COMPLETE REPORT

Application of chromatography-mass spectrometry for analysis of *Aquilaria crassna* essential oils

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Dr. Patcharee Pripdeevech
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บทคัดย่อ

องค์ประกอบสารระเหยจากน้ำมันหอมระเหยกฤษณาพันธุ์ *Aquilaria crassna* จากส่วนต่าง ๆของประเทศไทยถูกวิเคราะห์ด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโทเมตรี,เทคนิคการสกัดด้วยวัฏภาคของแข็งในระดับจุลภาค-แก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี และเทคนิคแก๊สโครมาโทกราฟี 2 มิติ จากการทดลองพบองค์ประกอบจำนวน 18 องค์ประกอบในน้ำมันหอมระเหยกฤษณาพันธุ์ *A. crassna* จากเชียงราย องค์ประกอบหลักที่พบคือ hexadecanoate, guaia-1(10),11-dien-15-ol, karanone, cyclocolorenone และ jinkoh-eremol น้ำมันหอมระเหยกฤษณาพันธุ์ *A. crassna* จากเชียงใหม่มี 28 องค์ประกอบโดยมีองค์ประกอบหลักคือ hexadecanoate, kusunol, jinkoh-eremol, epoxybulnesene และ β-agarofuran ในขณะที่พบ 30 องค์ประกอบในน้ำมันหอมระเหยกฤษณาพันธุ์ *A. crassna* จากระยอง โดยมี hexadecanoate, β-agarofuran, kusunol, dehydrojinkoh-eremol และ 9,11-eremophiladien-8-one องค์ประกอบหลัก นอกจากนี้วิเคราะห์สารระเหยง่ายในน้ำมันหอมระเหยกฤษณาพันธุ์ *A. crassna* ด้วยเทคนิคการสกัดด้วยวัฏภาคของแข็งในระดับจุลภาค-แก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี พบองค์ประกอบระเหยง่ายจำนวน 74 องค์ประกอบ องค์ประกอบหลักที่พบได้แก่ β-agarofuran, 4-phenyl-2-butanone, furfural, benzaldehyde ส่วนองค์ประกอบรองที่มีกลิ่นหอมได้แก่ (*E*)-α-bergamotene, α-humulene, α-bulnesene, α-agarofuran, nor-ketoagarofuran, epoxybulnesene, agarospirol, jinkoh-eremol, kusunol, acorenone B, selina-3,11-dien-14-al and 9,11-eremophiladien-8-one จากการศึกษาพบว่าองค์ประกอบเหล่านี้อาจเป็นสารที่ก่อให้เกิดความหอมในน้ำมันหอมระเหยกฤษณาพันธุ์ *Aquilaria crassna* การแยกสารระเหยอย่างชัดเจนพบในการประยุกต์ใช้เทคนิคแก๊สโครมาโทกราฟี 2 มิติ ซึ่งให้ผลของลายนิ้วมือที่แตกต่างกันอย่างมีนัยสำคัญสำหรับตัวอย่างน้ำมันหอมระเหยกฤษณาทั้ง 3 ตัวอย่าง

Abstract

Volatile components from the essential oils of *A. crassna* from different parts of Thailand were analyzed by gas chromatography-mass spectrometry (GC-MS), solid phase microextraction-gas chromatography-mass spectrometry (SPME-GC-MS) and comprehensive two-dimensional gas chromatography (GC×GC). 18 components was identified from the essential oil of *A. crassna* from Chiang Rai with the major components being hexadecanoate, guaia-1(10),11-dien-15-ol, karanone, cyclocolorenone and jinkoh-eremol. *A. crassna* oil from Chiang Mai yielded 28 identified compounds with the key components being hexadecanoate, kusunol, jinkoh-eremol, epoxybulnesene and β-agarofuran while 30 volatile compounds from *A. crassna* from Rayong were identified, with hexadecanoate, β-agarofuran, kusunol, dehydrojinkoh-eremol and 9,11-eremophiladien-8-one as the main constituents. 74 aroma-active components which included unidentified components were characterized by using the SPME-GC-MS technique. The major aroma components included β-agarofuran, 4-phenyl-2-butanone, furfural and benzaldehyde while the minor aroma notes were attributed to (*E*)-α-bergamotene, α-humulene, α-bulnesene, α-agarofuran, nor-ketoagarofuran, epoxybulnesene, agarospirol, jinkoh-eremol, kusunol, acorenone B, selina-3,11-dien-14-al and 9,11-eremophiladien-8-one were considered to be the important aroma impact compounds for the characteristic aroma of agarwood essential oils. The clear separation of the volatiles in all samples, demonstrated by the application of GC×GC, resulted in significantly different fingerprints for the three samples of agrawood essential oils.

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Agarwood is the resinous heartwood from *Aquilaria* trees, large evergreens native to Southeast Asia. The trees occasionally become infected with mold and begin to produce an aromatic resin in response to the fungus attack. The resin is commonly known as “jinko” in Japanese and as “aloeswood”, “agalloch” or “eaglewood” in English [1]. Agarwood and its essential oils are economically important natural products used for the production of incense, perfumes, and traditional medicines throughout Asia [2, 3]. Additionally, its essential oils had great cultural and religious significance in ancient civilizations around the world. The aroma of agarwood is a complex mixture of many volatile constituents which give it unique and elegant oriental aroma characters [4-6]. In recent decades, agarwood has usually been harvested from *A. malaccensis*; *A. agallocha* and *A. secundaria* are synonyms for *A. malaccensis* [7, 8]. Other agarwoods can also be collected from *A. crassna* and *A. sinensis* plants.

The volatile odor components of agarwoods and its essential oils have been investigated by many researchers. Meier et al. [8] analyzed the volatile constituents from *A. malaccensis* using gas chromatography-mass spectrometry (GC-MS). Agarospirol and jinkoh-eremol were identified as the major constituents with anisyl acetone as a minor component. Ishihara et al. [6] identified oxygenated guaiane, eudesmane derivatives and oxo-agarospirol as the major sesquiterpene components in Vietnamese agarwoods. Moreover, other components have also been reported as the constituents of agarwoods such as sesquiterpenes of eremophilane [9-11], prezizaane-type [9], 2(2-phenylethyl)chromone derivatives [12-14], diepoxy tetrahydrochromones, oxidoagarochromones etc [15]. In these previous reports most researchers focused on the study of the chemical composition of *A. malaccensis* species for which sesquiterpenes and chromone derivatives were found to be the major constituents. However, the volatile compounds and aromas of other agarwood species have not yet been investigated.

GC-MS has been a powerful tool for the identification and quantification of volatile constituents in essential oils. However, this technique cannot specifically identify those compounds which are odor-active; that is, which have a sensory perceptual impact. Gas chromatography-olfactometry (GC-O) is the appropriate method for the analysis of aroma-active components, distinguishing the aroma character of essential oils by combining chromatographic separation with human sensory detection [16]. In GC-O, a human assessor describes the aroma character and quality when an individual aroma is detected. It should be noted that compounds present in high concentrations often provide little of no aroma activity whereas components found at trace concentrations may have intense aroma activity [17].

Solid-phase microextraction (SPME), introduced by Pawliszyn et al. [18], provides a fast, efficient, solvent-free alternative extraction technique. The method establishes equilibrium among the sample matrix, the headspace above the sample and a polymer-coated fused fiber. The adsorbed volatiles are then desorbed from the fiber in an injection port of gas chromatograph for analysis. Due to its sensitivity, reproducibility and high concentration capability, SPME has been widely used for extracting the volatile components from plant material [19-22].

Comprehensive two-dimensional gas chromatography (GC×GC) is a relatively new but powerful technique successfully used for the separation of the volatile constituents in highly complex samples such as petroleum [23] environmental samples [24, 25], and essential oils [26, 27]. This multidimensional gas chromatography (GC) technique is characterized by the combination of two columns with different separation mechanisms coupled via a modulator interface. The cryogenic modulator provides zone compression of all individual components from the first column, focusing and re-injecting each collected zone into the second column. The chromatographic deconvolution of many co-eluting components in the first column is achieved in the second column. The technique offers greater sensitivity and resolution, as well as the inherent benefits of improved identification of the better-separated components. An early GC×GC application to essential oils reported by Marriott et al. [28] used two column sets, comprising phases BPX5/BPX50 and BPX5/BP20, respectively, to analyze the components of commercial vetiver oil. The results revealed the effectiveness of BP20 (polyethylene glycol) over the BPX50 (50% phenyl methyl polysilphenylene siloxane) phase in the second column for the separation of components having different polarities.

1.2 Scopes of study

In the present study, the chemical compositions of the essential oils of *A. crassna* obtained from different parts of Thailand were identified by using SPME-GC-MS, GC–MS and confirmed by the linear retention indices. The use of GC×GC with a longitudinally modulated cryogenic system (LMCS) was also employed to clearly differentiate between the fingerprints of these oil samples.

1.3 Expected output

International publication

CHAPTER 2

MATERIALS AND METHODS

2.1 Plant material and chemicals

Stem wood chips of *A. crassna* chips were collected in Rayong, Chiang Mai and Chiang Rai province, Thailand. The wood chips of all plants were dried for 10 days under the shade and then pulverized into a fine powder using a blender (AIM 5CF Double ribbon blender, CapPlus technologies, USA) before being subjected to simultaneous distillation and extraction (SDE).

2.2 Extraction of the Agarwood Oil

The extraction of agarwood oil was carried out in a modified Likens-Nickerson SDE apparatus for 48 h. Each blended wood sample (200 g) was put into a 2,000 ml round-bottom flask and 750 ml of distilled water added. Dichloromethane (150 ml) was added to a 250 ml round-bottom flask. Both flasks were then connected to the main SDE apparatus and additional dichloromethane and distilled water added into the central arm. The flask containing dichloromethane was heated using a water bath at 50 ºC and the flask containing wood and distilled water was heated using a paraffin oil bath at 200 ºC. Following extraction, the distillate in the 250 ml flask was dried over anhydrous sodium sulfate and concentrated using vacuum rotary evaporation and stored in headspace vials.

2.3 Solid Phase Microextraction (SPME)

The SPME sampling apparatus with a SPME fibre assembly holding a 1.0 cm fused-silica fiber was purchased from Supelco (Bellefonte, PA, USA). A 50/30 μm divinylbenzene-carboxen-polydimethylsiloxane (DVB-CAR-PDMS), 65 μm polydimethylsiloxane-divinylbenzene (PDMS-DVB) and 100 μm polydimethylsiloxane (PDMS) fibre were chosen to extract the odour volatiles of agarwood essential oil in this study. The fibres were mounted in the manual SPME holder and preconditioned for 10 min in a GC injection port set at 230 °C and cooled to room temperature before collecting the headspace volatiles in a vial. For each extraction, each oil sample (0.5 ml) was placed into a 10 ml headspace vial sealed with a silicone septum and a plastic cap. By insertion through the septum of the sample vial, the fibre was exposed to the sample headspace for 30 min prior to desorption of the volatiles into the splitless injection port of the GC-MS for 5 min.

2.4 Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS analysis was performed with an HP model 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an HP model 5975C mass-selective detector. The capillary column was HP-5 ms (30 m × 0.25mm i.d., 0.25 μm film thickness, Agilent Technologies, Palo Alto, CA, USA). The oven temperature was initially held at 40 °C hold for 4 min and then increased at a rate of 5 °C/min to a final temperature of 230 °C which was maintained for 5 min. The injector temperature was 230 °C. Purified helium was used as the carrier gas at a flow rate of 1 ml/min. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 30-550. The electron multiplier voltage was 1059 V. The ion source and analyzer temperatures were set at 230 °C and 200 °C, respectively. Identification of volatile components was performed by comparison of their retention indices, relative to C_6-C_{19} *n*-alkane mixture (ASTM D2887, Supelco, Bellefonte, PA, USA) and comparison of the mass spectra of individual components with the reference mass spectra in the W8N05ST databases. Results are presented in terms of percent relative peak areas as no external or internal standards were used in this work.

2.5 Comprehensive two-dimensional gas chromatography (GC×GC)

A gas chromatograph, model HP 6890, equipped with an FID detector and an HP 6890 series auto sampler was used for the GC×GC–FID experiments and was operated at 100 Hz data acquisition. The GC was retrofitted with a longitudinally modulated cryogenic system, LMCS (Chromatography Concepts, Doncaster, Australia). CO_2 was employed as the cryogen, which was thermostatically controlled at about –20 °C for the duration of each run. The injection temperature was 250 °C with an injection volume of

1.0 μl in the split mode with a split ratio of 100:1. The injection and detector temperature were operated at 250 °C. Hydrogen gas was used as the carrier gas at a flow rate of 1.5 ml min-1. The GC was operated in the constant flow mode. The column set for GC×GC analysis consisted of two capillary columns which were serially coupled by a zero-dead-volume fitting. The columns are available from SGE International (Ringwood, Australia). The GC×GC column set BPX5/BP20 was 5 % phenyl polysilphenylene-siloxane connected to a polyethylene glycol phase, which separates most components according to boiling point rather than polarity.

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CHAPTER 3
RESULTS AND DISCUSSION

Essential oils of *A. crassna* obtained from Chiang Rai, Chiang Mai and Rayong province extracted using a modified Likens-Nickerson apparatus appeared as yellow viscous liquids with percentage yields of 0.34, 0.61 and 0.81 (w/w), respectively. These essential oils were subjected to detailed GC-MS analysis in order to identify the volatile constituents. Overall, 31 volatile constituents were identified from the three agarwood oil samples. The volatile components identified by GC-MS with their relative area percentages and retention indices are summarized in Table 1. The different ecology and planting conditions presented significant variability in their essential oil compositions although similar terpene components and derivatives were found among the three samples. A total of 18 constituents representing 88.19% of the total peak area were identified in the essential oil of *A. crassna* planted in Chiang Rai province. The major components were hexadecanoate (55.65%), guaia-1(10),11-dien-15-ol (6.53%), karanone (4.90%), cyclocolorenone (4.71%) and jinkoh-eremol (4.22%). *A. crassna* oil from Chiang Mai yielded 28 identified constituents representing 84.11% of the total peak area with the major components hexadecanoate (37.96%) followed by kusunol (6.40%), jinkoh-eremol (5.64%), epoxybulnesene (4.90%) and β-agarofuran (3.85%), respectively. Thirty volatiles of *A. crassna* from Rayong province representing 84.65% of the total peak area were identified. The major components were hexadecanoate (13.38%), β-agarofuran (10.34%), kusunol (8.20%), dehydrojinkoh-eremol (7.34%) and 9,11-eremophiladien-8-one (6.29%), respectively. Our results are different from most published studies. Agarospirol was found to be the predominant constituent in the *A. malaccensis* essential oil as was reported by Meier et al. [8]. In other studies, guaia-1(10),11-dien-oic acid and 2-(2-(4-methyoxyphenyl)ethyl)chromone were represented as the key constituents in the smoke and acetone extracts of *A. malaccensis* as reported by Ishihara et al. [5, 6]. Dihydrokaranone and *rel*-(1R,2R)-9-(isopropyl-2-methyl-8-oxatricyclo[7.2.1.01,6]dodeca-4,6-diene[10] were also found as major components in *A.*

malaccensis essential oil. The quantitation of the chemical compositions of agarwood essential oils may be correlated with different environmental, ecological conditions and genetic factors.

The GC-MS chromatograms of *A. crassna* essential oil from Rayong using the three different SPME fibres are shown in Fig. 1. The PDMS fibre showed a poor efficiency in extracting the light volatiles, but it showed better efficiency for heavy volatiles than the efficiency of the other two fibres. The affinity of SPME fibres for extracting volatiles is based on the "like dissolve like" concept and the thickness of the selected fibres. Basically, non-polar fibres are expected to be effective in extracting non-polar compounds, whereas polar fibres are suitable for polar component extraction. As the Fig. 1 indicates, differences among the volatiles profiles using the three different fibres under the same conditions were demonstrated. The quantity of individual compounds detected can differ significantly depending upon the response factor of each SPME fibre. It is clear that DVB-CAR-PDMS extracted the highest numbers of volatile components compared to the PDMS-DVB and PDMS fibres under the same conditions. Although the PDMS-DVB fibre also has an intermediary polarity, the efficiency of the extraction of *A. crassna* volatiles decreased slightly as seen by the lower number of volatiles depicted in Fig. 1. Therefore the DVB-CAR-PDMS was chosen for extracting the volatile constituents of *A. crassna* essential oils obtained from Chiang Rai and Chiang Mai, respectively. Notable differences in the volatile components among the three different oil samples using the DVB-CAR-PDMS fibre were demonstrated. The identified volatiles and their relative peak area percentages of the SPME extracts among these oils are listed in Table 2. The relative (%) amount of individual compounds can differ significantly depending on the environmental, ecological conditions and genetic factors of each sample. A total of 74 volatiles were identified among the three SPME extracts of agarwood oils. Thirty-two volatile constituents were identified in the SPME extract of *A. crassna* essential oil from Chiang Rai; the majority of the constituents, representing 85.92% of the relative peak area, were comprised of the dominant components β-agarofuran (32.79%), hexanal (9.77%), 4-phenyl-2-butanone (9.53%), heptanol (4.87%) and benzaldehyde (3.38%). Forty-one constituents from the SPME extract of *A. crassna* essential oil from Chiang Mai, representing 87.91% of the relative peak area, were identified. The principal volatiles were found to be β-agarofuran (25.60%), 4-phenyl-2-butanone (18.45%), camphor (12.88%), furfural (5.48%) and menthol (4.76%). For the SPME extract of *A. crassna* essential oil from Rayong, 62 components (84.19%) were identified with the major components being β-agarofuran (41.12%), benzaldehyde (8.35%), 4-phenyl-2-butanone (5.38%), furfural (5.12%) and 2-ethyl hexanol (2.08%). As can be seen, components with high boiling points were detected by GC-MS in agrwood essential oils whereas the SPME extracts of agrwood essential oils contained components with low boiling points. This finding was in good agreement with the results obtained from Meier et al. [8], Ishihara et al. [5, 6] and Näf et al. [4] that found terpenoids as the major components of agarwood essential oil. Loss of highly volatile compounds in the GC-MS analysis might have resulted from their rapid evaporation during the sample preparation process and their extremely low concentrations. SPME extracts of agarwood oils were richer in highly volatiles components with lower boiling points; SPME is more efficient for the extraction of light terpenes.

All the volatile components from the SPME extracts displayed aroma activities which were generally of low to high intensity and any single constituent contributed little to the total aroma among these oils. Additionally, their aroma properties were similar to the overall aroma of the agarwood essential oils according to the combination and relative balance of a same group of aroma-active compounds which produced woody, nutty and burnt aroma. Aroma character of all volatiles is also listed in Table 2. Beta-agarofuran, exhibiting woody and nutty notes, was the most intense aroma-active component in all SPME extracts of agarwood essential oils. Woody, nutty and burnt notes could also be correlated with (*E*)-α-bergamotene, α-humulene, α-bulnesene, α-agarofuran, nor-ketoagarofuran, epoxybulnesene, agarospirol, jinkoh-eremol, kusunol, acorenone B, selina-3,11-dien-14-al and 9,11-eremophiladien-8-one although some components were detected with low concentration. In addition, major components including 4-phenyl-2-butanone, furfural and benzaldehyde were considered to be the important contributors to the overall aroma of agarwood oils as indicated by their high peak areas. 4-phenyl-2-butanone is likely responsible for the key notes of floral, jasmine, herbal, and fruity with balsam aroma whereas furfural and benzaldehyde were responsible for almond, fruity, powdery and nutty notes. The intense sweet and floral notes that were detected in the SPME extract of *A. crassna* essential oil from Rayong province can be correlated with xylene, 1,3,5-cycloheptatriene, cinnamol, (3*E*)-3-hepten-2-one, butyl butanoate, 4-hydroxyacetophenone, 2-ethyl hexanol and decanol. The strong fruity note also found in the SPME extract of *A. crassna* essential oil from Rayong can be correlated with hexanol, allyl butanoate, 2-heptanone, 2-heptanol, methyl hexanoate, 5-methyl-3-heptanone, (4*Z*)-heptenol and 2-undecanone while the concentrated camphoraceous note found in the SPME extracts of *A. crassna* essential oils from Chiang Rai and Chiang Mai correlates with 3-methyl cyclohexanone, camphor, (*Z*)-3-pinanone, *p*-cresol and isobornyl formate. Heptanal and *p*-vinylguaiacol were responsible for weak rancid notes in the SPME extracts of all oil samples. It is noted that *A. crassna* essential oil from Rayong province may contain sweet, floral and fruity aromas stronger than found in essential oils from Chaing Rai and Chiang Mai province which are strongly camphoraceous.

In general, GC×GC systems consist of non-polar and polar phase columns in first and second dimensions, respectively. This arrangement is commonly applied for the separation of the components in *A. crassna* oils. In this study, the conventional combination (BPX5/BP20) was employed. The resulting GC×GC-FID contour plots obtained by the three *A. crassna* oils obtained from Chiang Rai, Chiang Mai and Rayong province are shown in Fig. 2. The component separation in the column set was based on boiling point and polarity in first and second columns, respectively. As seen in the contour plots in Fig. 2, at least 55, 68 and 93 individual components of *A. crassna* oils obtained from Chiang Rai, Chiang Mai and Rayong, respectively, were resolved. This indicates that a better resolution was achieved by the use of column set, in which many overlapping peaks were resolved in the 2nd dimension, allowing additional volatile components to be detected. The differentiations of the chemical composition among three samples by the column set are present as contour plots shown in Fig. 2. Nevertheless, using GC×GC, more compounds were found and separated compared to those obtained by GC-MS. Grouping of the various components are highlighted in the circled areas: A includes the monoterpenes, B includes the sesquiterpenes, and C includes the oxygenated sesquiterpenes. Although similar fingerprint patterns were exhibited in all essential oil profiles, the number of oxygenated monoterpenes (region A) of *A. crassna* essential oil from Rayong was found to be significantly higher compared to that of the *A. crassna* essential oil profile from Chiang Mai and Chiang Rai, respectively. The similar profiles of volatile sesquiterpenes (region B) in all essential oils are shown, while numbers of oxygenated sesquiterpenes (region C) of *A. crassna* essential oil from Rayong province were higher than that obtained from the essential oil of *A. crassna* from Chiang Mai and Chiang Rai, respectively.

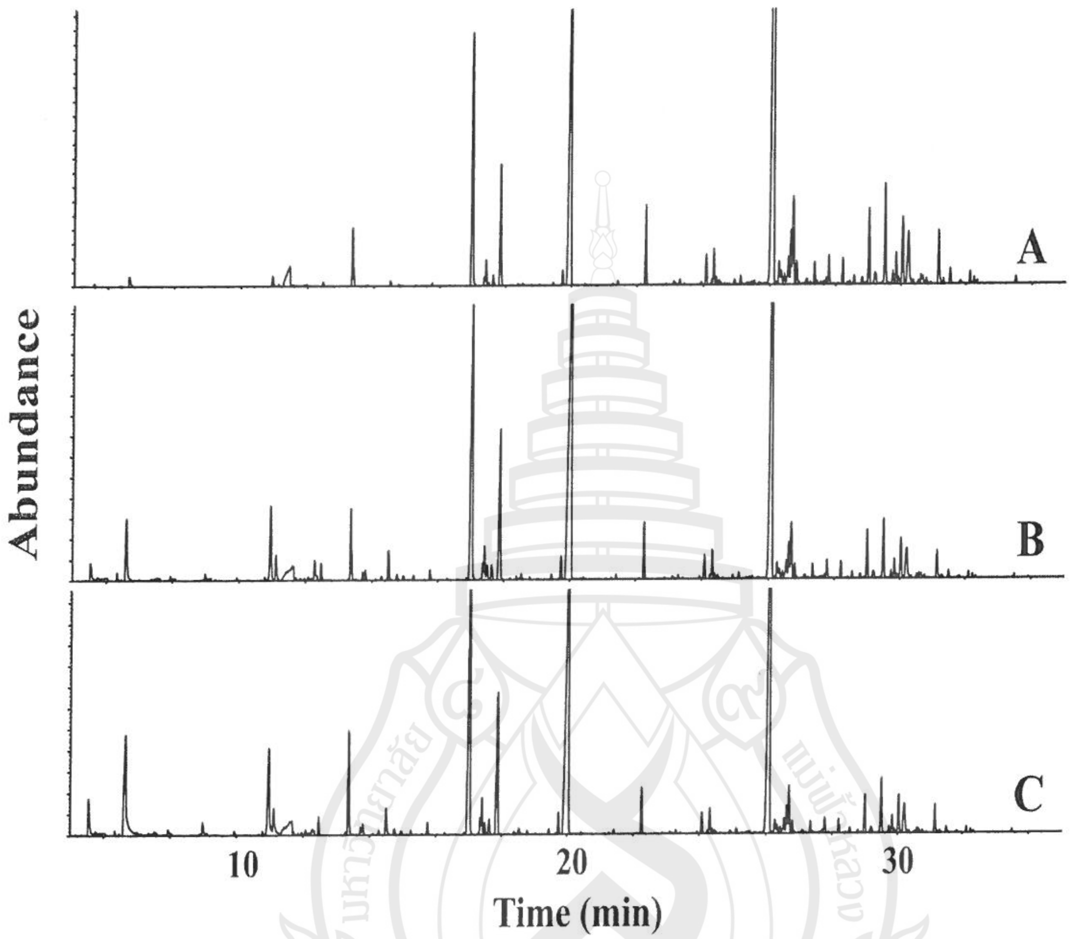

Fig. 1. SPME-GC-MS chromatograms of *A. crassna* essential oil from Rayong Province by PDMS (A), PDMS-DVB (B) and DVB-CAR-PDMS fibers (C)

Table 1. Volatile constituents and their relative peak area percentage obtained from *A. crassna* essential oils obtained from Chiang Rai, Chiang Mai and Rayong province.

Components	I^a	**Relative peak area (%)**		
		CRI	**CMI**	**RYG**
β-agarofuran	1476	0.36	3.85	10.34
p-methoxybenzylacetone	1504	0.03	0.19	0.47
α-agarofuran	1540	-	-	0.20
nor-ketoagarofuran	1602	-	0.70	1.54
epoxybulnesene	1624	1.28	4.90	3.04
γ-eudesmol	1679	0.17	0.88	4.22
agarospirol	1630	0.61	2.22	5.77
4-(-hydroxy-3-methoxyphenyl)-2-butanone	1665	-	0.37	0.45
jinkoh-eremol	1672	4.22	5.64	4.47
kusunol	1678	3.87	6.40	8.20
valerianol	1679	-	-	0.33
dehydrojinkoh-eremol	1680	0.51	2.29	7.34
selina-3,11-dien-9-one	1683	0.08	0.37	1.12
acorenone B	1715	-	-	1.09
rotundone	1719	-	0.44	1.22
3-thujopsanone	1725	-	1.19	-
selina-3,11-dien-9-ol	1731	0.32	0.23	2.85
(*E*)-nerolidol acetate	1738	-	0.39	0.11
selina-3,11-dien-14-al	1746	-	0.68	0.08
9,11-eremophiladien-8-one	1751	0.13	0.43	6.29
selina-3,11-dien-14-ol	1760	-	0.31	0.22
cyclocolorenone	1763	4.71	3.38	3.43
selina-4,11-dien-14-al	1766	-	0.12	0.28
methyl tridecanoate	1771	0.45	0.29	0.79
β-eudesmol acetate	1776	-	0.84	2.24
epi-α-bisabolol acetate	1779	2.52	1.39	0.45
guaia-1(10),11-dien-15-ol	1781	6.53	3.25	1.93
karanone	1822	4.90	3.57	1.54
oxo-agarospirol	1830	-	0.26	1.18
hexadecanoate	1842	55.65	37.96	13.38
(*Z*)-9-octadecanoic acid	1856	1.85	1.60	0.08

[a] Kovats indices using a HP-5MS column, CRI; Chiang RAi, CMI; Chiang Mai, RYG; Rayong

Table 2. Aroma-active components, relative peak area percentage of SPME extracts obtained from *A. crassna* essential oils from Chiang Rai, Chiang Mai and Rayong province.

Components	I^a	**Odor description**	**Relative peak area (%)**		
			CRI	**CMI**	**RYG**
dimethyl sulfide	727	sulfurous, vegetable	-	-	0.67
2-hexanol	738	green, bitter, almond, mushroom	-	-	0.13
1,3,5-cycloheptatriene	749	sweet	-	-	0.30
pentanol	777	green, fruity	-	-	0.13
hexanal	786	green, grassy	9.77	1.36	-
furfural	838	almond-like	1.05	5.48	5.12
o-xylene	854	sweet	-	-	0.12
m-xylene	862	sweet	-	-	0.16
hexanol	873	fruity, green, sweet, herbal, mild-woody	-	-	1.49
allyl butanoate	877	fruity, green, pineapple, sweet, waxy	-	-	0.43
cinnamol	890	sweet, balsamic, floral	-	-	1.69
2-heptanone	892	banana, cinnamon, spicy, fruity	-	-	0.44
heptanal	898	fruity, fat, citrus, rancid	3.11	0.45	0.16
2-heptanol	905	fruity, herbaceous, sweet, oily	-	-	0.44
methyl hexanoate	923	fruity, fresh, sweet	-	-	1.02
hexyl formate	927	green, fruity	-	0.11	0.57
(*3E*)-3-hepten-2-one	934	sweet, fruity, cheesy, green, woody	-	-	0.40
5-methyl-3-heptanone	935	mild, fruity	-	-	0.03
3-methyl cyclohexanone	945	camphoreous	0.62	0.12	-
benzaldehyde	956	almond, fruity, powdery, nutty	3.38	3.39	8.35
5-methyl furfural	958	sweet, caramel, spice, coffee, bitter almond	-	1.22	0.71
heptanol	963	green, fruity	4.87	-	0.27
(*4Z*)-heptenol	964	green, grassy, fruity	-	-	0.35
3-octanone	974	musty, mushroom, green, vegetative	-	-	0.22
2-octanone	977	musty, cheese-like	0.87	0.10	0.20
2-amyl furan	984	fruity, green, vegetable	0.96	0.17	0.72

Table 2 (continued)

Components	I^a	Odor description	Relative peak area (%)		
			CRI	**CMI**	**RYG**
butyl butanoate	988	sweet, fruity, fresh	-	-	0.37
hexyl acetate	996	fruity, herbaceous	-	0.42	0.72
limonene	1006	fresh, citrus, orange-like	0.59	0.08	-
1,8-cineole	1010	mint, sweet	0.31	2.92	-
2-ethyl hexanol	1024	sweet, floral (rose-like)	-	-	2.08
salicylaldehyde	1037	medicinal, spicy, cinnamon like	0.25	0.23	0.30
4-hydroxybenzaldehyde	1048	sweet, nutty, almond, woody	0.14	-	-
2-butyl thiophene	1057	fruity floral milky	0.09	0.04	0.19
acetophenone	1059	sweet, orange, coumarinic	0.37	0.89	0.81
methyl cyclohexane carboxylate	1061	fruity	0.14	-	-
octanol	1063	citrus, waxy, green, aldehydic	-	0.18	0.57
p-cresol	1066	camphoraceous, minty, powdery, nutty	-	0.13	0.43
o-guaiacol	1080	smoky, spicy, medicinal, woody	-	0.12	1.36
2-nonanone	1084	fruity, sweet, waxy, green, herbaceous	-	-	0.73
nonanal	1097	sweet, melon	1.36	0.31	0.31
isophorone	1102	woody, sweet, green, camphoreous, fruity	-	0.06	-
methyl octanoate	1118	waxy, green, sweet, orange, aldehydic	-	-	0.38
octyl formate	1122	orange, fruity, rose	-	-	0.15
(*Z*)-*p*-mentha-2,8-dien-1-ol	1125	fresh, mint	-	-	0.41
camphor	1128	camphoraceous	0.38	12.88	-
isoborneol	1146	camphoraceous, sweet, musty	-	0.41	-
borneol	1147	pine, woody, camphor	-	0.36	-
4-hydroxyacetophenone	1154	sweet, floral	-	-	0.37
(*Z*)-3-pinanone	1158	cedar, camphoreous	-	-	0.21
menthol	1159	peppermint, cool, woody	0.28	4.76	
decanal	1194	sweet, aldehydic, orange, waxy, citrus	0.41	0.07	0.19

Table 2 (continued)

Components	I^a	**Odor description**	**Relative peak area (%)**		
			CRI	**CMI**	**RYG**
isobornyl formate	1221	medicinal, camphoreous, minty, woody	-	0.11	
4-phenyl-2-butanone	1240	floral, jasmine, herbal, fruity, balsam	9.53	18.45	5.38
decanol	1261	floral, sweet, orange	-	-	0.20
p-vinylguaiacol	1281	sweaty, cheese, rancid	-	-	0.11
2-undecanone	1293	waxy, fruity, fatty, pineapple	-	-	0.08
methyl geranate	1321	floral, fruity, woody, camphor	1.15	-	0.17
β-elemene	1398	herbal, waxy, fresh	-	0.05	0.10
(*E*)-α-bergamotene	1427	woody, warm	-	-	0.07
α-guaiene	1441	sweet, woody, balsam, peppery	-	0.13	0.21
α-humulene	1456	woody	-		0.20
β-agarofuran	1476	woody, nutty	32.79	25.60	41.12
α-bulnesene	1521	woody, warm	2.13	0.38	0.30
α-agarofuran	1540	woody, nutty	2.85	0.42	0.41
nor-ketoagarofuran	1602	woody, burnt	0.34	0.98	0.90
epoxybulnesene	1624	woody, warm, nutty	1.55	1.52	0.35
γ-eudesmol	1679	waxy, sweet	0.14	0.22	0.05
agarospirol	1630	spicy, peppery, woody	0.52	0.47	0.09
jinkoh-eremol	1672	woody, burnt	2.56	1.08	0.14
kusunol	1678	woody, burnt	3.11	1.23	0.26
acorenone B	1715	warm, spicy, woody	0.22	0.82	0.17
selina-3,11-dien-14-al	1746	woody	-	0.13	0.06
9,11-eremophiladien-8-one	1751	smoke, woody	0.09	0.08	0.11

[a] Kovats indices using a HP-5MS column, CRI; Chiang RAi, CMI; Chiang Mai, RYG; Rayong

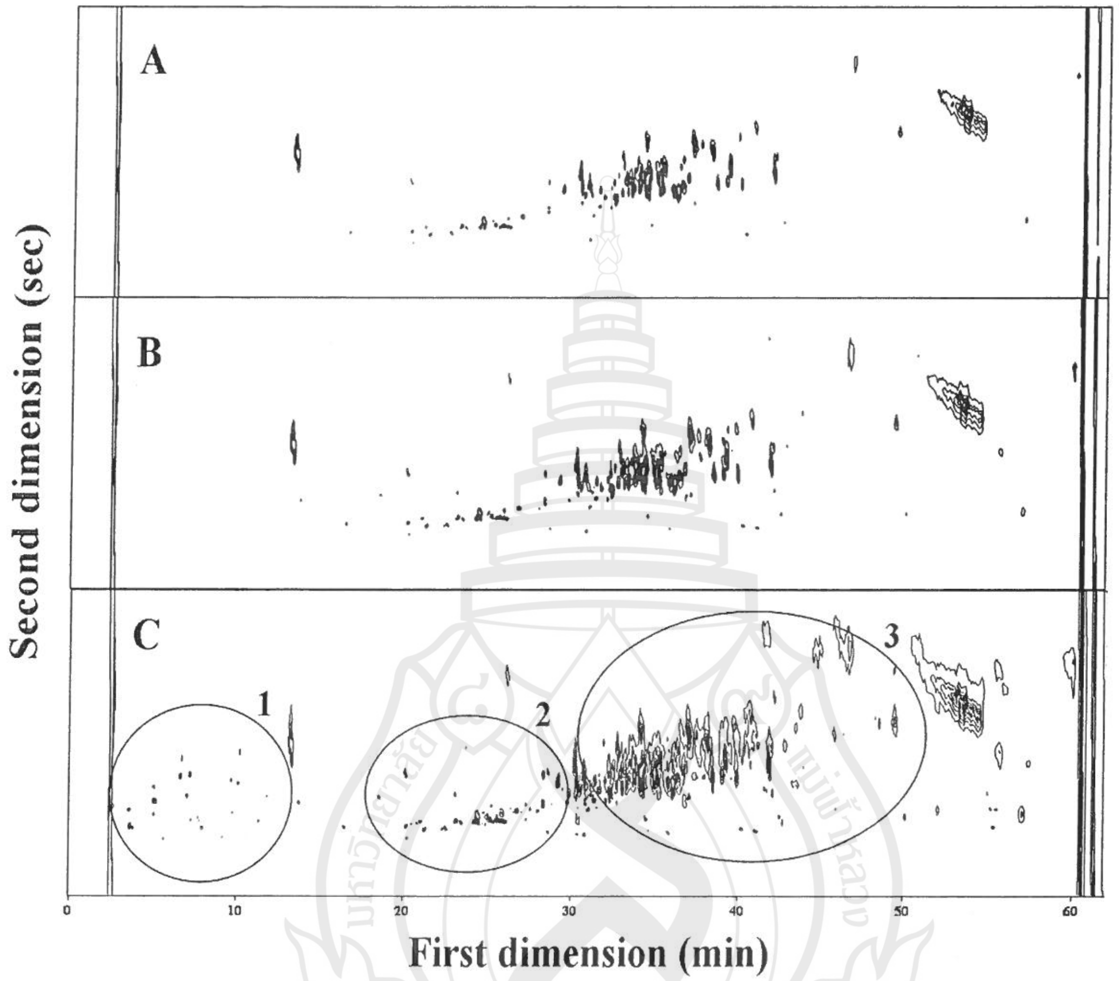

Fig 2. The contour plots of the volatile component profiles of: A) *A. crassna* essential oil from Chiang Rai, 2) *A. crassna* essential oil from Chiang Mai and 3) *A. crassna* essential oil Rayong. The components are grouped into 3 groups: monoterpenes (A), sesquiterpenes (B) and oxygenated sesquiterpenes (C).

CHAPTER 4

CONCLUSION

GC-MS was successfully utilized for the identification of components presented in high concentrations but SPME-GC-MS was a crucial tool for investigating key aroma-impact components in the essential oils. In this study, key aroma-active components of the essential oils of *A. crassna* from different parts of Thailand represent various terpenic constituents. Beta-agarofuran was found to be the most important aroma contributor to the three agarwood oil samples based on its high aroma property and concentration. 4-phenyl-2-butanone, furfural and benzaldehyde were also important to the overall aroma of all agarwood oils which are responsible for floral, jasmine, herbal, fruity and almond aromas. It can be concluded that the essential oil of *A. crassna* from Rayong province has sweet, floral and fruity aromas stronger than those of *A. crassna* essential oils from Chiang Rai and Chiang Mai, respectively, due to its high levels. Although the chemical compositions of all essential oils of *A. crassna* were similar, both oil samples had significant differences in their major constituents, as determined by GC–MS. In addition, GC×GC separation was utilized to monitor the profiles of both samples and good resolution was exhibited using a combination of non-polar and polar columns. Thus, this technique could be very useful for quality control during the industrial production of these essential oils.

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Curriculum Vitae

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