes No.333 ,100 mL per box. Then three of 5x10 cm TLC silica plates were immersed in each stainless steel box with $AgNO_3$ solution and labeled as “A”, “B”, and “C”. Each box was left immersed for 5, 10, and 15 minutes, respectively. A retention factor and ability of separation were considered.

2.3.3.4 Oven-Drying time

The silver nitrate solution of 2.0010 g was prepared in a 500-mL beaker. It was then diluted with 80 mL of milli-Q water and added 320 mL of methanol into the solution with a final volume in 400 mL 2.0% $AgNO_3$ (w/v). Three of No.333 stainless steel boxes were prepared and filled with 100 mL of 2.0%. $AgNO_3$ (w/v) solution. Three of 5x10 cm^2 TLC silica gel plates were prepared and immersed in each stainless steel box with $AgNO_3$

solution label as “D”, “E”, and “F”. After 15 minutes all of nine TLC silica gel plates were taken out from the solution. Then the impregnated-TLC plates batch “D”, “E”, and “F” were dried in the oven at 110℃ for 10, 15, and 20 minutes respectively. These are used to compare due to a different time, outcome from heated and ability of separation were considered.

2.3.4 Impregnated-TLC sample application and development

Both silica TLC plates and impregnated-TLC plates were spotted with SD, S61, SW, and S63 , by using 0.5µl at 0.5 cm above the bottom edge of the plates. The plates were then waited for 10 minutes to get the sample dried. For developing the TLC plates, 6.4 cm diameter cylindrical glass were used. Dichloromethane was used as a mobile phase in a volume of 10mL as shown in **Figure 2.1**. It was developed until the solvent ran up at 0.5 cm below the upper edge of the plates. Vanillin was then used to stain the plates. After it was waited for vanillin to dry out, the plates were heated on a hotplate at 95℃ or higher until the spots occurred and became visible with great color intensity.

Figure 2.1: Impregnated-TLC development

2.3.5 Retention factor calculation

TLC plates that were successfully developed and showed acceptable resolution were transferred to the photographing area. Then, the images of TLC plates were taken. After the TLC plate images were exported into the computer, the ImageJ program was used to calibrate to the distance of the plates and calculated into centimeters, then calculated the distance as retention factors.

2.3.6 Color intensity

The MATLAB program was obtained from Ms. Phawida Yodthongchai (who studies in classifying the type of agarwood oils) used to determine the intensity of colors spotted from the succeeded TLC plates with the suitable $AgNO_3$ impregnated approach.

2.3.7 Gas chromatography-mass spectrometry (GC-MS) conditions

GC-MS was used to determine the difference of compound ranges that were developed by a solvent between a silica TLC and a $AgNO_3$Impregnated-TLC. The S63 sample was used. Plates were prepared as **Figure 2.2**, and 50µl of the S63 sample was spotted on the 3cm-band and developed. Each plate was then transferred into 20 mL vials respectively, which started from fraction 1 to fraction 5 and sealed with the vial caps. Furthermore, the

essential oil was extracted using headspace-solid phase microextraction (HS-SPME) fiber for 15 min extraction time and desorbed into GC-MS injection port for 5 min desorption time. The operating conditions of GC-MS considered were HP-5 MS capillary column (30m x 0.25mm), Inlet mode (splitless mode), Carrier gas (helium), Flow rate (1mL / min), Injector temperature (220℃), Temperature range (40-240℃), Ionization: EI (70 eV) and Mass range (30-300 Da).

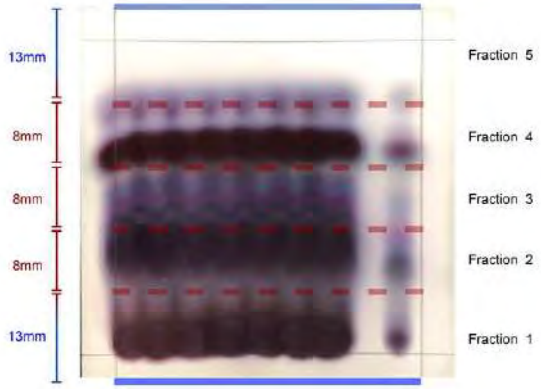

Figure 2.2: TLC plate composition

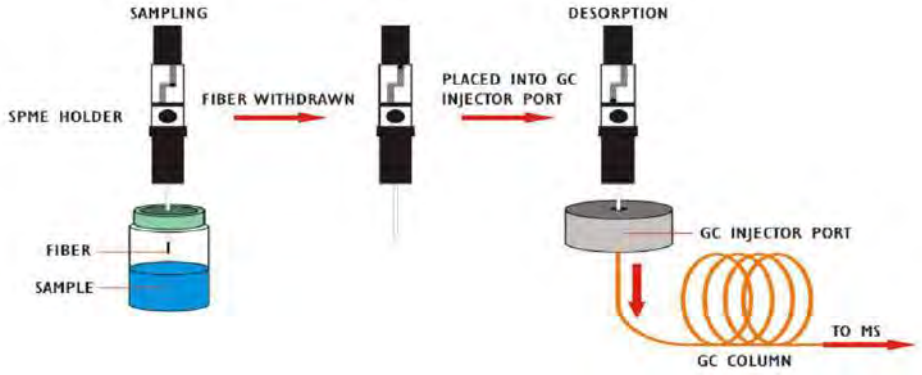

Figure 2.3: Headspace-solid phase microextraction diagram

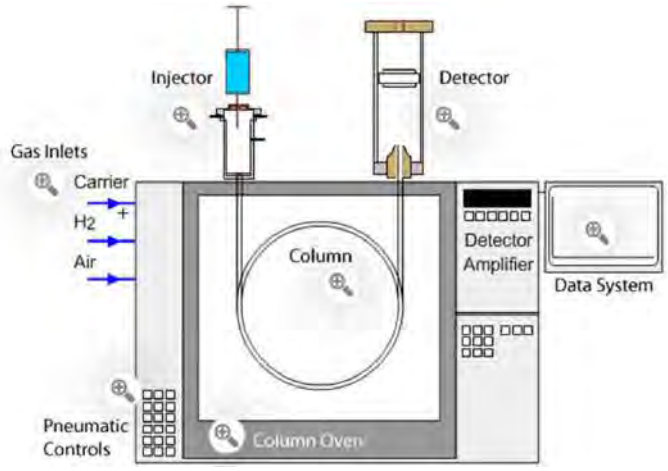

Figure 2.4: Gas chromatography-mass spectrometry diagram

Chapter 3
Results and discussion

3.1 Study of the various metal-ion types

To identify the suitable metal ions that can deliver different selectivity on the separation, three different solutions of $AgNO_3$, $CuSO_4$, and $NiSO_4$ were adopted in this study. **Figure 3.1a-3.1d** demonstrates the spot with the same area, difference in metal-ion impregnated ($AgNO_3$, $CuSO_4$ and $NiSO_4$) and a bare silica TLC plate in the same amount of concentration. In **Figures 3.1c** and **3.1d**, TLC impregnated with $CuSO_4$ and $NiSO_4$ shows a separation in a most polarized area (in the box) that the solvent can carry out, the number of spots is similar as a bare silica TLC. It was obvious that $CuSO_4$ and $NiSO_4$ did not show a different separation of the polarity component. Moreover, TLC impregnated with $AgNO_3$ significantly resulted in separation of polarity components as **Figure 3.1b**, the number of spots had expanded in separation which means that $AgNO_3$ can provide better separation of polarity components than the others.

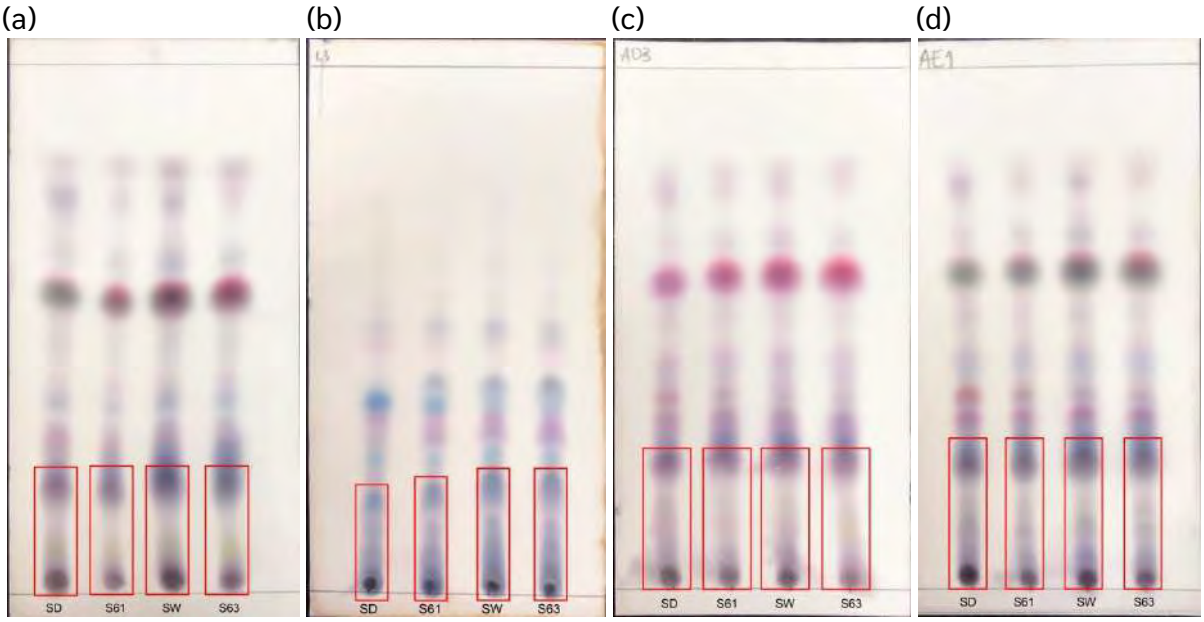

Figure 3.1: Impregnated TLC (a) Bare silica TLC; (b) 1% $AgNO_3$ solution; (c) 1% $CuSO_4$ solution; and (d) 1% $NiSO_4$ solution

3.2 Effect of solvent ratio (methanol to milli-Q)

As shown in **Figure 3.1b**, impregnated TLC with silver nitrate had shown a good separation for polarity components. The impregnation methods were various experimental conditions were investigated with $AgNO_3$ impregnated approach. By varying the solvent ratios between methanol and milli-Q (50:50, 60:40, 70:30, and 80:20) with the same amount of 1% $AgNO_3$ solution(w/v), **Figures 3.2a-3.2d** indicate that retention factors of the spots with the same area are similar in a range of 0.41 - 0.47 (sd=0.01). However, the results are similarly the same. By the way, the less amount of water was more efficient in order to avoid precipitation, so 80:20 of methanol to milli-Q was used.

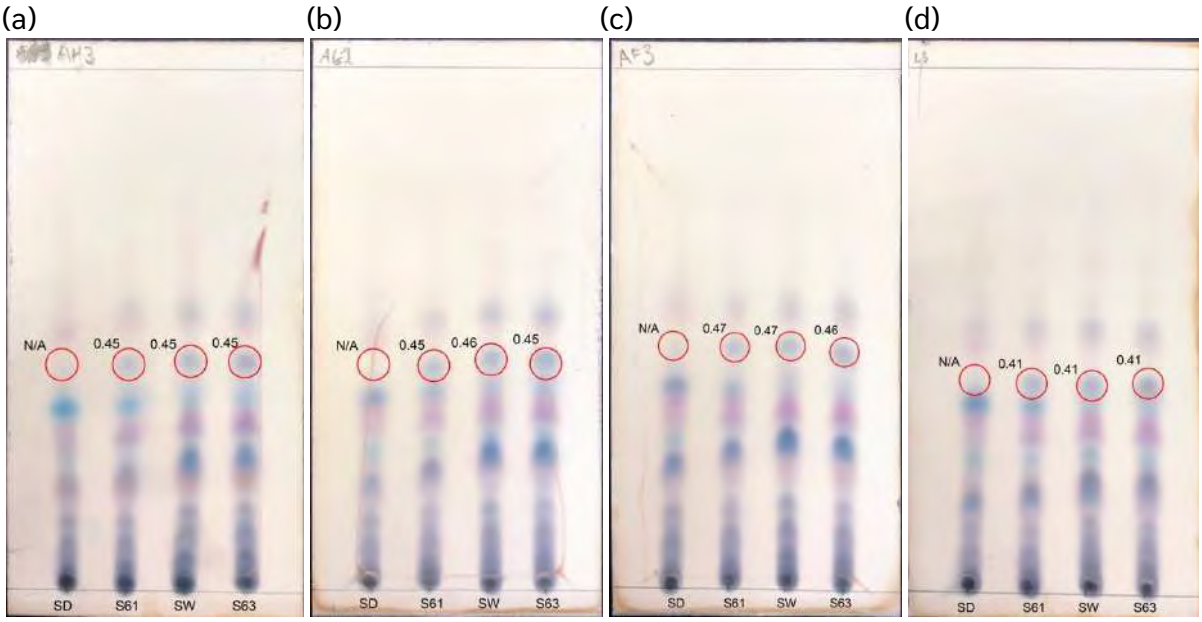

Figure 3.2: Solvent ratio (methanol:milli-Q) (a) 50:50; (b) 60:40; (c) 70:30; and (d) 80:20

3.3 Effect of $AgNO_3$ concentrations

Different concentration of $AgNO_3$ solution (0.3%, 0.5%, 0.7%, 1%, and 2%), were used to discover the effect of impregnated-TLC with $AgNO_3$. 2% (w/v) indicates that the marked spots, the retention factors of 0.39-0.40 (sd=0.01) were not well separated and the separation is too brief compared to the other concentrations (**Figure 3.3e**). 1% (w/v) has a good separation but the marked spots with the retention factors of 0.49-0.50 (sd=0.01) were not clearly separated (**Figure 3.3d**). Moreover, the retention factors of 0.50-0.51 (sd=0.01) in 0.5% (w/v)(**Figure 3.3b**) showed two separated spots except for the sample SD because SD sample was baked oil but S61, SW, and S63 were not with the same process and just difference in manufactured date, compared to 1% and 2% concentration at the same marked spots. 0.3% (w/v) showed the best separation of polarity components in general but according to the characteristics of baked and unbaked samples the retention factors of 0.59-0.61 must be the focus (**Figure 3.3a**). 0.7% (w/v)(**Figure 3.3c**) also showed the similar separation result as 0.5% (w/v)**.** However, 0.5% (w/v) is continuously the minimum $AgNO_3$ concentration that was used to impregnate TLC.

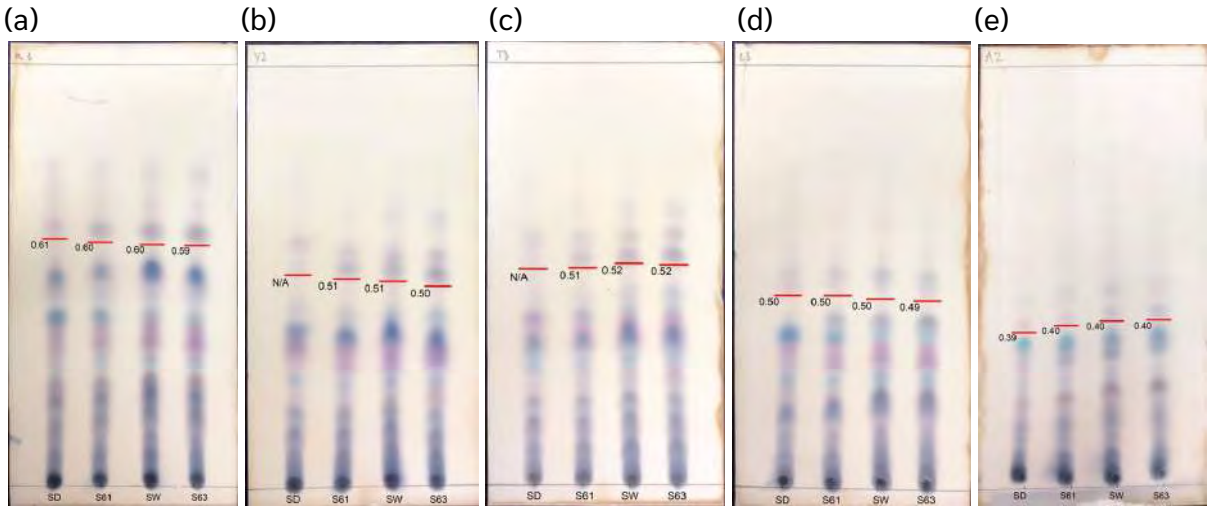

Figure 3.3: Difference concentration of $AgNO_3$ (a) 0.3%; (b) 0.5%; (c) 0.7%; (d) 1%; and (e) 2%

3.4 Impregnated time

The results of varying impregnated time are shown in **Figures 3.4a-3.4b** where 5 minutes showed unclear separation of target spots; the target spots which are circles known between unbaked and baked agarwood essential oils. **Figures 3.4b and 3.4c** showed the same separation, compared to the retention factors in a range of 0.50 to 0.51 (sd=0.01). It is similarly the same position. However, 10 minutes is selected for development.

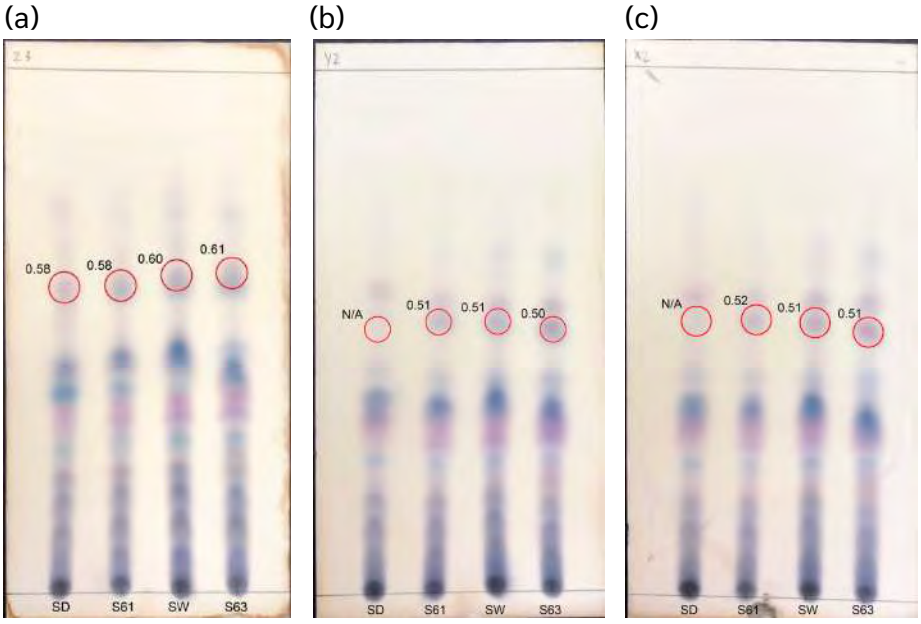

Figure 3.4: Impregnated time of TLC (a) 5 minutes; (b) 10 minutes; and (c) 15 minutes

3.5 Drying plates time

It is obvious that the longer drying time gives a clear plate at the lower edge which samples spotted were located as demonstrated in **Figure 3.5c** compared to **Figure 3.5b and 3.5a**. As a result, time does not affect the separation.

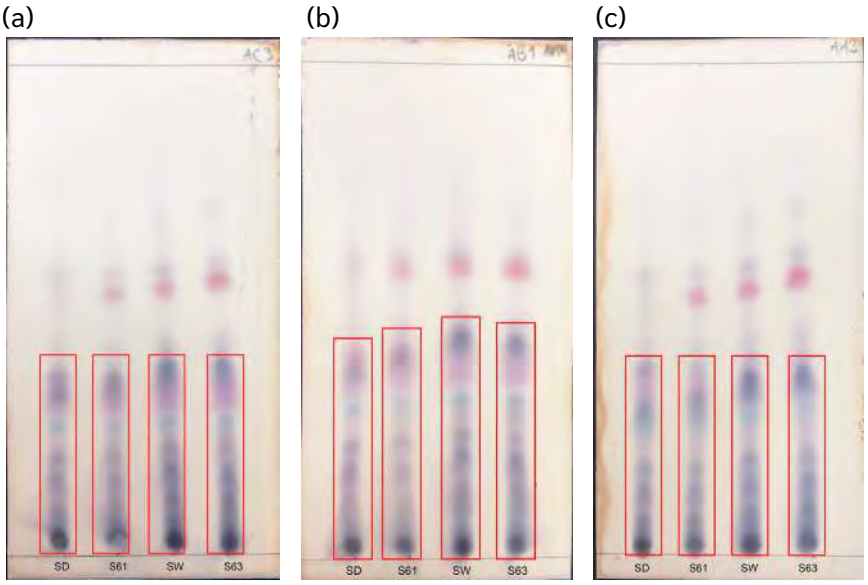

Figure 3.5: Drying time of TLC in oven at 110℃ (a) 5 minutes; (b) 10 minutes; and (c) 15 minutes

3.6 Suitable condition

According consideration in various condition, the result of suitable condition for this experiment were using a 80:20 (methanol:milli-Q) ratio of 0.5% $AgNO_3$ (w/v), then immersed the plates in solution for 10 minutes and dry in the oven at 110℃ for 15 minutes. From the result, **Figures 3.6a** and **3.6b** show significantly difference in separation as a TLC impregnated with silver nitrate give a high number of spots than a bare silica TLC plate which indicate that silver nitrate is a good stationary phase due to tune the selectivity toward components and the high polarity which can be pulling down the components that might be unsaturated or complex compounds to the lower plate.

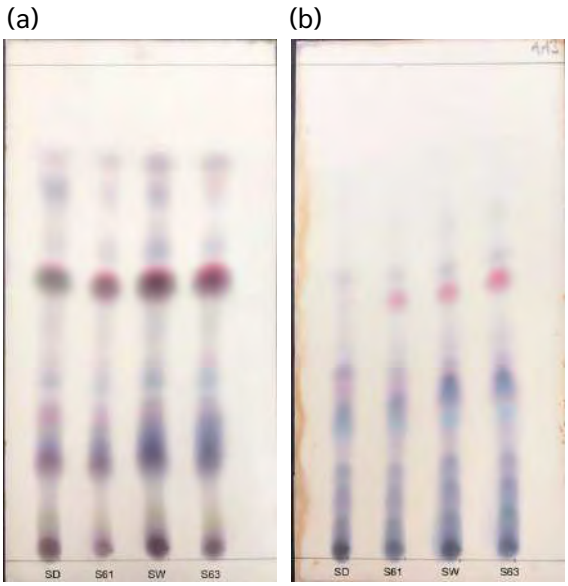

Figure 3.6: Developed TLC plates in different stationary phase (a) a bare silica TLC; and (b) a TLC impregnated with silver nitrate

3.7 GC-MS analysis

The S63 samples was chosen as one of the samples to study compound selectivity between developed samples with a bare silica and an impregnated TLC after separation then cut into 5 fractions as prepared and SPME analyzed by GC-MS showing the volatile compounds in **Table 5-Table 9**. In these tables, the white rows demonstrate sample S63 compounds identical with the developed impregnated TLC, the orange rows present sample S63 compounds developed with silica TLC and the green rows indicate that both sample S63 developed with impregnated TLC and silica TLC have the same compounds. As a result, Modification silver nitrate as a stationary phase had changed in compound selectivity as shown in **Table 5-Table 9**. for example of epi-γ-Eudesmol which can be found on the fraction 1 of the impregnated TLC (**Table 5**) while the same compound was found in the fraction 4 of the bare silica TLC. As **Figure 3.7** shows the structure of epi-γ-Eudesmol, silver ions might be more easily attracted to the OH- group than a silica gel. On the other hand, γ-HIMACHALENE can be found on fraction1 on both the bare silica and impregnated TLC plates (**Table 5**). Based on the structure of this compound (**Figure 3.8**), the similar retention factors obtained between these two plates indicate that the Ag-impregnated TLC might form similar interactions with the unsaturated compounds as silica gel-TLC.

Figures 3.9-3.13 showed SPME GC-MS result for the impregnated TLC and Silica TLC in the same fraction. As a result, the S63 sample that was developed with impregnated TLC gave a higher abundance than developing with silica TLC because for instance in **Figure 3.9**, the red spectrum gave a higher abundance than the black one. The high influence anion in a salt was analysed in headspace caused the higher abundance in the spectrum.

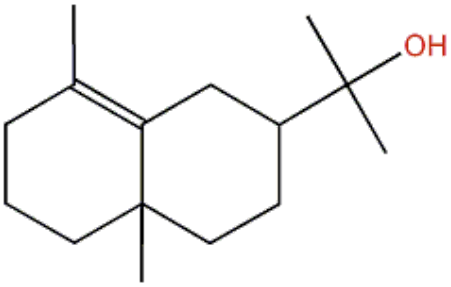

Figure 3.7: The structure of epi- γ -Eudesmol

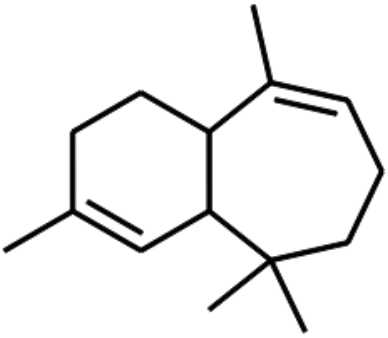

Figure 3.8: The structure of γ -HIMACHALENE

Table 5: Compounds from GC-MS of fraction 1

Compound name	Time	Retention Index		Area	Match	R Match
		EXP	RI			
Isoquinoline, 3-methyl	20.060	1312	1318	5614167	519	665
2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)	24.994	1468	1471	26714586	617	648
γ-HIMACHALENE	25.350	1480	1477	1331909	600	669
γ-HIMACHALENE	25.541	1486	1477	3556447	617	636
Dihydro-β-agarofuran	25.806	1495	1496	996648	516	698
3-Buten-2-one, 4-(2,2,3-trimethyl-6-methylenecyclohexyl)	25.912	1498	1490	1642972	596	647
β-Guaiene	26.245	1509	1490	1890361	606	647
β-Vatirenene	27.167	1541	1540	4185793	594	747
Furopelargone A	28.696	1593	1588	7286780	518	565
Khusimone	29.320	1615	1605	4338682	648	713
L-Leucine, N-(n-propyloxycarbonyl)-, n-propyl ester	29.664	1628	1640	1257021	494	610
β-Acorenol	29.776	1632	1649	1597256	630	784
γ-Eudesmole	30.001	1640	1631	3062647	637	735
γ-Eudesmol	30.245	1649	1631	4500690	642	708
epi-γ-Eudesmol	30.780	1668	1662	8527772	584	682
Costal	31.434	1691	1691	1059314	556	673
(1R,7S,E)-7-Isopropyl-4,10-dimethylenecyclodec-5-enol	31.567	1696	1695	3315105	584	635
Carnegine	32.223	1721	1727	772576	551	587
2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	32.594	1735	1730	2871213	626	688
Octanal, 2-(phenylmethylene)-	32.800	1743	1755	18555831	781	816
Octanal, 2-(phenylmethylene)-	33.450	1768	1755	841432	557	675
4a,5-Dimethyl-3-(1-methylethylidene)-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone	34.262	1798	1817	2457714	625	671
Isopropyl myristate	34.889	1823	1827	1057132	540	649
β-Vetispirene	25.638	1489	1495	1704428	579	740
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	25.731	1492	1507	1205201	529	610
Benzohydroxamic acid, 2TMS derivative	26.150	1506	1494	1432250	-	-

White: Compounds on impregnated TLC
Orange: Compounds on bare silica TLC
Green: Compounds on both type of TLC

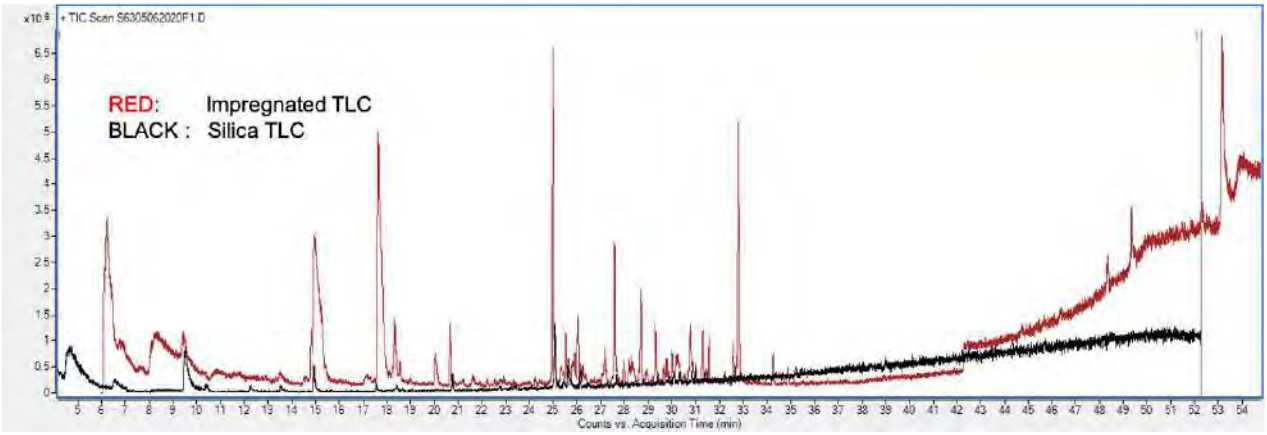

Figure 3.9: GC-MS overlapped spectrums in fraction 1 (Impregnated TLC and Silica TLC)

Table 6: Compounds from GC-MS of fraction 2

Compound name	Time	Retention Index		Area	Match	R Match
		EXP	RI			
Quinoline, 2-methyl	20.044	1312	1311	4750275	536	683
2-Hexenoic acid, 2-[(trimethylsilyl)oxy]-, trimethylsilyl ester	20.671	1331	1338	5941590	486	575
Isolongifolene	22.903	1400	1390	3587451	673	694
(3E)-4,4-Dimethyl-3-(3-methyl-3-butenylidene)-2-methylenebicyclo-[4.1.0]heptane	24.414	1449	1459	3845206	554	607
2,5-di-tert-Butyl-1,4-benzoquinone	24.992	1468	1466	17566631	663	695
β-Guaiene	25.427	1482	1490	4520894	657	689
β-Vetispirene	25.547	1486	1495	9438974	999	999
Benzohydroxamic acid, 2TMS derivative	26.050	1503	1494	10245597	503	543
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	26.243	1509	1507	1372315	619	660
Eremophila-1(10),11-diene	26.371	1514	1499	2398504	599	644
1-Naphthalenol, 1,2,3,4,4a,5,6,7-octahydro-4a,5-dimethyl-3-(1-methylethenyl)	27.169	1541	27.169	12326121	773	806
Spathulenol	28.425	1584	1577	12422948	625	681
1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)	28.696	1593	1583	4145558	502	523
Name: 2-Allyl-1,4-dimethoxy-3-methyl-benzene	28.889	1600	1601	6176800	544	660
Ethanone, 1-[(1S,3aR,4S,7R,7aR)-octahydro-3a,7a-dimethyl-4,7-methano-1H-inden-1-yl]	29.111	1608	1589	4473032	573	683
γ-Eudesmol	29.320	1615	1631	33477795	770	791
γ-Eudesmol	29.660	1628	1631	14713438	755	817
Agarospirol	29.773	1632	1645	51431457	884	910
Hinesol	29.870	1635	1635	11516763	572	833
Agarospirol	30.004	1640	1645	43073941	689	747
Valerianol	30.237	1648	1661	72935354	845	866
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	30.902	1672	1690	2361997	592	667
Zizanal	31.299	1687	1680	2605125	579	666
2,2,6-Trimethyl-1-[3-methylbuta-1,3-dienyl]-7-oxabicyclo[4.1.0]heptan-3-ol	31.408	1691	1692	2101729	575	607
β-Nootkatol	31.552	1696	1712	3712980	587	782
Guaiac acetate	32.214	1721	1727	947869	518	624
β-Nootkatol	32.319	1725	1712	1214067	613	725
2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)] undec-4-en-3-one	32.583	1735	1730	2388918	672	748
Octanal, 2-(phenylmethylene)	32.795	1743	1755	27458535	791	820
Octanal, 2-(phenylmethylene)	33.459	1768	1755	1111164	665	775
2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4a,5-dimethyl-3-(1-methylethylidene)-, (4ar-cis)	34.270	1799	1817	6058557	653	669
Isopropyl myristate	34.892	1823	1827	2291401	658	751
6-(Dimethylamino)purine, TMS derivative	35.154	1834	1850	373540	518	588

Compound name	Time	Retention Index		Area	Match	R Match
		EXP	RI			
2-Isopropyl-N-methylaniline, N-trimethylsilyl	20.768	1334	1316	997226	479	507
Cycloseychellene	23.096	1406	1418	2677067	549	650
α-Cedrene	23.341	1414	1411	400491	529	668
4-tert-Butylaniline, N-pentafluoropropionyl	23.830	1430	1440	269100	476	546
2,6-Diethylaniline, N-trimethylsilyl	24.139	1440	1447	864255	521	560
Patchoulene	24.341	1447	1467	1122857	502	583
Pheneturide	24.669	1458	1465	544507	415	542
γ-Selinene	25.084	1471	1479	63157312	537	568
Chamigrene	25.128	1473	1476	3503354	630	755
4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	25.440	1483	1492	4951447	673	743
β-Vetispirene	25.635	1489	1495	7592918	751	787
γ-Selinene	25.738	1492	1479	5086185	603	640
Dihydro-β-agarofuran	25.914	1498	1496	2408023	640	758
2,3,3-Trimethyl-2-[(1E)-3-methyl-1,3-butadienyl]	25.996	1501	1517	1404559	543	655
(+)-Cycloisolongifol-5-ol	26.137	1506	1519	3950745	499	739
β-Himachalene	26.398	1515	1500	1305444	477	525
Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)	26.465	1517	1533	2078370	526	699
Guaia-9,11-diene	27.054	1537	1521	6151920	557	599
cis-Eudesm-6-en-11-ol	27.765	1561	1571	3664725	570	613
β-Iraldeine	28.777	1596	1584	2874330	605	643
2-Allyl-1,4-dimethoxy-3-methyl-benzene	28.987	1603	1601	1163100	443	619
γ-Eudesmole	30.085	1643	1631	19509909	684	729
(-)-Isolongifolol, methyl ether	31.383	1690	1672	14852279	545	554
Eremophila-1,11-dien-9-one	31.625	1698	1694	3910545	535	602
β-Santalol	32.392	1727	1715	2319289	465	611
4a,5-Dimethyl-3-(1-methylethylidene)-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone	34.351	1802	1817	8151319	651	792
DL-Valine, N-methyl-N-(3-chloropropoxycarbonyl)-, pentyl ester	39.433	2009	2012	3491489	510	589

White: Compounds on impregnated TLC
Orange: Compounds on bare silica TLC
Green: Compounds on both type of TLC

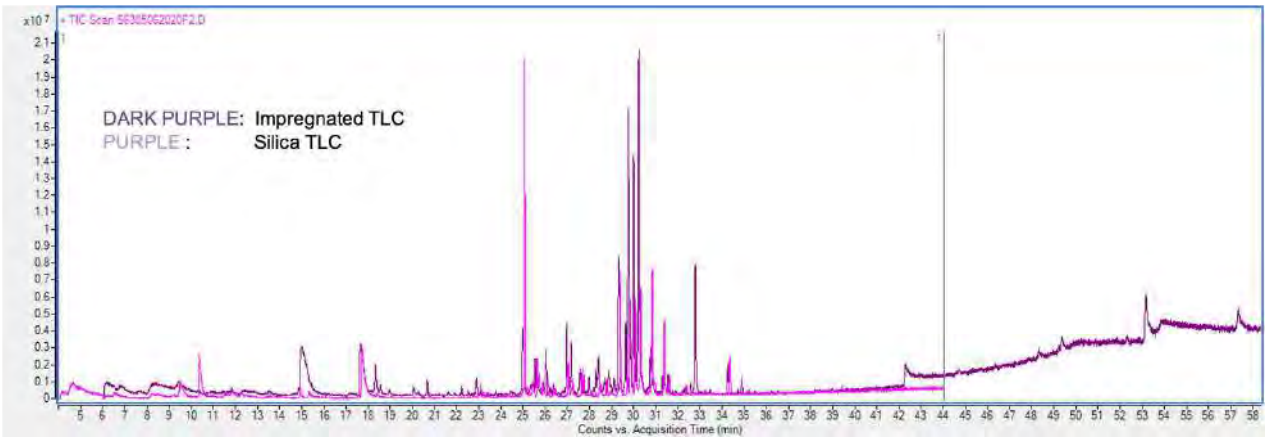

Figure 3.10: GC-MS overlapped spectrums in fraction 2 (Impregnated TLC and Silica TLC)

Table 7: Compounds from GC-MS of fraction 3

Compound name	Time	Retention Index EXP	Retention Index RI	Area	Match	R Match
Isoledene	21.636	1361	1375	2592083	511	616
Ylangene	22.216	1379	1372	1794891	573	631
α-Gurjunene	23.140	1408	1409	7095961	741	784
Patchoulene	24.992	1468	1467	17566631	508	636
Dihydro-β-agarofuran	25.427	1482	1496	4520894	579	673
3,5,11-Eudesmatriene	25.547	1486	1495	9438974	598	620
Isogermafurene	25.899	1498	1498	2251462	527	548
β-Eudesmene	26.050	1503	1486	10245597	644	674
Isoshyobunone	26.243	1509	1520	1372315	684	664
Dihydro-β-agarofuran	26.371	1514	1496	2398504	530	672
1,3-Benzodioxole, 4-methoxy-6-(2-propenyl)	26.610	1522	1519	810618	629	699
4a,7-Methano-4aH-naphth[1,8a-b]oxirene, octahydro-4,4,8,8-tetramethyl	27.163	1541	1544	54903434	672	710
cis-Eudesm-6-en-11-ol	27.678	1558	1571	39804547	556	722
Spathulenol	28.352	1582	1577	11982173	607	644
Isoaromadendrene epoxide	28.697	1593	1589	94107962	629	716
6-Methyl-2-[4-methylcyclohex-3-en-1-yl]hepta-1,5-dien-4-ol	29.165	1610	1608	4769837	494	649
γ-Eudesmole	29.814	1615	1631	65379412	807	879
γ-Eudesmol	29.652	1627	1631	10374585	727	774
Agarospirol	29.773	1632	1645	54142387	861	903
γ-Eudesmol	30.004	1640	1631	33428377	739	746
Valerianol	30.235	1648	1661	51527932	807	849
β-Acorenol	30.294	1650	1649	4279905	626	722
β-Acorenol	30.566	1660	1649	2153357	582	631
δ-Cadinol	30.675	1664	1645	1705317	562	649
8-Isopropyl-1,5-dimethyltricyclo[4.4.0.02,7]dec-4-en-3-one	30.890	1672	1687	10950204	587	740
Ylangenal	31.147	1681	1675	3384619	631	668
8-Isopropyl-1,5-dimethyltricyclo[4.4.0.02,7]dec-4-en-3-one	31.298	1687	1687	20117878	551	680
α-Costal	31.447	1692	1695	7047471	579	701
1(2H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-4a,5-dimethyl-3-[1-methylethenyl]-, [3S-(3α,4aα,5α)]	32.582	1735	1755	26812544	656	692
Octanal, 2-(phenylmethylene)-	32.798	1743	1755	27263184	778	804
Octanal, 2-(phenylmethylene)-	33.440	1767	1755	2260150	591	704
Isovalerenesol	34.265	1799	1788	6242880	514	619
Succinic acid, 2,2,3,3-tetrafluoropropyl 2,6-dimethylnon-1-en-3-yn-5-yl ester	34.704	1816	1815	479504	444	588
Isopropyl myristate	34.898	1824	1827	2895061	646	745

Compound name	Time	Retention Index EXP	Retention Index RI	Area	Match	R Match
Cinnamyl alcohol, pentafluoropropionate	20.126	1314	1298	401136	391	480
Name: 2-Isopropyl-N-methylaniline, N-trimethylsilyl	20.363	1321	1316	665495	471	487
2-Isopropyl-N-methylaniline, N-trimethylsilyl	20.762	1334	1316	969296	482	503
1H-Indole, 1,3-dimethyl	21.330	1351	1371	556847	446	644
β-Longipinene	22.765	1396	1403	3424152	624	655
(±)-β-Isocomene	23.006	1404	1412	1633900	566	658
1,2,9,10-Tetradehydroaristolane	23.212	1410	1435	1662185	530	555
α-Bergamotene	23.344	1415	1435	2574071	636	741
Prezizaene	23.826	1430	1444	1250855	594	705
α-Guaiene	24.055	1438	1429	1570872	577	726
α-Elemene	24.226	1443	1462	160335	442	544
4-tert-Butylaniline, N-pentafluoropropionyl	24.427	1450	1440	234001	416	530
(-)-Aristolene	24.670	1458	1453	1031011	438	546
2,6-Di-tert-butylbenzoquinone	25.106	1472	1471	248374342	685	694
4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydronaphthalene	25.203	1475	1492	6244347	676	730
(+)-Valencene	25.353	1480	1492	3392663	495	661
Eudesma-4(14),7(11)-diene	25.434	1482	1479	8449190	687	726
β-Vetispirene	25.638	1489	1495	20431325	765	785
(+)-Valencene	25.730	1492	1492	5214300	663	815
2,3,3-Trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone	25.993	1501	1517	2605977	595	654
Guaia-1(10),11-diene	26.122	1505	1505	6036437	627	734
Cubebol	26.399	1515	1515	3313402	490	545
(-)-α-Panasinsen	26.465	1517	1527	3023662	608	697
Kessane	26.735	1526	1537	795359	463	632
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, trans	26.963	1534	1544	837493	637	710
Guaia-9,11-diene	27.058	1537	1521	2145037	583	685
Selina-3,7(11)-diene	27.172	1541	1542	2371047	556	657
2-(4a,8-Dimethyl-1,2,3,4,4a,8a-hexahydro-2-naphthalenyl)-2-propanol	27.257	1544	1548	4703294	592	687
Rosifoliol	28.781	1596	1600	11419063	498	662
Cuminyl alcohol, tert-butyldimethylsilyl ether	29.136	1609	1610	848264	427	538
(Z)-Ethyl 3-(4-methoxyphenyl)acrylate	30.314	1651	1670	2479567	465	529
Widdrenal	31.630	1699	1724	2935643	449	600
2,2,7,7-Tetramethyltricyclo[6.2.1.0(1,6)]undec-4-en-3-one	32.675	1738	1730	1921900	542	672
4a,5-Dimethyl-3-(1-methylethylidene)-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone	34.352	1802	1817	1309151	435	580

White: Compounds on impregnated TLC
Orange: Compounds on bare silica TLC
Green: Compounds on both type of TLC

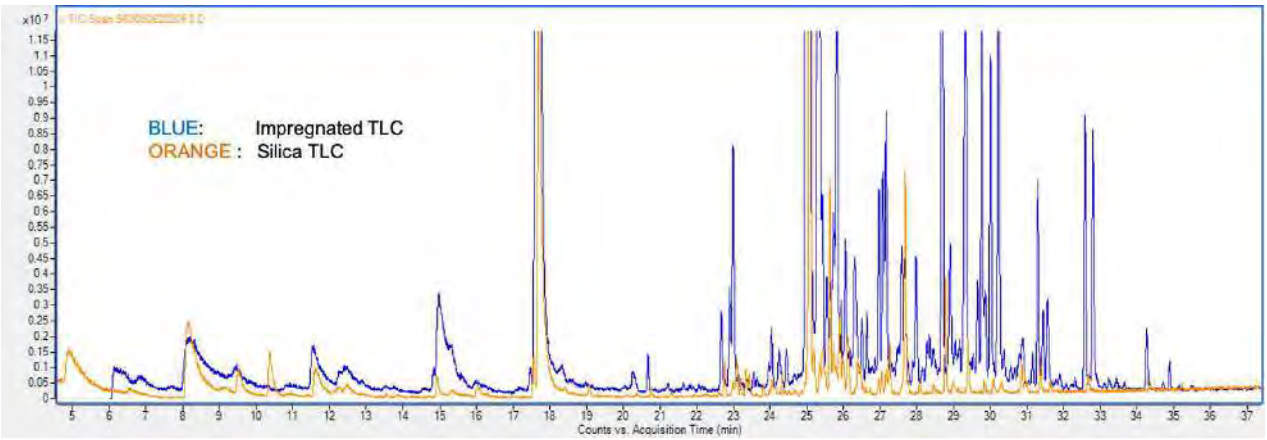

Figure 3.11: GC-MS overlapped spectrums in fraction 3 (Impregnated TLC and Silica TLC)

Table 8: Compounds from GC-MS of fraction 4

Compound name	Time	Retention Index		Area	Match	R Match
		EXP	RI			
Quinoline, 2-methyl	19.765	1303	1311	8852499	598	690
Naphthalene, 1,2,3,4-tetrahydro-1,8-dimethyl	20.578	1328	1318	6757347	999	999
Cyclosativene	21.218	1348	1368	54391046	683	756
β-Elemen	22.103	1375	1391	31801855	699	705
β-Isocomene	22.676	1393	1412	127803883	696	755
Di-epi-α-cedrene	23.122	1407	1400	52105412	742	753
Caryophyllene	23.262	1412	1419	189552576	670	673
α-Gualene	23.727	1427	1439	61367842	722	801
Guaia-6,9-diene	23.977	1435	1443	130500531	705	719
(-)-Aristolene	24.152	1441	1453	52725185	659	684
Aristolene	24.591	1455	1453	44440619	659	684
2,6-Di-tert-butylbenzoquinone	25.107	1472	1471	6.335E+09	660	672
γ-HIMACHALENE	25.290	1478	1477	67554783	658	684
Aristolochene	25.381	1481	1487	147816865	787	818
β-Vetispirene	25.609	1488	1495	1.868E+09	874	912
Dihydro-β-agarofuran	25.850	1496	1496	227296262	863	902
α-Bulnesene	26.042	1502	1505	217317048	827	881
Nootkatene	26.243	1509	1511	17988891	677	784
Valencene	26.384	1514	1494	240234253	791	795
1,1,5,6-Tetramethyl-1,2-dihydronaphthalene	26.561	1520	1511	25256899	602	712
(4aR,8aS)-4a-Methyl-1-methylene-7-(propan-2-ylidene) decahydronaphthalene	26.877	1531	1544	58285070	821	837
Lilial	26.970	1534	1533	35406008	681	749
Selina-3,7(11)-diene	27.074	1538	1542	96932129	826	841
1-Naphthalenol, 1,2,3,4,4a,5,6,7-octahydro-4a,5-dimethyl-3-(1-methylethenyl)	27.179	1541	1553	46695346	720	770
1aR,4S,7R,7aS,7bR)-1,1,4,7-Tetramethyl-1a,2,3,4,6,7,7a,7b-octahydro-1H-cyclopropa[e]	27.990	1569	1577	71790866	514	611
Isoaromadendrene epoxide	28.714	1594	1589	458963966	629	725
Isolongifolen-9-one	29.312	1615	1628	55795361	641	718
α-Coracalene	29.488	1622	1623	9245770	529	686
2-Furoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	29.766	1631	1633	13097547	440	575
10-Hydroxycalamenene, trans	30.361	1653	1673	10147683	506	544
Ziza-6(13)-en-3α-ol	30.578	1661	1677	8663363	522	581
α-Costal	31.443	1692	1695	5288125	656	697
1,1'-Biphenyl, 3,4-diethyl-	31.883	1708	1692	4658105	543	588
Octanal, 2-(phenylmethylene)-	32.795	1755	1755	30563272	810	832
2-Aminonaphthalene, N-trimethylsilyl-	33.103	1738	1738	3453650	595	622
4a,5-Dimethyl-3-(1-methylethylidene)-4,4a,5,6,7,8-hexahydro-2(3H)-naphthalenone	34.261	1817	1817	1838883	599	641
Isopropyl myristate	34.897	1827	1827	3633352	659	724

Compound name	Time	Retention Index		Area	Match	R Match
		EXP	RI			
Naphthalene, 1-methyl	19.856	1306	1307	1937451	569	673
2-Isopropyl-N-methylaniline, N-trimethylsilyl	20.683	1331	1316	2102459	484	502
α-Cubebene	21.099	1344	1351	1199727	478	567
Naphthalene, 1,2-dihydro-1,5,8-trimethyl	21.301	1351	1354	5605745	516	525
Ylangene	21.630	1461	1372	1742301	499	607
3-((3R)-2,3-Dimethyltricyclo[2.2.1.02,6]heptan-3-yl)propan-	21.830	1367	1343	986342	496	667
Ylangene I	21.954	1371	1372	2645874	628	693
Ylangene II	22.264	1380	1372	6145272	543	642
Ylangene III	22.448	1386	1372	1179323	483	599
(-)-β-Elemene I	22.769	1396	1391	38441750	703	709
(-)-β-Elemene II	23.005	1303	1391	28345377	691	722
1,2,9,10-Tetradehydroaristolane	23.216	1310	1435	17378257	647	662
cis-α-Bergamotene	23.353	1315	1415	22726904	799	820
4-(2,4,4-Trimethyl-cyclohexa-1,5-dienyl)-but-3-en-2-one	23.428	1417	1423	21379585	642	650
1,2,9,10-Tetradehydroaristolane	23.637	1424	1435	3337671	589	638
Khusimene	23.820	1430	1451	13153823	623	638
α-Elemene	24.251	1444	1462	2707329	540	556
α-Himachalene	24.333	1447	1449	9613743	510	578
Patchoulene	24.439	1450	1467	1939849	595	640
Humulene	24.508	1452	1454	2200165	615	820
p-Benzoquinone, 2,6-di-tert-butyl	25.220	1476	1471	1.873E+09	691	715
(+)-Valencene	25.756	1493	1492	58838875	810	854
3,5,11-Eudesmatriene	26.043	1490	1495	3766920	564	618
α-Cuprenene	26.537	1519	1509	3446832	694	709
trans-Calamenene	26.646	1523	1529	2665868	476	530
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR,8aS)	26.964	1534	1544	18254131	779	812
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR,8aS)-I	27.061	1537	1544	8757934	616	737
Guaia-3,9-diene	27.854	1564	1556	4155141	565	645
Isolongifolene, 4,5,9,10-dehydro	27.987	1569	1544	2481934	424	536
β-Copaen-4α-ol	28.076	1572	1586	8999373	428	644
Khusimone	28.553	1588	1605	3180191	576	678
Isoaromadendrene epoxide	28.797	1597	1589	110266585	591	672
epi-γ-Eudesmol	30.660	1667	1662	2161130	488	590
β-Acorenol	30.757	1667	1649	972785	434	534
Cedr-8-en-15-ol	30.960	1674	1682	2563266	448	685
Ylangenal	31.233	1684	1675	541648	489	641
2-Furoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	31.533	1695	1714	2284304	424	600
Valerenal	32.670	1738	1716	34671808	612	618

White: Compounds on impregnated TLC
Orange: Compounds on bare silica TLC
Green: Compounds on both type of TLC

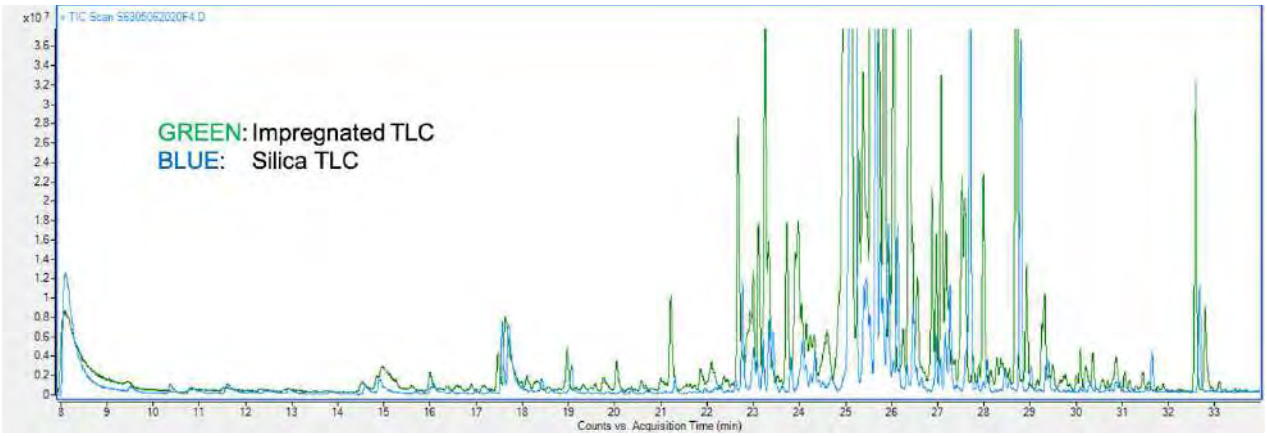

Figure 3.12: GC-MS overlapped spectrums in fraction 4 (Impregnated TLC and Silica TLC)

Table 9: Compounds from GC-MS of fraction 5

Compound name	Time	Retention Index		Area	Match	R Match
		EXP	RI			
Decane, 2,3,5,8-tetramethyl	20.443	1324	1318	3850473	629	733
Heptylcyclohexane	20.887	1338	1346	8292271	756	796
Benzene, 1-(1-methylethenyl)-4-(1-methylethyl)	21.232	1348	1361	26777051	553	642
Tridecane, 2-methyl	21.649	1361	1364	12086790	694	824
Tridecane, 3-methyl	21.858	1368	1371	13878784	653	793
Dodecane, 2,6,10-trimethyl	22.063	1374	1366	15258882	794	904
β-Patchoulene	22.161	1377	1381	7109592	730	809
Tetradecane	22.813	1397	1400	62329014	773	907
α-Cedrene	23.127	1407	1411	12614754	783	821
α-Cedrene	23.252	1412	1411	28703213	623	658
β-Copaene	23.899	1433	1432	10330676	640	667
Clovene	24.151	1441	1441	31365350	752	773
Patchoulene	24.607	1451	1467	3187867	631	667
α isomethyl ionone	24.761	1461	1481	19318709	585	619
γ-HIMACHALENE	24.995	1468	1477	26902900	650	680
α-Elemene	25.371	1480	1480	12640499	704	737
β-Vetispirene	25.552	1486	1495	22870674	742	795
Pentadecane	25.884	1497	1500	12943728	693	835
Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene	26.230	1509	1507	2177827	589	631
α-Cuprenene	26.381	1514	1509	72384749	762	770
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, trans	26.960	1534	1544	3253727	635	703
α-Calacorene	27.102	1539	1542	8260603	522	706
1-Naphthalenol, 1,2,3,4,4a,5,6,7-octahydro-4a,5-dimethyl-3-(1-methylethenyl)	27.993	1569	1553	30493389	635	642
1,1'-Biphenyl, 2-methoxy	28.150	1575	1574	1166478	612	653
1-Penten-3-one, 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)	28.695	1593	1584	9093117	640	696
Hexadecane	28.808	1597	1600	1253246	576	672
Naphthalene, 2-butyl-	29.501	1622	1611	2788404	510	557
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)	30.859	1671	1674	6832430	742	778
Naphthalene, 1,6-dimethyl-4-(1-methylethyl)	31.049	1678	1674	4208141	660	674
Valerenal	32.592	1735	1716	5148348	679	688
Octanal, 2-(phenylmethylene)	32.798	1743	1755	15312917	821	853
Pyrimethanil	34.146	1794	1793	1958078	555	691
Isopropyl myristate	34.894	1823	1827	3363230	632	701

Compound name	Time	Retention Index		Area	Match	R Match
		EXP	RI			
1H-Indene, 1-ethylidene-	19.845	1305	1315	8158054	752	888
Quinoline, 2-methyl-	20.131	1314	1311	8749639	569	662
Naphthalene, 6-ethyl-1,2,3,4-tetrahydro-	20.368	1322	1339	17867714	509	576
1-Iodo-2-methylnonane	20.530	1327	1320	2436224	608	800
Name: 3-Buten-2-ol, 4-phenyl-	20.687	1332	1329	8240572	557	664
α-Cubebene	20.861	1337	1351	1918514	529	607
Benzene, (1-methylheptyl)-	21.090	1344	1358	9836158	532	594
Naphthalene, 1,2-dihydro-1,5,8-trimethyl	21.304	1351	1354	29224603	597	602
Longicyclene	21.733	1364	1374	10052666	577	614
Ylangene	21.829	1367	1372	3384036	678	739
Ylangene I	21.960	1371	1372	21423057	743	777
Cyperene	22.257	1380	1399	40784144	583	617
(-)-β-Elemene	22.635	1392	1391	19839507	730	764
Isocaryophyllene	22.793	1397	1406	209224343	722	725
(E)-β-Isocomene	23.022	1404	1412	116775110	758	768
trans-α-Bergamotene	23.369	1415	1435	263420947	702	762
1H-Cyclopropa[a]naphthalene, 1a,2,6,7,7a,7b-hexahydro-1,1,7,7a-tetramethyl	23.661	1425	1440	10814595	673	737
Caryophyllene	23.830	1430	1419	63834521	659	663
α-Guaiene	24.090	1439	1439	195257288	810	860
3[E]-4,4-Dimethyl-3-(3-methyl-3-butenylidene)-2-methylenebicyclo[4.1.0]heptane	24.257	1444	1455	33937418	633	637
α-Guaiene I	24.430	1450	1439	20367547	693	748
Humulene	24.516	1453	1454	7104448	599	717
α-Guaiene II	24.682	1458	1439	60126916	736	783
Naphthalene, 1,2,3,4,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, (1R,7S,8aS)	25.062	1470	1476	544598316	784	863
Eudesma-4(14),7(11)-diene	25.245	1476	1479	287313350	830	832
Naphthalene, 1,2,3,4,4a,5,6,7-octahydro-4a,8-dimethyl-2-(1-methylethenyl)	25.493	1484	1492	363120799	852	853
β-Vetispirene I	25.953	1499	1495	38288116	470	536
α-Bulnesene	26.167	1597	1505	258382399	814	851
(-)-Nootkatene	26.356	1513	1511	38414068	821	842
α-Bulnesene	26.498	1518	1505	183059806	772	779
β-Vatirenene II	26.667	1524	1540	28942262	490	568
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR,8aS)-	26.988	1535	1544	106896606	824	842
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR,8aS)- I	27.085	1538	1544	50252855	764	770
Selina-3,7(11)-diene	27.194	1542	1542	70731507	835	845
1-Naphthalenol, 1,2,3,4,4a,5,6,7-octahydro-4a,5-dimethyl-3-(1-methylethenyl)	27.304	1546	1553	155700057	844	853
β-Vatirenene III	27.442	1550	1540	10177699	576	639
Sesquirosefuran	27.754	1561	1553	601043625	521	624
Gleenol	27.841	1564	1586	3456019	508	605
β-Copaen-4α-ol	28.455	1585	1586	32511265	493	529
6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	29.037	1605	1608	10373045	631	737
α-Corocalene	29.233	1612	1623	2794214	494	595
6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hepta-1,5-dien-4-ol	29.339	1616	1608	16944566	590	673

White: Compounds on impregnated TLC
Orange: Compounds on bare silica TLC
Green: Compounds on both type of TLC

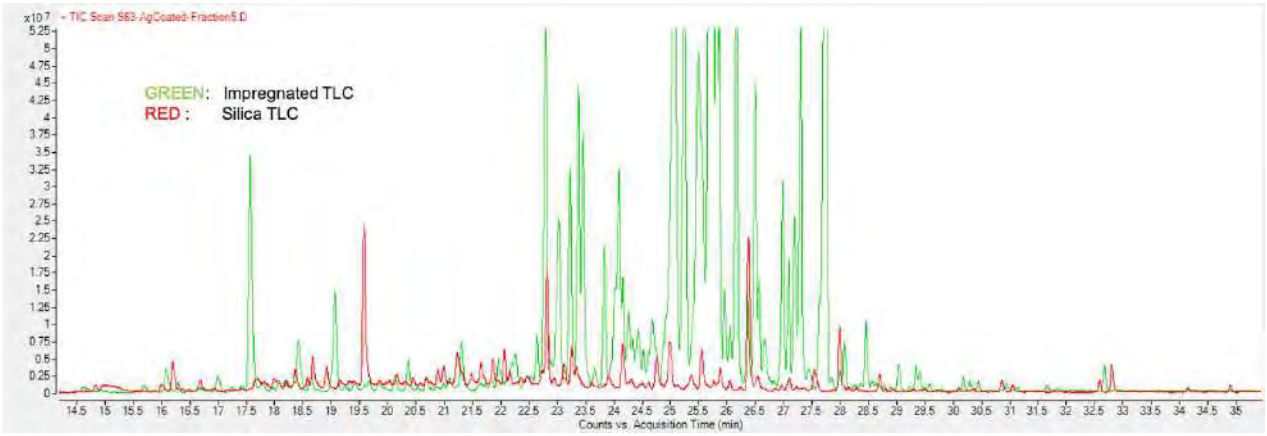

Figure 3.13: GC-MS overlapped spectrums in fraction 5 (Impregnated TLC and Silica TLC)

3.8 Potential application

In this section the potential application of our developed method was demonstrated. The fingerprints of the different agarwood oil were present by measuring the color intensity of each separated spot on TLC read by ImageJ. The separation result of sample SD (baked sample) was compared to the remaining three samples in **Figure 3.14**. The SD sample did not show a pink spot having the retention factors in a range of 0.53 to 0.55 (sd=0.01). From **Figure 3.15**, the color intensity spectra demonstrated that our developed method might be useful for qualitative analysis of the agarwood oil product from different processes.

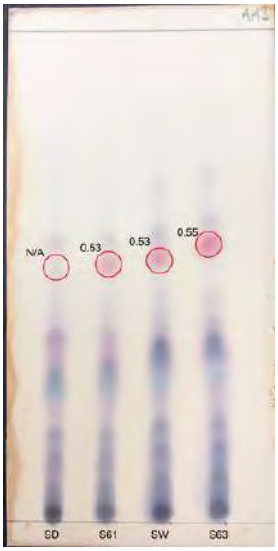

Figure 3.14: A suitable condition TLC plate shown the spots between baked and unbaked sample

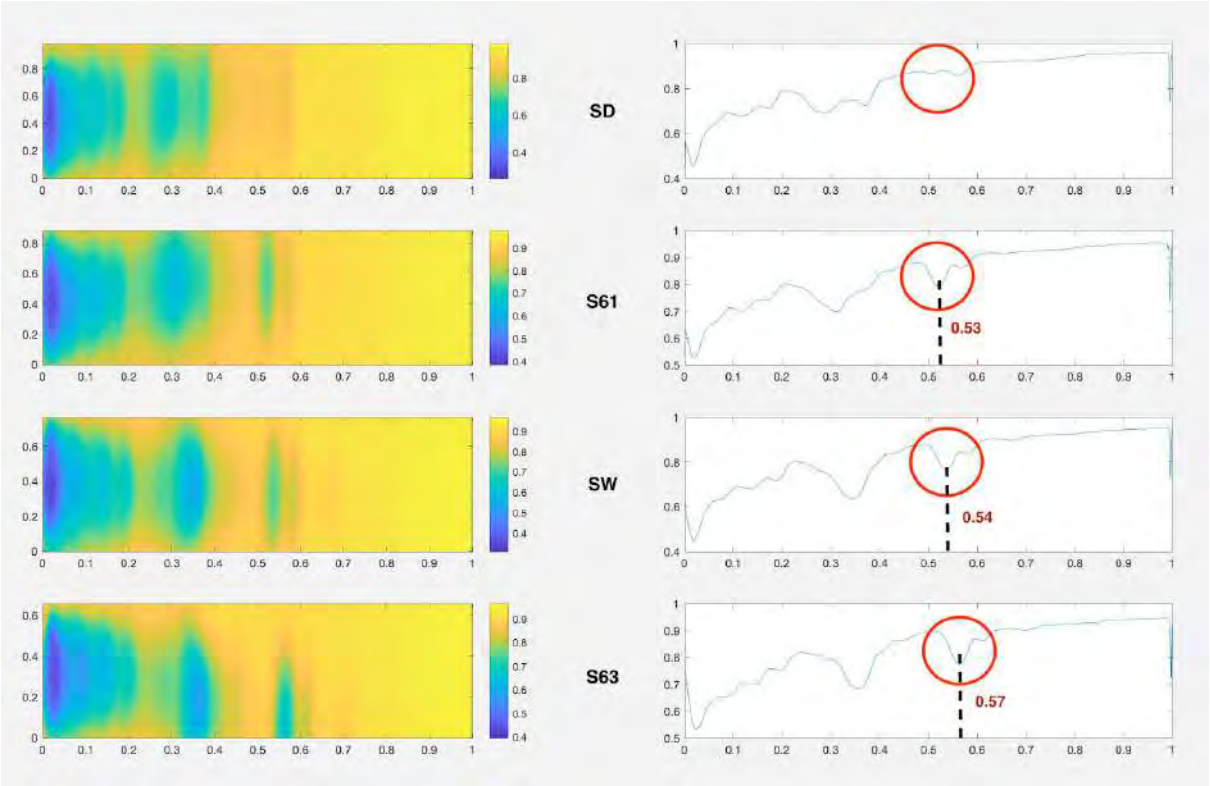

Figure 3.15: TLC color intensity spectrum shown missing peak between baked and unbaked sample

Chapter 4
Conclusions

In this project, agarwood essential oil was analyzed by modifying TLC with metal ion methods to determine the various characteristics of adulterants, species, and manufacturing methods. Silver nitrate was used to modify stationary phase to tune the selectivity of the stationary phase and increase efficiency in separation in the complex compounds in agarwood oil. Therefore, impregnation conditions were considered by solvent ratio, concentration, immersed time, and drying time. 0.5% $AgNO_3$ (w/v) in a ratio of 80:20 (methanol:milli-Q) immersed 10 minutes in $AgNO_3$ solution and dried in the oven for 15 minutes (110℃) was the optimum circumstance to separate baked and unbaked oil samples. GC-MS was used to identify the compound selectivity between impregnated TLC and a bare silica TLC. Lastly, ImageJ was used as a potential application to identify as a fingerprint and might be compared with the standard solution which is easier to identify in further oils.

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Biography

Mr. Pongrapee Ieosuwan was born on August 5th , 1998 in Samutprakarn, Thailand. He lives at 198/108 Bangpleeyai, Banplee, Samutprakarn, Thailand 10540. He graduated from Assumption Samutprakarn School in 2017 and continued studying bachelor degree at Bachelor of science in Applied chemistry (BSAC), Major of Industrial Chemistry and Management, Chulalongkorn university.

Contact information:
pngrapee.cngs@hotmail.com

Mr. Ekkapob Imkool was borned on October 11th, 1998 in Bangkok, Thailand. He lives at 660 Thanon Rama IV, Khwaeng Maha Phruttharam, Khet Bang Rak, Bangkok, Thailand 10500. He graduated from Satit Bilingual School of Rangsit University in 2017 and continued his studies at the Department of Chemistry, Faculty of Science, Chulalongkorn university, with an interest in Industrial Chemistry and Management.

Contact information:
6033848023@student.chula.ac.th