EFFECTS OF EXTRACTION METHODS ON YIELD AND CHEMICAL COMPOUNDS OF GAHARU (*AQUILARIA MALACCENSIS*)

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SULAIMAN N, IDA IDAYU M, RAMLAN AZ, NUR FASHYA M, NOR FARAHIYAH AN, MAILINA J & NOR AZAH MA. 2015. Effects of extraction methods on yield and chemical compounds of gaharu (*Aquilaria malaccensis*). Different extraction methods affect the yield and chemical compounds of gaharu oil. Hydrodistillation, Soxhlet and accelerated solvent extraction (ASE) methods were studied for their extraction yields and percentages of five specific chemical compounds, namely, 3-phenyl-2-butanone, α-guaiene, α-agarofuran, 10-epi-γ-eudesmol and agarospirol. Chemical compounds were analysed using gas chromatography–mass spectrometer (GC–MS). Results showed that ASE method exhibited the highest oil recovery (2.12%) compared with Soxhlet (1.67%) and hydrodistillation (0.18%). The total percentage of the five chemical compounds were higher in gaharu oil extracted using ASE (21.60%), followed by Soxhlet (9.51%) and hydrodistillation (5.08%) methods. However, hydrodistillation exhibited fewer chemical compounds (only three of five, i.e. α-agarofuran, 10-epi-γ-eudesmol and agarospirol) compared with Soxhlet and ASE methods. ASE was the most suitable method to obtain high amount of gaharu oil containing the five chemical compounds with highest total compound percentage. ASE reduced extraction time, making it a practical method compared with Soxhlet and hydrodistillation.

Keywords: Hydrodistillation, Soxhlet extraction, accelerated solvent extraction

INTRODUCTION

Gaharu is a natural plant resin which accumulates in the plant species of four genera from the family Thymelaeaceae, namely, *Gyrinops, Aetoxylon, Gongystylis* and, more commonly, *Aquilaria*. These plants grow widely in South and South-East Asian countries. In Asia, 15 species of gaharu produce oil. This resinous wood is also known as agarwood, eaglewood or aloeswood depending on the ethnic and country. In Malaysia, the plant is known as karas and its resinous wood, gaharu. The species of *Aquilaria* and *Gyrinops* are the major gaharu sources in Malaysia and Indonesia. Oil extracted from gaharu wood obtained from different locations vary in quality (Nor Azah et al. 2008). Gaharu may be classified into various grades, namely, A, B, C and D. Grading of gaharu is usually based on its physical properties, formation and unique scent. In Malaysia, oil is mostly produced from grade C gaharu wood using hydrodistillation method.

Gaharu and its essential oil are valued for their sweet and strong odour and medicinal benefits. The resinous wood and oil of gaharu are extremely expensive due to low yield during extraction and resinous wood formation rarely occurs in wild and young trees. Generally, the price for good quality gaharu can reach up to RM10,000 per kg^{-1} depending on the grade of the resinous wood. Different qualities of gaharu oil are sold at RM50 to RM200 for every 12 g (Chiew 2005).

Gaharu oil can be extracted using several methods including hydrodistillation, solvent extraction, supercritical fluid carbon dioxide extraction (SFE), phytosol extraction and maceration. Hydrodistillation requires 7–10 days

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and consumes high energy (Wetwitayaklung et al. 2009). A major problem in hydrodistillation extraction method is it destroys the heat-sensitive essential oil compounds. SFE is a good alternative but is expensive. SFE has the capability to prevent undesired reactions such as hydrolysis, oxidation and degradation of essential oil compounds (Anklam et al. 1998).

The objective of the current research was to determine the yield and quality of gaharu oil extracted via hydrodistillation, Soxhlet and accelerated solvent extraction (ASE) methods. In addition, composition of some chemical compounds in gaharu oils was evaluated to determine the oil quality. In this report, we present the percentages of five major chemical compounds in gaharu oil extracted via three different extraction methods.

MATERIALS AND METHODS

Pretreatment of raw material

Grade C gaharu wood sample was obtained from naturally-inoculated plants from producers in Teluk Emas, Malacca. The gaharu wood was completely dried (80 °C for 6 hours) before treatment. The dry material was ground in a hammer mill to the desired powder size range by passing through 40-mesh screen but retained on 60-mesh. Mean diameter of the wood particles was determined by mechanical sieving separator.

Extraction of gaharu

Soxhlet method

Soxhlet apparatus was used for solvent extraction of gaharu oil. In a preliminary study, n-hexane was identified as suitable solvent to extract non-polar chemical compounds of gaharu oil (Aleksovski et al. 1998). In order to study the effects of solvent extraction on yield of gaharu oil, extraction time was chosen as parameter. Gaharu powder (10 g) was inserted into a thimble and 300 mL of n-hexane was prepared in a round bottom flask. Soxhlet extractor was connected to a round bottom flask and temperature was set to boiling point of n-hexane, i.e. 68.7 °C. The extraction was heated for about 6 hours, i.e. until a clear extract was obtained indicating maximum extraction was achieved. The extract solutions were dried in vacuum rotary evaporator at 70 °C. Gaharu oil obtained was weighed and samples were kept in amber bottles and refrigerated (5 °C) until further analyses (Md-Nazrul et al. 2009).

Hydrodistillation method

A total of 200 g of ground gaharu were put in a round bottom flask filled with 2000 mL of deionised water for up to 14 days (Md-Nazrul et al. 2009, Pornpunyapat et al. 2011). Raw material at 60-mesh and optimum water ratio of 1:10 minimised the effects of particle size and water ratio on extraction yield. For hydrodistillation, the gaharu powder and water mixture was filled into an extraction vessel (Pornpunyapat et al. 2011). Extraction time was the only parameter evaluated in the hydrodistillation method. Samples were continuously distilled at various durations, namely, 24, 48 and 72 hours after the mixture had reached 100 °C and ambient pressure. The liquid–liquid extraction performed using n-hexane solvent and extracted oils were dried using anhydrous sodium sulphate. Excess solvent was removed using vacuum rotary evaporator at 70 °C. Gaharu oil obtained was separated and its yield determined. Samples were kept in amber bottles and refrigerated (5 °C) until further analyses.

Accelerated solvent extraction method

The ASE method by Dawidowicz et al. (2008) was employed due to its capability to extract at high temperatures of up to 200 °C. The ASE system was equipped with a solvent controller unit and extractions were performed using n-hexane as solvent. Dried and ground gaharu sample (10 g) was placed in the extraction cell (100 cm^3). During extraction, temperature was set from 140 to 200 °C, time from 50 to 99 min and percentage of solvent flush volume from 40 to 100%. General full factorial design was used to analyse the relationship between extraction parameter and extraction yield. When extraction process was completed, the extract solution was dried in vacuum rotary evaporator at 70 °C. The resultant extract was placed into glass vials, sealed and stored at 5 °C to prevent any possible degradation.

Determination of chemical compounds in gaharu oil

The compositions of volatile fractions of gaharu oils were determined using gas chromatography–mass spectrometry (GC–MS) with fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Samples were injected in split mode, using pressure controlled helium as carrier gas and electron energy at 70 eV. The temperature of the injector and detector were maintained at 250 and 300 °C respectively. The column oven temperature was programmed from 60 °C (after 10 min) to 230 °C at rate of 3.0 °C min^{-1} and the final temperature was held for 5 min. Compounds were identified by matching their mass spectra with those of pure compounds (Mailina et al. 2011).

RESULTS AND DISCUSSIONS

Yield of gaharu oil using different extraction methods

Soxhlet extraction

Figure 1 shows the yield of gaharu oil extracted using Soxlet extraction method. Yield of oil increased linearly with duration of extraction. Extraction time was stopped at 16 hours because the solution obtained in the thimble was clear, indicating that oil was fully extracted from the raw material. The extract obtained was brownish clear with high viscosity. The scent of gaharu oil extracted via Soxlet extraction method was strongly woody and sweet. Since the reference of *A. malaccensis* was limited, we compared our results with those of other *Aquilaria* species. Oil yield obtained in this study was comparatively lower than that reported by Habibur et al. (2012) who found that extraction yield from the wood of *A. agallocha* was 2.3% using n-hexane solvent. Extraction from *A. crassna* gave 2.0% of extractives content and most of the compounds were semi-polar compounds (Eka-Novriyanti et al. 2010).

Hydrodistillation extraction

Gaharu oil yields obtained by hydrodistillation extraction are presented in Figure 2. Maximum extraction yield obtained using this method was 0.18% at 72 hours of extraction. The gaharu oil was dark green and of low viscosity (by visual observation). The scent of gaharu oil extracted via hydrodistillation was strongly woody and bitter sweet at 72 hours extraction. From the data, extraction time at 48 hours was sufficient to obtain maximum yield since it had insignificant changes compared with extraction at 72 hours.

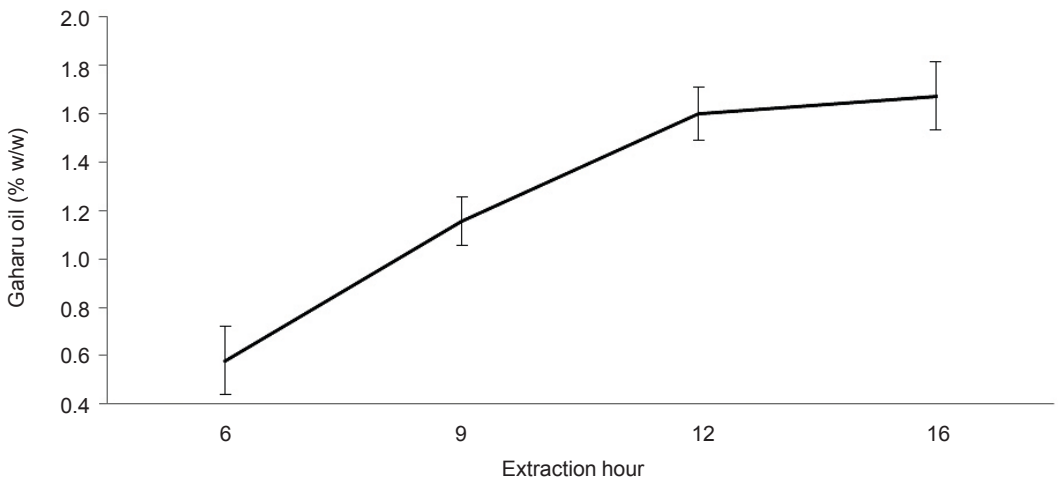

Figure 1 Yield of gaharu oil using Soxhlet extraction; yield is reported as mean ± standard error of mean

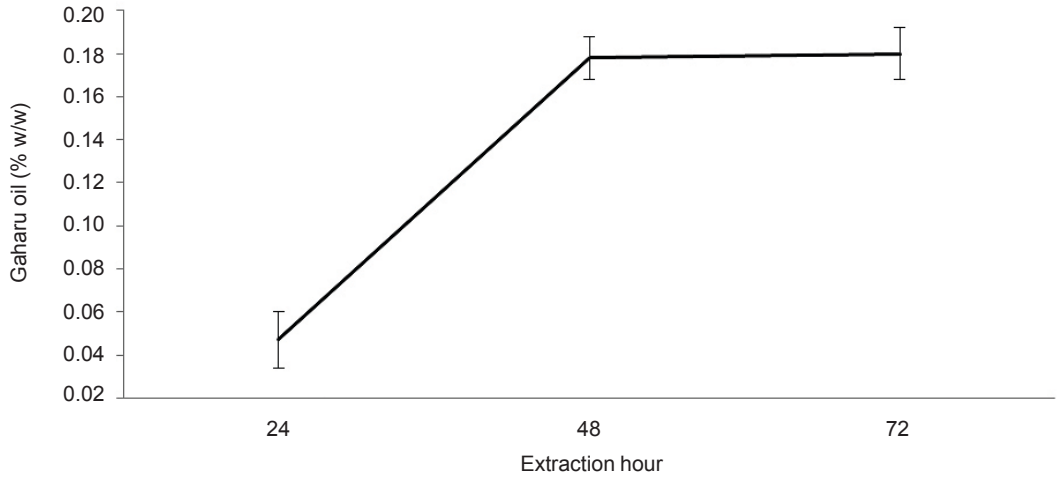

Figure 2 Yield of gaharu oil using hydrodistillation extraction; yield is reported as mean ± standard error of mean

Accelerated solvent extraction

Results of the experiment based on full factorial design are presented in Table 1. The percentage of oil yield obtained using this extraction method was between 0.56 and 2.28%. Based on analysis of variance (ANOVA) in Table 2, the extraction duration and solvent volume were found to have the greatest effect on extraction yield ($p < 0.05$). The r^2 value was 99.26%, indicating that the data were reliable and could be used for optimisation. Oil yield of 2.12% was achieved at optimum point of 150 °C and 90 min extraction duration using 90% solvent volume. Oil produced was dark brown with burning smell. The dark brown colour indicated that the oil was incorporated with other unwanted compounds including plant pigment, resin and latex in the trunk that reduced the quality of the gaharu oil.

Comparison of gaharu oil yields via three different extraction methods

From the three extraction methods, ASE gave the highest yield of gaharu oil with the recovery percentage of 2.12% followed by Soxhlet (1.67%) and hydrodistillation methods (0.18%). ASE required shorter extraction time but had higher oil recovery compared with Soxhlet and hydrodistillation methods. In ASE method, optimum oil recovery was achieved at 150 °C in only 90 min. On the other hand, Soxhlet extraction at 70 °C required longer extraction time of 16 hours. The hydrodistillation method required 72 hours at 100 °C to achieve maximum oil recovery. The oils were of different colours depending on the type of extraction methods, i.e. hydrodistillation—dark green, Soxhlet extraction—light brown and ASE—dark brown.

Comparison of chemical compounds in gaharu oil extracted via different extraction methods

Composition of the volatile fraction of gaharu oil was determined by GC–MS and the result is presented in Figure 3. The identification of five chemical compounds (3phenyl-2-butanone, α-guaiene, α-agarofuran, 10-epi-γ-eudesmol and agarospirol) was measured by percentage of relative area in chromatogram. There were different trends for chemical distribution and percentage of chemical compounds in gaharu oil. The contents of all compounds were higher in oil extracted using ASE method (21.60%), followed by Soxhlet (9.51%) and hydrodistillation (5.08%) methods. The highest chemical compound was 10-epi-γ-eudesmol obtained when using ASE method. Soxhlet and hydrodistillation methods exhibited the highest agarospirol compound in gaharu oil. Both ASE and Soxhlet methods

Table 1 Yield of gaharu oil using accelerated solvent extraction by full factorial design

Temperature (°C)	Extraction (min)	Volume of solvent (%)	Gaharu oil (% w/w)
141	69	63	0.556
145	73	42	0.781
150	56	49	0.727
152	46	46	0.763
160	90	86	2.281
174	79	41	0.876
187	53	47	0.798
189	85	57	1.539
197	49	85	1.136
200	56	89	1.582

Table 2 Analysis of variance in accelerated solvent extraction method

Source	Sum of squares	df	Mean square	F value	p > F
Model	2.58	7	0.37	38.28	0.0257
x_1	0.49	1	0.49	5.09	0.1527
x_2	0.80	1	0.80	83.42	0.0118
x_3	0.63	1	0.63	65.74	0.0149
x_1x_2	9.856E-003	1	9.856E-003	1.02	0.4183
x_1x_3	0.020	1	0.020	2.13	0.2823
x_2x_3	0.19	1	0.19	19.66	0.0473
$x_1x_2x_3$	1.047E-003	1	1.047E-003	0.11	0.7731
Residual	0.019	2	09.638E-003		
Cor total	2.60	9			
r^2	0.9926				

df = Degree of freedom, x_1 = temperature, x_2 = duration, x_3 = volume of solvent, Cor = correlation

showed the presence of all five chemical compounds. Only three chemical compounds, namely, α-agarofuran, 10-epi-γ-eudesmol and agarospirol were detected in hydrodistillation method. This was due to the higher heating process of the raw material which destroyed certain chemical compounds (Handa 2008). However, the presence of nitrogen gas in ASE helped reduce the oxidation of the compound at high temperature.

CONCLUSIONS

Gaharu oils extracted via the three different methods revealed that ASE presented the highest oil recovery (2.12%) compared with Soxhlet (1.67%) and hydrodistillation (0.18%). The percentage of chemical compounds was highest in gaharu oil extracted using ASE method (21.60%), followed by Soxhlet (9.51%) and hydrodistillation (5.08%) methods. ASE and Soxhlet methods were able to extract all five compounds (3phenyl-2-butanone, α-guaiene, α-agarofuran, 10-epi-γ-eudesmol and agarospirol) compared with hydrodistillation method that only had three compounds in the oil (α-agarofuran, 10-epi-γ-eudesmol and agarospirol). ASE had the highest percentage of total chemical compounds in gaharu oil. Of the three methods tested, ASE could reduce extraction time, making it a practical method compared with Soxhlet and hydrodistillation.

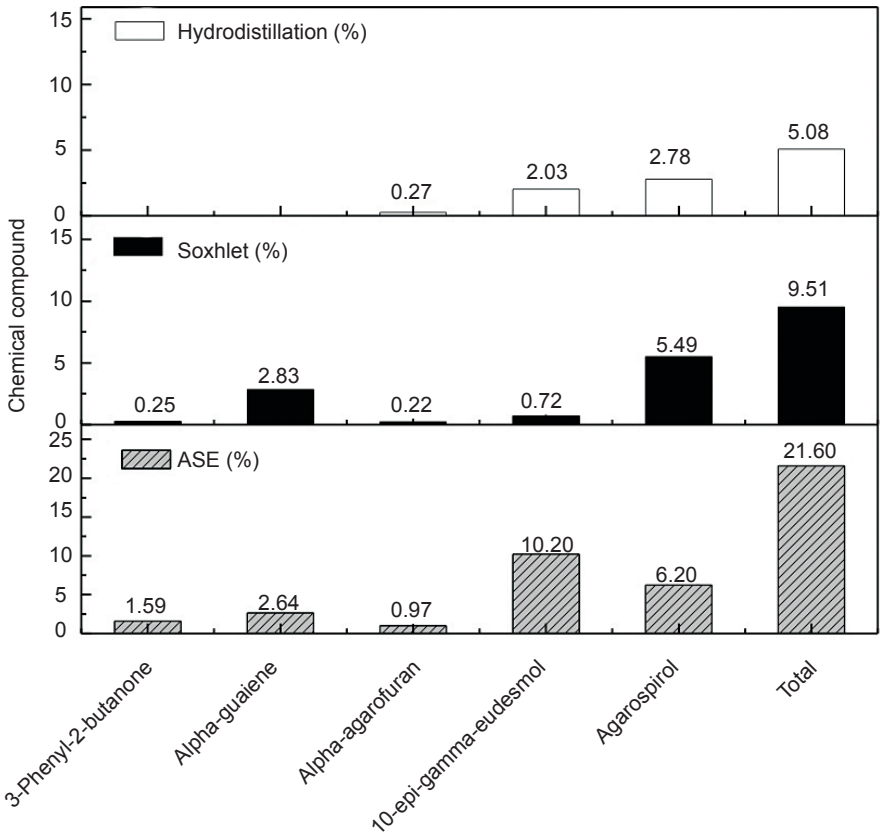

Figure 3 Percentages of five chemical compounds in gaharu oils obtained by ASE Soxhlet and hydrodistillation methods

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REFERENCES

Aleksovski S, Sovova H, Urapova B & Poposka F. 1998. Supercritical CO_2 extraction and Soxhlet extraction of grape seed oil. *Bulletin of the Chemists and Technologists of Macedonia* 17: 129–134.

Anklam E, Berg H, Mathiasson L, Sharman M & Ulberth F. 1998. Supercritical fluid extraction (SFE) in analysis: a review. *Food Additives and Contaminants* 15: 729–750.

Chiew H. 2005. Brewing gold. http://www.thestar.com.mystory/?file=%2F2005%2F8%2F9%2Flifefocus%2F1158517&sec=lifefocus.

Dawidowicz AL, Rado E, Wianowska D, Mardaromicz M & Jan Gawdzik. 2008. Application of PLE for the determination of essential oil components from *Thymus vulgaris* L. *Talanta* 76: 878–884.

Eka-Novriyanti, Santosa E, Syafii W, Turjaman M & Sitepu IR. 2010. Antifungal activity of wood extract of *Aquilaria crassna* Pierre ex Lecomte against agarwood-inducing fungi, *Fusrium solani*. *Journal of Forestry Research* 7: 155–165.

Habibur R, Vakati K & Eswaraiah MC. 2012. In-vivo and in-vitro antiflammatory activity of *Aquilaria agallocha* oil. *International Journal of Basic Medical Sciences and Pharmacy* 2: 7–10.

Handa SS. 2008. An overview of extraction technology for medicinal and aromatic plants. Pp 1–58 in Handa SS et al. (eds) *Extraction Technologies for Medicinal and Aromatic Plants*. International Centre for Science and High Technology, Trieste.

Mailina J, Nor Azah MA, Fadzureena J et al. 2011. Identification of volatile constituents from fresh sample of *Baeckea Fruitescens* L and their distilled oils. Pp 41–45 in Mastura M et al. (eds) *Harnessing The Tropical Herbal Heritage: Recent Advances in R&D and Commercialisation. Proceedings of the Seminar on Medicinal and Aromatic Plant (MAPs 2010)*. 3–4 August 2010, Kepong.

Md-Nazrul IB, Jaripa B & Md Nurul HB. 2009. Analysis of essential oil of eaglewood tree (*Aquilaria agallocha* Roxb) by gas chromatography mass

spectrometery. *Bangladesh Journal Pharmacology* 4: 24–28.

Nor Azah MA, Chang YS, Mailina J et al. 2008. Comparison of chemical profiles of selected gaharu oils from Peninsular Malaysia. *Malaysian Journal of Analytical Sciences* 12: 338–340.

Pornpunyapat J, Chetpattananondh P & Tongurai C. 2011. Mathematical modeling for extraction of essential oil from *Aquilaria crassna* by hydrodistillation and quality of agarwood oil. *Bangladesh Journal Pharmacology* 6: 18–24.

Wetwitayaklung P, Thavanapong N & Charoenteeraboon J. 2009. Chemical constituents and antimicrobial activity of essential oil and extract of heartwood of *Aquilaria crassna* obtained from water distillation and supercritical fluid carbon dioxide extraction. *Silpakron University Science and Technology Journal* 3: 25–33