CHEMOMETRIC STUDY OF SELECTED AGARWOOD OILS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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NOR AZAH MA, ISMAIL N, MAILINA J, TAIB MN, RAHIMAN MHF & MUHD HAFIZI Z. 2014. Chemometric study of selected agarwood oils by gas chromatography–mass spectrometry. Agarwood oils are concentrated volatile aromatic compounds mainly produced by the distillation of agarwood (*Aquilaria* spp.). Currently, grading of agarwood oil is done by trained personnel based on colour and odour. This technique is done manually and limited to sample repeatability. In this study, the chemical compositions of several selected agarwood oil samples were analysed by gas chromatography and gas chromatography–mass spectrometry. Their pattern recognition profiles were examined by chemometric analysis using principal component analysis (PCA). The samples were categorised into two groups according to their quality: group A (high quality) and group B (unknown quality). At least 43 chemical compounds were identified from both groups. From PCA, six significant compounds were obtained, i.e. 4-phenyl-2-butanone, α-guaiene, ar-curcumene, 10-epi-γ-eudesmol, β-dihydroagarofuran and valencene. Correlation analysis revealed significant and high correlation between groups A and B. This is due to samples from group B showing similarity to major compounds found in group A.

Keywords: Chemical compounds, correlation, high quality, gaharu oil, principal component analysis

INTRODUCTION

Aquilaria belongs to the family Thymelaeaceae. The resinous part of *Aquilaria* spp. is known as gaharu, agarwood, eaglewood, oudh, oud, kanankoh, kyara, jinkoh and kalambak. There are 19 species of *Aquilaria* found in the Asean region (Naef 2011), while the Malaysian gaharu comes from *A. malaccensis, A. beccariana, A. hirta* and *A. microcarpa*. Agarwood has many uses—from tree trunks, branches, chips, flakes of uniform quality powder to essential oils (Wetwitayaklung et al. 2009). Many researchers have highlighted that agarwood oil is beneficial as incense, perfumery and for traditional medicines (Barden et al. 2000, Wetwitayaklung et al. 2009, Naef 2011, Pripdeevech et al. 2011). Agarwood oils are traded worldwide especially in United Arab Emirates, Saudi Arabia, China and Japan. They are traded differently depending on their quality. Usually the grading of agarwood oil is done based on its physical appearance such as colour and odour. High grade oil has dark colour, strong odour and high fixative properties, thus making the aroma long lasting. The supreme oil may cost from RM200 to RM2000 per tola (12 mL) (Lim et al. 2010).

Agarwood oils are mainly made up of a complex mixture of sesquiterpene hydrocarbons, oxygenated sesquiterpenes and their chromone derivatives (Ishihara et al. 1991, Tamuli et al. 2005, Nor Azah et al. 2008, Naef 2011). The compounds identified from agarwood include α-agarofuran, β-agarofuran, 10-epi-γ-eudesmol, agarospirol, jinkohol, jinkohol II and valerianol. Agarwood oils may come in various colours ranging from yellowish to deep brownish and it is difficult to differentiate them using colour (Nor Azah et al. 2009). Thus, it is necessary to investigate the compounds of oils via gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). The technique is a combination of GC to separate the mixture of components and mass spectroscopy to characterise every compound (Tajuddin et al. 2011).

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Chemometrics is an analysis of getting information from multivariate chemical data by applying statistical or mathematical techniques (Pérez et al. 1998). Several techniques may be employed in chemometric study such as Z-score, artificial neural network (ANN), boxplot and k-nearest neighbour (Nor Azah et al. 2013, Nurlaila et al. 2013a, b). However, among them, principal component analysis (PCA) is one of the famous methods (Pérez et al. 1998). PCA is a quantitatively rigorous method to reduce data from a set of data with many variables to a new set of variables called principal components (Tabachnick et al. 2001). Each principal component is a linear combination of the original variables and orthogonal to one another. PCA was used as a pattern recognition method to classify the essential mint oils from different geographic origin (Marengo et al. 1991). This method was found successful in giving good description of spill samples in oil weathering assessment (Malmquist et al. 2007). Besides, PCA was applied to select significant data coming from the electronic nose sensor for classification system of agarwood and agarwood oil (Hidayat et al. 2010, Muhammad Sharfi et al. 2010). This shows that PCA is one of the preferred tools in data analysis for essential oil study.

Little research has been carried out to characterise the quality of agarwood oils in Malaysia. PCA demonstrated five significant components explaining a total of 40 to 99% of variance in agarwood oils (Nor Azah et al. 2012). In addition, agarwood quality differentiation via HS–SPME–GCMS analyses, Z-score and ANN techniques, which are related to chemometrics have been published (Nurlaila et al. 2013a, b). Therefore, the proposed PCA in this study would bring significant impact on the economical exploitation of agarwood oil and its products. This study was further carried out to identify the significant compounds from similar samples in earlier works and the correlation between the different grades of oil.

MATERIALS AND METHODS

Plant materials

Agarwood oil samples were obtained from reliable agarwood suppliers (Fragrance Asia Sdn Bhd, Jannatul Maqwa Sdn Bhd, HWP Aquilaria Sdn Bhd, Infobiotech Sdn Bhd and Sarina Agarwood Sdn Bhd). The oils were obtained by hydrodistillation. One agarwood oil sample (LA) was obtained from Laos (courtesy of MA Rasadah, Forest Research Institute Malaysia). Samples were labelled as TG, MA1, LA, KB, JBD, MA2 and MA. Samples TG, MA1, JBD and MA2 were graded as high quality oils (group A). The other three agarwood oils, namely, LA, KB and MA were of unknown quality and categorised under group B. The correlation between them was analysed.

Chemical analysis

The GC analysis was carried out using gas chromatograph equipped with flame ionisation detector and fused silica capillary column (25 m × 0.25 mm; 0.25 µm film thickness). The carrier gas was helium. The temperatures for injector and detector were set at 220 and 280 °C respectively. The oven temperature was programmed from 60 (10 min) to 230 °C at 3 °C min^{-1} and finally held at 230 °C for 10 min. The volume of oil injected was 1 µL. The retention indices of the components were determined relative to the retention times of a series of n-alkanes.

The apparatus for GC–MS analysis was programmed initially at 60 °C for 10 min, then 230 °C for 10 min at 3 °C min^{-1}. Helium was used as carrier gas at a flow rate of 1 mL min^{-1} and the ion-source temperature was programmed at 280 °C. The chemical compounds were identified by matching them to the mass spectral library (HPCH2205.L; Wiley7Nist05.L; NIST05a.L). The results of the peak areas were expressed as peak area counts or relative percentage.

Chemometric analysis

PCA is used in this study in order to reduce all chemical compounds identified to a significant number of compounds (Malmquist et al. 2007, Mustafa et al. 2010). The method is more economical rather than using all the compounds for analysis. Pearson correlation (also known as Spearman correlation) was used to study the correlation between the two groups (groups A and B). Pearson correlation was used to explore the strength of relationship between two variables (Pallant 2011). The following guidelines recommended by Pallant (2011) were used to determine the strength of the relationship between two variables, i.e. groups A and B.

Small: r = 0.10 to 0.29
Medium: r = 0.30 to 0.49
Large: r = 0.50 to 1.00

where r = correlation coefficient. Using SPSS software analysis, the correlation coefficient r as well as PCA was automatically computed.

RESULTS AND DISCUSSION

At least 43 chemical compounds were identified in agarwood oil samples from both groups (Table 1). The chemical composition of the agarwood oils was mainly made up of sesquiterpenes and its oxygenated derivatives. The major components detected were aromadendrene (25.9%), 10-epi-γ-eudesmol (9.7%) and agarospirol (9.3%) found in TG sample; 10-epi-γ-eudesmol (10.9%) and γ-eudesmol (8.2%) in MA1 sample; agarospirol (24.2%), aromadendrene (9.8%) and 10-epi-γ-eudesmol (7.6%) in LA sample; γ-eudesmol (30.4%) and 10-epi-γ-eudesmol (7.1%) in KB sample; γ-eudesmol (14.8%) and 10-epi-γ-eudesmol (8.1%) in JBD sample; γ-eudesmol (12.9%) and 10-epi-γ-eudesmol (8.3%) in MA2 sample as well as 10-epi-γ-eudesmol (20.6%) in MA sample. Two of the chemical compounds, namely, γ-eudesmol and 10-epi-γ-eudesmol were predominantly present in four samples (i.e. MA1, KB, JBD and MA2). The chemical composition or abundance (%) patterns of chemical compounds extracted by GC–MS are shown Figure 1. Six extreme peaks with relative percentage composition above 15% were observed. The peaks belonged to compounds no. 10, 25, 26, 27, 31 and 32 which corresponded to aromadendrene in TG oil (25.9%), 10-epi-γ-eudesmol in MA oil (20.6%), γ-eudesmol in KB oil (30.4%), agarospirol in LA oil (24.2%), α-eudesmol in TG oil (17.3%) and valerianol in TG oil (19.4%). These chemical compounds signified them as dominant in the agarwood oils in this study.

Nevertheless, all the compounds found in the oils were grouped automatically corresponding to six principal components using PCA. The grouping was done according to their characteristics and similarities. To be specific, each component was represented by a certain chemical compound. In this study, the six components were labelled as components 1, 2, 3, 4, 5 and 6 and they were represented by 4-phenyl-2-butanone, α-guaiene, ar-curcumene, 10-epi-γ-eudesmol, β-dihydroagarofuran and valencene respectively (Table 2). The scree plot of the PCA (Figure 2) explained 13.84, 10.38, 8.83, 4.95, 3.74 and 2.62% of the variance for components 1, 2, 3, 4, 5 and 6 respectively. The observation of variance and its component as shown by the scree plot in this study is a norm as practised by other researchers (Malmquist et al. 2007, Mustafa et al. 2010).

The results from the correlation analysis showed that there was significant and high correlation (r = 0.987) between both grades (Table 3). This was due to the presence of similar major chemical compounds between groups A and B. These referred to 4-phenyl-2-butanone (0.7% in LA, 2.7% in KB and 1.2% in MA), α-guaiene (0.3% in LA and 0.6% in KB), 10-epi-γ-eudesmol (7.6% in LA, 7.1% in KB and 20.6% in MA) and β-dihydroagarofuran (0.5% in LA, 1.3% in KB and 0.04% in MA) as shown in Table 2. Since most of the significant compounds found in group B oil were also found in group A oil and they belonged to similar sesquiterpene compounds, it was clear that the Pearson correlation was reliable and could be used to predict the strong relationship between both groups of oils.

CONCLUSIONS

GC–MS together with chemometric approach could be used to identify the chemical composition of agarwood oil of all the chemical components. 4-Phenyl-2-butanone, α-guaiene, 10-epi-γ-eudesmol, γ-eudesmol, β-dihydroagarofuran and valencene were commonly found. PCA method was reliable and useful in generating significant compounds towards classification of high quality agarwood oils.

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Table 1 Relative percentage composition of agarwood oils extracted by GC and GC–MS

No.	Compound	RI	Relative percentage composition						
			TG	MA1	LA	KB	JBD	MA2	MA
1	2-Phenylpropanal	1102	–	–	0.7	–	–	–	–
2	Benzaldehyde	1109	1.7	1.7	0.7	–	–	–	–
3	Benzeneacetonitrile	1134	1.4	1.7	–	–	–	–	–
4	4-Phenyl-2-butanone	1241	–	–	0.7	2.7	0.8	1.3	1.2
5	α-Funebrene	1402	–	0.3	–	–	–	–	–
6	α-Gurjunene	1403	–	–	–	–	–	2.8	1.2
7	β-Copaene	1430	–	0.9	–	–	–	–	–
8	α-Guaiene	1433	–	0.4	0.3	0.6	0.1	0.8	–
9	γ-Elemene	1434	–	–	1.0	–	–	–	–
10	Aromadendrene	1443	25.9	1.3	9.8	–	–	0.9	–
11	Valencene	1469	–	1.4	–	–	–	–	–
12	γ-Gurjunene	1472	–	0.4	–	–	0.2	-	0.4
13	β-Agarofuran	1474	4.3	1.7	2.0	1.0	1.0	1.4	5.0
14	ar-Curcumene	1479	–	0.6	–	0.6	1.2	0.8	0.6
15	α-Muurolene	1496	–	–	–	–	1.2	2.4	–
16	γ-Guaiene	1499	–	–	0.7	–	–	–	–
17	β-Dihydroagarofuran	1502	–	2.7	0.5	1.3	1.1	2.9	0.04
18	α-Agarofuran	1547	3.5	1.7	1.4	1.5	1.8	1.5	2.7
19	Elemol	1550	–	–	0.5	1.8	–	–	1.0
20	Dodecanoic acid	1553	–	–	0.5	–	–	–	–
21	γ-Vetivenene	1553	–	–	0.4	0.9	–	–	–
22	β-Vetivenene	1554	–	–	0.4	–	–	–	–
23	Spathulenol	1577	–	–	0.4	–	–	1.6	–
24	β-Gurjunene	1600	–	–	1.1	–	–	0.5	–
25	10-Epi-γ-eudesmol	1621	9.7	10.9	7.6	7.1	8.1	8.3	20.6
26	γ-Eudesmol	1635	–	8.2	–	30.4	14.8	12.9	3.1
27	Agarospirol	1639	9.3	–	24.2	2.1	–	–	–
28	Alloaromadendrene epoxide	1639	–	1.2	–	–	–	1.7	–
29	Guaia-3,9-dien-11-ol	1648	–	4.9	–	–	–	–	–
30	β-Eudesmol	1649	–	4.1	8.4	–	–	–	–
31	α-Eudesmol	1652	17.3	–	–	–	–	–	–
32	Valerianol	1656	19.4	–	14.1	2.3	–	–	1.7
33	β-Costol	1672	2.0	–	–	–	–	–	–
34	α-Bisabolol	1683	–	2.9	–	–	–	1.2	–
35	Selina-3,11-diene-9-one	1687	–	–	1.0	0.4	–	0.3	–
36	Cyperotundone	1695	–	–	1.7	–	–	–	–
37	10-nor-Calamenen	1702	2.8	–	–	–	–	–	–
38	Selina-3,11-dien-14-ol	1735	–	–	1.8	–	7.1	–	–
39	Aristolone	1762	–	–	0.6	–	–	–	–
40	Hexadecanoic acid	1874	–	7.0	5.7	4.5	2.7	–	2.6
41	Thujopsenal	1887	2.1	–	–	–	1.0	–	–
42	Cyclohexadecanolide	1933	–	1.1	–	–	–	–	–
43	Dihydrocolumellarin	1962	2.8	3.0	0.4	1.9	1.8	2.3	2.6

RI = retention index; identification of chemical compounds were based on mass spectra library database; GC = gas chromatography, GC–MS = gas chromatography–mass spectrometry; TG, MA1, LA, KB, JBD, MA2 and MA are codes for studied samples

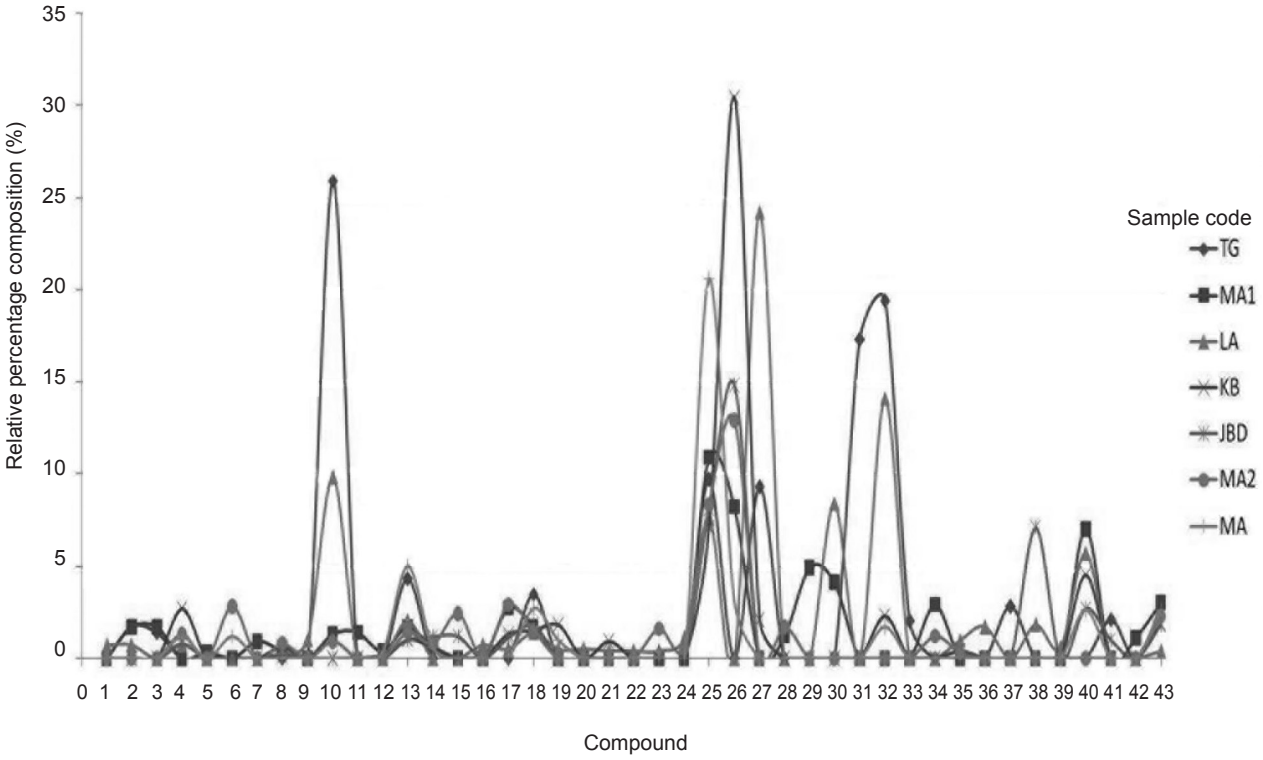

Figure 1 The relative percentage composition for chemical compounds of agarwood oils; refer to Table 1 for compound names

Table 2 Relative percentage composition of selected agarwood oils obtained by principal component analysis

Compound	RI	Group A				Group B		
		TG	MA1	JBD	MA2	LA	KB	MA
4-Phenyl-2-butanone	1241	–	–	0.8	1.3	0.7	2.7	1.2
α-Guaiene	1433	–	0.4	0.1	0.8	0.3	0.6	–
ar-Curcumene	1479	–	0.6	1.2	0.8	–	0.6	0.6
10-Epi-γ-eudesmol	1621	9.8	10.9	8.1	8.3	7.6	7.1	20.6
β-Dihydroagarofuran	1502	–	2.7	1.0	2.9	0.5	1.3	0.04
Valencene	1469	–	1.4	–	–	–	–	–

RI = retention index; TG, MA1, JBD, MA2, LA, KB and MA are codes for studied samples

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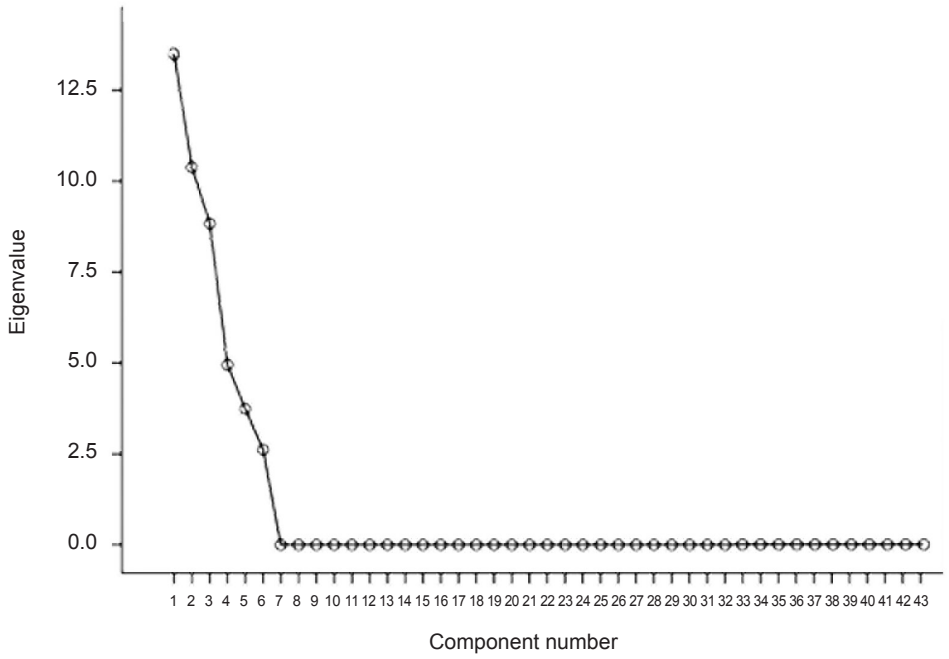

Figure 2 The scree plot of agarwood samples using principal component analysis

Table 3 The correlation r between groups A and B of the agarwood oils

Group	Test	Group A	Group B
Group A	Pearson correlation	1	0.987**
	p-value (2-tailed)		0.000
Group B	Pearson correlation	0.987**	1
	p-value (2-tailed)	0.000	

** Correlation is significant at the 0.01 level (2-tailed)

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