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Enhancement of Essential Oil from Agarwood by Subcritical Water Extraction and Pretreatments on Hydrodistillation

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***Abstract*—**The traditional method for essential oil extraction from agarwood (*Aquilaria Crassna*) is to soak it in water and follow with hydrodistillation. The effect of various agarwood pretreatments: ethanol, acid, alkaline, enzymes, and ultrasound, and the effect of subcritical water extraction(SWE) was studied to compare with the traditional method. The major compositions of agarwood oil from hydrodistillation were aroma compounds as follow: aristol-9-en-8-one (21.53%), selina-3, 7(11)-diene (12.96%), τ-himachalene (9.28%), β-guaiene (5.79%), hexadecanoic acid (4.90%) and guaia-3,9-diene (4.21%). Whereas agarwood oil from pretreatments with ethanol and ultrasound, and SWE got fatty acid compounds. Extraction of agarwood oil using these pretreatments could improve the agarwood oil yields up to 2 times that of the traditional method. The components of the pretreated sample with diluted acid (H_2SO_4) at pH 4 gave quite similar results as the traditional method. Therefore, the enhancement of essential oil from agarwood depends on requirement of type of extracted oil that involved extraction methods.

***Keywords*—**Agarwood, *Aquilaria Crassna*, Hydrodistillation, Subcritical Water Extraction

I. Introduction

AQUILARIA CRASSNA, (Agarwood) is one of the highly valuable fragrant woods that containing economically important essential oil. It is an unique aroma product. In Thailand, local name of agarwood is also called "Mai Krisana", is widely cultivated in Chanthaburi and Trat provinces. Since, agarwood oil is high prices product and high demand. In 2001, the organization of conserve the agarwood (CITES; The Convention on International Trade in Endangered Species of Wild Fauna and Flora) govern the conservation and utilization of tropical species and determine resource of agarwood [1]. It is a tropical tree that distributes in rain forest Southeast (Cambodia, Laos, Thailand and Vietnam) [2]. The components of essential oil have revealed various kinds of compound that consist of the major sesquiterpenes compounds (γ-selinene,selina-3, 11-dien-9-one, selina-3, 11-dien-14-al) and derivate choromones [1]. It is a member of Thymceleceae family and Magnliopsida class. The conventional methods for extraction of essential oil from agarwood performed either by hydrodistillation or steam distillation.

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The procedure for production of essential oil from agarwood start with immersion of the agarwood in water about 5days followed by distillation at boiling temperature (100 ^oC) for 5 days.

The yield of essential oil after this method is about 0.2% (W/W) [3]. Therefore, the conventional methods have the major disadvantages of the operating process such as long extraction time (total about 15-20 days) and high energy consumption on the other hand, agarwood oil is expensive product with sell price approximately 3,000 – 4,000 bath for 1 tola of oil (1 tola = 12.5 CC.) [4]. The high price of agarwood essential oil and high demand of this product motivate researcher to develop new extraction methods for higher yield, shorter extraction time and lower processing costs. Enzymes usually show a remarkable specificity for the biocatalysts. They have been catalogued by the Enzyme Commission of the International Union of Biochemistry: for example, Ligases Transferases, Oxidoreductase, Hydrolases and Isomerases as followed by function and promote give reactions. They are applied for several industries: agriculture, chemical process, pharmaceutical and petroleum industries [5] such as fruit and vegetable juices extraction [6], olive oil extraction [7]. Generally, the cell wall of plants is composed of cellulose, hemicellulose, lignin and pectin. Hydrolases such as cellulases, hemicellulases and pectinases break down the cell wall [8]. Besides, many researchers have attracted increasing interest to apply enzyme for cleavage the strong linkages of plant polysaccharides [9]. Agarwood is a strong woody structure and act as a barrier for essential oil extraction. Therefore, using of the cellulase, xylase and other digestive enzymes in this work could be easier to extract and might enhances the yield of agarwood oil. Ultrasonic extraction is proved to be economical and more effective. The cavitations during ultrasonic extraction have advantage due to ability to penetrate the cellular wall, reduce the particle size and increase the mass transfer between the cell walls and solvent [10-11]. It is used as alternative extraction technique to increase the extraction yield effectively and decrease the extraction time [12-13]. In recent years, subcritical water extraction (SWE) is known as a powerful alternative methods for extraction. It is a new technique based on the use of water at temperatures that above its normal boiling point at 100^oC and more than 5 bar, but below its critical point at 374^oC, 220 bar. Water is one of the solvent that is cheap, safe, environmental friendly. First period of the using of subcritical water, it has been applied to solve the pollution problem including treatment of organic pollution in soil, sludge [14].

Afterward, other researchers have been used to extract the natural products such as active antioxidant compound from rosemary [15], anthocyanins from red grape skin [16], essential oil from *Coriandrum Sativum L*. [17], essential oil from *Eucaliptus globules* [18] and lignans from whole flaxseed [19]. Another researcher reported that subcritical water can be avoiding the cuticular wax for extraction of essential from natural plants [20].

The major advantages of SWE are shorter extraction time, higher quality of extracted. Therefore, the objective of this work was to study the agarwood oil yield from various pretreatments on hydrodistillation such as aqueous ethanol, dilute acid/alkaline solution, technical enzymes, and ultrasound. Moreover, the subcritical water extraction was investigated and compared the oil yield with traditional method.

II. Materials and Methods

A. Raw Materials

Aquilaria crassna (agarwood) used in this study was cultivated by artificial method to stimulate the resin production. This agarwood was purchased from Bo-rai, Trat province. The plant material was dried in the oven, cut into small pieces and grounded to size of 3.0 mm. Carbon Dioxide, purity 99.9%, was purchased from Thai Industrial Gases Public Company Limited, Thailand. Ethanol, analytical grade 99.9% was purchases from Merck & Co., Inc. Germany. Cellulase and xylase (Dr. Luca, Germany), alcalase and rohalase (AB-Enzyme, Germany) were used as technical enzyme.

B. Hydrodistillation

Two hundred grams (200 g) of the ground agarwood were soaked in round-bottle flask with 1 L of water at room temperature for 5 days. Then, hydrodisitllation was carried out at boiling temperature of water for 30 hours. The obtained agarwood oil in Clevenger-type apparatus was taken and stored at ambient temperature until analyze the chemical constituents.

C. Pretreatment with water and aqueous ethanol

Two hundred grams (200 g) of the ground agarwood were given in 1800 ml flask and added water, 50% ethanol (v/v) and 80% ethanol (v/v), respectively until 5 days. Then, the liquid phase were filtered with cloth filter and removed ethanol from liquid phase by using rotary evaporator. Then the pretreated sample was added in round-bottle flask until extracted essential oil by hydrodistillation.

D. Pretreatment with an acidic solution

Two hundred grams (200 g) of the ground agarwood were placed in a 2 L flask and added 1800 ml solution of water and 1N H_2SO_4 for adjust pH 2 and 4, respectively until 5 days. After that the pretreated sample was adjusted to pH 7 with 1N NaOH. Then the pretreated sample was added in round-bottle flask until extracted essential oil by hydrodistillation.

E. Pretreatment with an alkaline solution

Two hundred grams (200 g) of the ground agarwood were placed in a 2 L flask and added 1800 ml solution of water and 1N NaOH for adjust pH 10 until 5 days. After that the pretreated sample was adjusted to pH 7 with 1N H_2SO_4. Then the pretreated sample was added in round-bottle flask until extracted essential oil by hydrodistillation.

F. Pretreatment with technical enzymes

Two hundred grams (200 g) of the ground agarwood were mixed with 1800 ml of 1% H_2SO_4 and immediately autoclave at 121^{o}C for 15 minutes. After that the sample was cooled down until 55^{o}C. Finally, the sample was neutralized to pH 4.0-4.5 with 5N NaOH and mixed with 12 ml of enzyme mixture (5 ml cellulase, 5 ml xylase, 1 ml alcalase and 1 ml rohalase). The prepared sample was then incubated for 3 days at 55 ^{o}C in a water bath followed by using hydrodistillation. The extracted agarwood oil was stored at ambient temperature until analyze the chemical constituents.

G. Pretreatment with ultrasound

Two hundred grams (200 g) of the ground agarwood were placed in a 2 liter plastic flask and added 1800 ml of water. Then the soaking of sample was placed in an ultrasonic cleaning bath and operated at 44-48 KHz frequency. The sonication was performed 30 hour.

H. Subcritical Water Extraction (SWE)

Subcritical water extraction was carried out in pilot-plant scale equipment with 2 liter volume of extractor (SLB-Foodtech, Germany). Two hundred grams (200 g) of the ground agarwood were soaked in water during 5 days and then added in the extraction bag (20x50 cm.) and put into the extraction vessel. The total liquid phase (distillation water) was 4 L.The extractor was filled with 2 L of distillation water and remaining water was used to circulate extractive cycles in extractor. The SWE was conducted at temperature 100, 125 and 150^{o}C at constant pressure 6 bars during extraction time 30 hour, respectively. The flow rate was 40 ml/min. After extraction, the extracted agarwood oil was stored at ambient temperature until analyze the chemical constituents.

I. Essential oil analysis by GC-MS

Chemical constituents of extracted agarwood oils were analyzed by a Gas chromatograph (Triplus) equipped with Polaris mass spectrometer. The MS operating conditions were ionization voltage 70 eV. The column used was Zebron capillary, ZB-5 ms (30M x 0.25-mm i.d., film thickness 0.25 μm). Condition: split mode and injection of 1 μm. The GC-MS operating temperature was 60^{o}C for 5 min., ramp of 3^{o}C per min up to 240^{o}C and 240^{o}C for 1 min. Helium was used as the carrier gas. All experiments were performed 3 to 4 times and average values were reported.

III. Results and Discussions

A. Aqueous ethanol (50% and 80%) pretreatment

According to the low oil yield from traditional pretreatment (soaking in water), the effect of soaking in ethanol solvents

(50% and 80%v/v) as pretreatment on the yield of agarwood oil was investigated. The oil yield was 0.08, 0.20 and 0.21% (w/w) after soaking in water, 50% and 80% aqueous ethanol, respectively as shown in Table I.

TABLE I
THE AGARWOOD OIL YIELD (%) FROM DIFFERENT PRETREATMENT METHODS ON HYDRODISTILLATION AND EXTRACTION TIME 30 HOUR

Pretreatment method	Pretreatment time (hour)	Oil yield (%)	Oil appearance (ambient)
Water, pH 7	120	0.08	Yellow clear-liquid
50% Ethanol	120	0.20	Yellow clear-liquid
80% Ethanol	120	0.21	Yellow clear-liquid
H_2SO_4, pH 2	120	0.22	Yellow clear-liquid
H_2SO_4, pH 4	120	0.21	Yellow clear-liquid
NaOH, pH 10	120	0.20	Yellow clear-liquid
Mixed enzyme	120	0.21	Brown yellowish clear-liquid
Ultrasound	35	0.20	Yellow solid

The agarwood oil obtained after ethanol solvent pretreatment exhibited yellow clear liquid as Ramlan, M.F.[21] reported that agarwood oil is soluble in alcohol. The increasing of ethanol percent did not significantly affected to the higher oil yield after 50% ethanol. The chemical compositions of agarwood oil indicated in Table III and the major compounds demonstrated in Fig. 1.

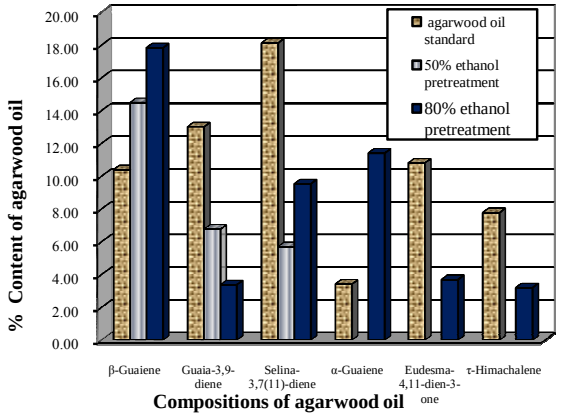

Fig. 1 the major compositions of extracted agarwood oil from water, 50% and 80% of aqueous ethanol pretreatment

From Table III (the last page), there were three fatty acid compounds; oleic acid ethyl ester (9.05%), hexadecanoic acid, ethyl ester (7.13%) and hexadecanoic acid (3.27%) in the extract. Both 50% and 80% aqueous ethanolic pretreatment resulted high amount of sesquiterpene hydrocarbon compounds in the extract. The 50% and 80% aqueous ethanol pretreatment were not suitable pretreatment for essential oil product. However, the amount of fatty acids were high which assessed in the antibacterial activity, could be considered as an additional therapeutic approach to improving oral health [22], display antioxidant properties and can help prevent atherosclerosis in rats [23].

B. Pretreatments with dilute acidic and alkaline solution

The agarwood oil yields from various pH (2, 4, 7 and 10) of dilute acidic and alkaline solution as pretreatment were 0.22, 0.21, 0.08 and 0.20%, respectively as shown in Table I. Grethlein,H.E. and Converse, A. [24] demonstrated that the acidic and alkaline hydrolysis methods have been used to degrade cell wall as chemical pretreatment and H_2SO_4 and NaOH widely used to hydrolysis under acid and alkaline condition. From this study, the acidic pretreatment (pH 2) can enhance agarwood oil yield more than alkali due to dilute sulfuric acid pretreatment can effectively hydrolyze cellulose and hemicelluloses [25]. These extracted oil were yellow clear liquid. The chemical compositions of agarwood oil indicated in Table III and the major compounds demonstrated in Fig. 2.

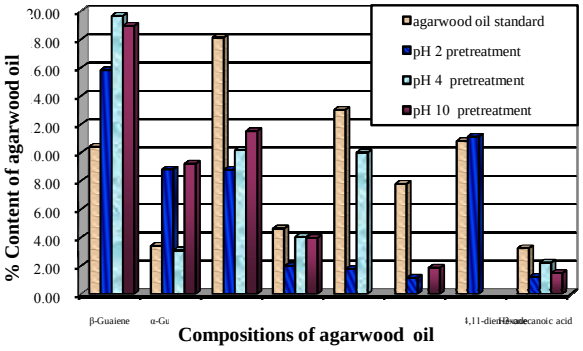

Fig. 2 the major compositions of extracted agarwood oil from acid and alkaline solution pretreatment.

Eight main components were identified as β-guaiene, α-guaiene, selina -3,7(11)-diene, δ-selinene, guaia-3,9-diene, τ-himachalene, eudesma-4,11-diene-3-one and hexadecanoic acid, respectively. The result showed that the component from dilute acid (H_2SO_4) at pH 4 pretreatment gave almost similar to soaking in water (traditional pretreatment). The higher amount of β-guaiene in extracted oil was remarkable from pretreated sample with acid at pH 2, 4 and base at pH 10 (15.82%, 19.65% and 18.94%, respectively).

C. Pretreatments with technical enzymes

The agarwood was pretreated with the mixture of cellulase and hemicellulase enzymes at pH 4.5 and temperature 55°C during 5 days before hydrodistillation for 30 hour as shown in Table I. Marek E. et al [26] reported that the application of enzymatic pretreatment could facilitate the essential oil extraction enhance the extraction yield. From the results obtained, the agarwood oil yield was up to 3 times (0.21%) higher than the sample without enzymatic pretreatment (0.08%). This indicated the advantage of apply technical enzymes in the agarwood oil extraction process. Technical

enzymes such as celulase and hemi-cellulase could degrade the woody plant cell wall materials (cellulose and hemi-cellulose) and improve the mass transfer during hydrodistillation [27].

The color of agarwood oil was intensive brown yellowish, clear liquid which compare to hydrodistillation without enzyme pretreatment. The chemical compositions of agarwood oil indicated in Table III and the major compounds demonstrated in Fig. 3.

From table III, sixteen components were detected in extracted agarwood oil. The main chemical composition of extracted agarwood oil from enzymatic pretreatment consisted of Dihydrokaranone (28.36%), β-guaiene (14.73%), δ-selinene (14.21%) and caryophyllene oxide (7.47%), respectively. The extraction of fatty acid was absent in enzymatic pretreatment. In this study, the extracted agarwood gave large proportion of oxygenated sesquiterpenes in pretreated sample with enzyme.

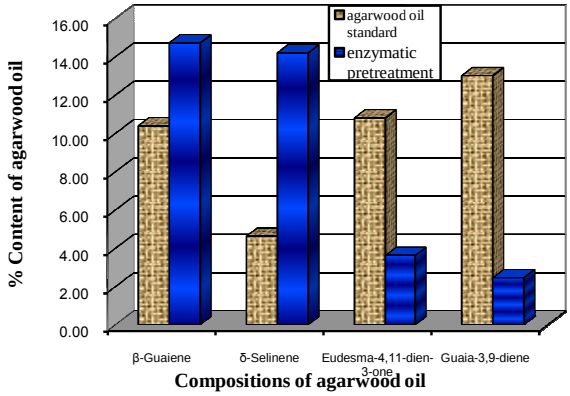

Fig. 3 the major compositions of extracted agarwood oil from technical enzymes pretreatment

From Fig. 3, the high amount of β-guaiene (14.73%) was found in enzymatic pretreatment. Whereas, the amount of β-guaiene in water pretreated sample was lower (10.40%) than in enzymatic pretreated sample. In contrary, eudesma-4,11-dien-3-one and guaia-3,9-diene were detectable at high levels (10.81% and 13.02%, respectively) by soaking in water but were presented at lower amounts of extracted oil (3.63% and 2.47%, respectively) in enzymatic pretreated sample. Also, it was clearly differences in agarwood oil compositions isolated by two pretreatment methods.

D.Pretreatments with ultrasound

The power of ultrasound was applied as pretreatment using 46 kHz and treatment time was 35 h before hydrodistillation. It indicated that the essential oil yield increased up to 0.20% and was distinct higher than the oil yield without ultrasound pretreatment (0.08%) as shown in Table I. The oil appearance was yellow solid. According to the cavitations' phenomena, the collapse of bubbles on the cell wall is expected to induce cell disruption together with a good penetration of the solvent into the cells, increase mass transfer and consequently improve extraction of compounds into the solvent medium [28-29]. These can lead to increase of extracted oil compare to oil extraction without ultrasound pretreatment. Therefore, the ultrasound was an effective pretreatment method to increase the oil yield. The chemical compositions of agarwood oil indicated in Table III and the major compounds demonstrated in Fig. 4.

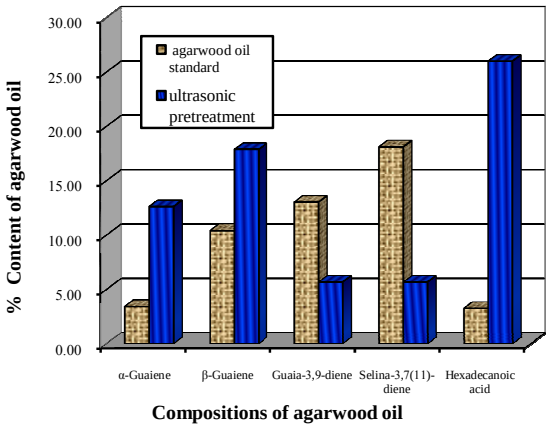

Fig. 4 the major compositions of extracted agarwood oil from ultrasound pretreatment

The effect of ultrasonic pretreatment on composition of extracted agarwood oil was presented in Fig. 4. It was obvious that the ultrasonic pretreatment showed extremely higher percentage of hexadecanoic acid (25.97%) as fatty acid in oil extract. The appearance of extracted essential oil was solid at ambient temperature because of high concentration of hexadecanoic acid. Comparison between chemical composition of essential oils from sample pretreated with ultrasound followed by hydrodistillation and agarwood oil standard showed distinct higher α-guaiene (12.61%) and β-guaiene (17.87%) but less guaia-3,9-diene and selina-3,7(11)-diene in ultrasonic pretreated extract.

1. Subcritical Water Extraction (SWE)

The agarwood oil was extracted by using subcritical water extraction (SWE) as a novel technique. The SWE treatment of sample without soaking in water could not be effective to extract agarwood oil at 125°C as shown in Table II. When the agarwood was soaked in water and extracted with various temperature (100, 125 and 150°C), the oil yield was 0.05, 0.10 and 0.06%, respectively.

TABLE II
THE AGARWOOD OIL YIELD (%) FROM SUBCRITICAL WATER EXTRACTION AT EXTRACTION PRESSURE 6 BAR AND EXTRACTION TIME 30 HOUR

Temperature (°C)	Pretreatment	Oil yield (%)	Oil appearance (ambient)
125	without	N.D.	-
100	Soaking in water	0.05	Yellow clear-liquid
125	Soaking in water	0.10	Yellow clear-liquid
150	Soaking in water	0.06	Yellow clear-liquid

TABLE III
THE CHEMICAL COMPOSITIONS OF EXTRACTED AGARWOOD OIL BY DIFFERENT METHODS WITH % RELATIVE CONTENT

Compound	% Relative content									
	Extraction method									
	STD	Water	50% ETOH	80% ETOH	pH 2	pH 4	pH 10	Enzyme	Ultra sound	SWE
β-Guaiene	10.40	5.79	14.49	17.84	15.82	19.65	18.94	14.73	17.87	22.47
Selina-3,7 (11)-diene	18.12	12.96	5.69	9.54	8.78	10.16	11.52	-	5.67	9.28
Guaia-3,9-diene	13.02	4.21	6.77	3.38	1.77	10.00	-	2.47	5.67	6.03
τ-Himachalene	7.78	9.28	-	3.20	1.61	-	1.87	-	-	-
α-Guaiene	3.42	3.90	-	11.40	8.81	3.09	9.21	-	12.61	7.31
δ-Selinene	4.63	-	-	-	1.98	4.03	3.96	14.21	-	-
Nootkatone	-	-	6.17	4.48	1.21	5.22	3.64	-	8.07	-
Eudesma-4,11-dien-3-one	10.81	3.01	-	3.69	11.12	-	-	3.63	-	-
Aristol-9-dien-8-one	10.19	21.53	-	7.74	-	-	6.90	-	-	-
Aristolene	-	-	-	9.05	-	-	-	3.12	7.12	10.84
α-Copaen-11-diol	-	-	-	5.80	6.14	6.88	7.43	-	-	-
Cedrane-8,13-diol	-	-	-	-	-	9.67	9.48	-	3.04	4.44
Iso-velleral	-	-	-	-	3.21	2.14	1.82	-	-	-
Agarospirol	-	-	-	-	7.09	8.93	10.17	-	-	-
α-Selinene	-	-	-	-	11.64	-	2.79	-	-	-
δ-Guaiene	-	-	-	-	1.16	-	-	-	9.53	-
β-Chamigrene	9.33	3.34	-	-	-	-	-	-	-	-
Cadina-3,9-diene	2.32	3.49	-	-	-	-	-	-	-	-
Tetraisopropylidene cyclobutane	1.97	-	-	-	-	-	-	-	-	-
5β,19-Cycloandrost-6-ene-3,17-dione	2.16	-	-	-	-	-	-	-	-	-
Caryophyllene	-	-	7.43	-	-	-	-	-	-	-
Vellerdiol	-	-	-	-	3.66	-	-	-	-	-
7,7-Dichloro bicyclohept-2-en-6-one	-	-	-	-	-	-	-	2.48	-	-
Cedr-8(15)-ene	-	3.54	-	-	-	-	-	1.30	-	-
Furanodiene	-	-	-	-	-	-	-	2.81	-	-
Caryophyllene oxide	-	-	-	-	-	-	-	7.47	-	-
1,5Diphenyl-3-pentanone	-	-	-	-	-	-	-	3.76	-	-
Dihydrokaranone	-	-	-	-	-	-	-	28.36	-	-
Longiverbenone	-	-	-	-	-	-	-	-	-	8.05
6-(1Hydroxy methylvinyl) -4,8a-dimethyl-3,5,6, 7,8,8ahexahydro-1H-naphthalen-2-one	-	-	-	-	-	-	-	3.68	-	-
4,6,6-Trimethyl-2-(3-methyl-buta-1,3-dienyl)-3-oxatricyclo [5.1.0.0.2,4] octane	-	-	-	-	-	-	-	-	-	6.41
5β-Guaia-7(11),10(14)-dien-8α-ol, 5,8-epoxy	-	-	-	-	-	-	-	-	-	1.98
Olean-12-ene-3,15,16,21,22,28-hexol	-	-	-	-	-	-	-	-	-	4.32
Fatty acid compounds:-										
Hexadecanoic acid	3.24	4.90	6.25	3.27	1.22	2.23	1.48	-	25.97	-
Hexadecanoic acid ethyl ester	-	-	13.22	7.13	-	-	-	-	-	8.71
Oleic acid ethyl ester	-	-	27.32	9.05	-	-	-	-	-	9.48
9,12- Octadecadienoic acid	-	-	-	-	-	-	-	-	-	6.80
Eicosanoic acid	-	-	2.50	-	-	-	-	-	-	-

Thus, the yield of agarwood oil increased with increasing temperature up to 125°C. Under this extraction condition, the main affect of SWE is on hydrogen bond of water molecule. The interactions between water molecules and hydrogen bond can disrupt three dimension network and reduce the polarity of water at high temperature. Hench, the dielectric constant values of water decrease lower than 80. The subcritical water as solvent will act similar to other organic solvents. So that it can increase the solubility of low polarity compounds [30-31]. Further, the increase of temperature to 150°C showed negative effect on agarwood oil yield. The decreasing of oil yield got high temperature that maybe thermal degradation of agarwood oil during long extraction time. Therefore, the maximum yield extraction condition from effect of subcritical water extraction (SWE) on agarwood oil yield was at 125 °C, 6 bar and 30 hour that was selected to analysis of the chemical composition of extracted agarwood oil by GC-MS. From Table III, thirteen components were identified with SWE extraxted agarwood oil. The most of SWE compounds were sesquiterpene hydrocarbons that included β-guaiene (22.47%), aristolene (10.84%), selina-3,7(11)-diene (9.28%), longiverbenone (8.05%) and α-guaiene (7.31%), respectively. The extracted oil comprised of fatty acid derivatives such as hexadecanoic acid ethyl ester (8.71%), oleic acid ethyl ester (9.48%), and 9,12-Octadecadienoic acid (6.80%).

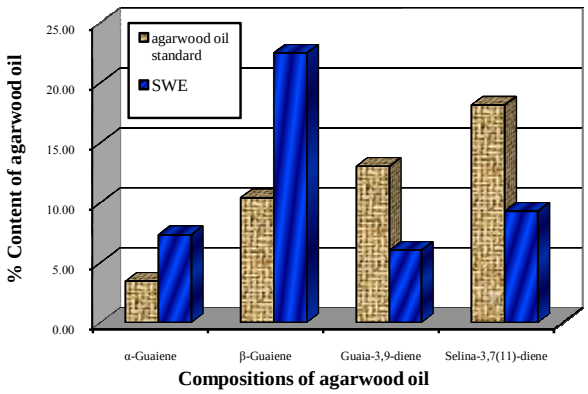

Fig. 5 the major compositions of extracted agarwood oil from subcritical water extraction

The major compounds of extracted agarwood oil were illustrated with hydrodistillation (agarwood oil standard) and subcritical water extraction (SWE) in Fig.5. Comparing between their agarwood oil, some sesquiterpene hydrocarbons in SWE oil were similar to hydrodistillation, nevertheless different percentage of amount. The SWE of agarwood oil contained higher amount of α-guaiene (7.31%), β-guaiene (22.47%) than hydrodistillation (3.42%, 10.40, respectively). In contrast, hydrodistillation revealed more amount of guaia-3,9-diene (13.02%), selina-3,7(11)-diene (18.12%) than SWE of agarwood oil (6.03%, 9.28%, respectively). Some oxygenated compounds were found in SWE essential oil but was absent in hydrodistilled essential oil such as 5,8-epoxy-5β-Guaia-7(11),10(14)-dien-8α-ol (1.98%), cedrane-8,13-diol (4.44%) and longiverbenone (8.05%).

IV. Conclusions

The traditional method for extraction of agarwood oil from *Aquilaria crassna* resulted low oil yield (0.08% w/w) and took about 10 days for extraction. This method was very time intensive and requires high energy consumption. New pretreatment methods include: aqueous ethanol, sulfuric acid, sodium hydroxide, enzymes and ultrasound followed by hydrodistillation could enhance the agarwood oil yields more than 2 times of traditional method. All extracted agarwood oil were divided into two main essential oil groups that consisted of sesquiterpene hydrocarbons (formula = $C_{15}H_{24}$) and oxygenated sesquiterpenes (composed of oxygen atom in molecule). In addition, fatty acid compounds were detectable in the extracted agarwood oil. The compositions of essential oil from subcritical water extraction (SWE) indicated differently with traditional method. However, the extracts obtained slightly different.

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