CLASSIFICATION OF AGARWOOD (GAHARU) BY RESIN CONTENT

MA Nor Azah*, S Saidatul Husni, J Mailina, L Sahrim, J Abdul Majid & Z Mohd Faridz

Forest Research Institute Malaysia, 52109 Kepong, Selangor Darul Ehsan, Malaysia

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NOR AZAH MA, SAIDATUL HUSNI S, MAILINA J, SAHRIM L, ABDUL MAJID J & MOHD FARIDZ Z. 2013. Classification of agarwood (gaharu) by resin content. Agarwood or gaharu is a fragrant resinous heartwood that can develop in diseased trees from the genus *Aquilaria* (Thymelaeaceae). The wood is often classified into various grades according to the colour, density, gaharu formation and scent. These parameters are very subjective and depend on individual perception. In this study, several grades of gaharu wood from natural sources and inoculated materials were evaluated for resin content and the data classified using Z-score transformation technique. The results demonstrated that resin content could be used as a guide for the classification of gaharu, thus assisting the industry to reduce bias judgement among traders.

Keywords: *Aquilaria,* wood extractives

NOR AZAH MA, SAIDATUL HUSNI S, MAILINA J, SAHRIM L, ABDUL MAJID J & MOHD FARIDZ Z. 2013. Pengelasan gaharu menggunakan kandungan resin. Gaharu merupakan teras kayu beresin dan berbau wangi yang boleh terbentuk di dalam pokok berpenyakit daripada genus *Aquilaria* (Thymelaeaceae). Kayu gaharu kerap dikelaskan kepada beberapa gred berdasarkan warna, ketumpatan, pembentukan gaharu dan bau. Parameter ini sangat subjektif dan bergantung kepada persepsi individu. Dalam kajian ini, beberapa gred kayu gaharu daripada sumber semula jadi dan bahan inokulasi dinilai kandungan resinnya dan datanya dikelaskan menggunakan teknik transformasi skor-Z. Keputusan menunjukkan yang kandungan resin boleh dijadikan panduan dalam pengelasan gaharu. Ini dapat membantu industri mengurangkan kekeliruan penggredan di kalangan pedagang.

INTRODUCTION

Gaharu which is also known as agarwood or aloeswood is a fragrant resinous heartwood that is usually derived from diseased timber of the genus *Aquilaria* (Thymelaeaceae). Gaharu can be formed through inducement process such as injury, cutting, pest or insect disturbance, microorganism, fire, chemical or colonisation (Dai et al. 2009). The strong scent of gaharu oil is unique and complex and highly favoured as perfumery, while the gaharu in powder form is used in incense and medicinal application (Naef 2011). However, the harvesting of gaharu from the forest is posing a threat to the gaharu-producing species. The highly lucrative prices of this commodity have attracted much interest among local and overseas traders. There is increasing demand for high grade gaharu in the global market. Furthermore, all species within the genus *Aquilaria* are now listed in Appendix II of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) including all gaharu-related products. This means that a permit is required for the export or import of gaharu. More importantly, for the past few years there has been increasing interest from the private sector to set up gaharu plantations. Despite the risk, some companies and individuals have proceeded to establish plantations with the belief that the inducement technology will enable the invaluable heartwood to be formed. However, without any injury or illness, trees may not produce gaharu. There is also uncertainty whether trees grown under cultivation and the use of artificial heartwood inducement techniques will produce gaharu of similar quality to that harvested from natural

**norazah@frim.gov.my*

trees. Taking these factors into consideration, the Forest Research Institute Malaysia (FRIM) has conducted studies on gaharu inducement, gaharu oil extraction and composition, *in vitro* propagation, karas cultivation and genetic diversity (Abdul Rashid & Ahmad Zuhaidi 2011).

Gaharu wood in Malaysia is often graded into different classes based on their colour, density, gaharu formation and unique scent. Currently, the grading of gaharu is based on individual perception and experience. Some gaharu wood has been graded into unspecified classifications, including according to origin or unique name in order to attract buyers. The price of gaharu is determined by its grade; the higher the grade the higher the price. From personal communication with gaharu traders, the price of gaharu premium grade (grade A) may fetch RM16,000 to RM20,000 per kg. The price for supreme or deluxe oil may range from RM400–RM2000/tola (1 tola = 12 mL) (Nor Azah et al. 2009). An alternative solution to overcoming the grading problem is through scientific approach. With this approach, the reliability and consistency of assessing the quality of gaharu wood chips from the wild and plantation can be assured. Attempts to classify gaharu according to the density of the black colour of greyscale images have been reported (Azma et al. 2007) but its potential application is uncertain. Another approach is through a signal-based classification system using electronic nose (E-nose) technology (Muhammad Sharfi et al. 2011, 2012) which has been developed by FRIM and is currently being patented. Nevertheless, in general, gaharu can be classified into four main categories, i.e. A, B, C and D (Lim et al. 2011) as shown in Table 1.

Chemical studies on gaharu oils from *Aquilaria* species including *A. malaccensis* have reported the presence of several sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, hydrocarbons and acids (Ishihara et al. 1991, 1993, Tamuli et al. 2005, Nor Azah et al. 2008). Some of the compounds which had been identified from gaharu included α-agarofuran, β-agarofuran, 10 epi-γ-eudesmol, agarospirol, jinkohol, jinkohol II and valerianol (Nakanishi et al. 1984, Ishihara et al. 1993, Nor Azah et al. 2009). Other studies reported the medicinal properties of *Aquilaria* sp. which included antinoceptive and anti-inflammatory (Zhou et al. 2008) as well as antimicrobe properties (Norzafneza et al. 2011). While a full review of the volatile and semi-volatile components of *Aquilaria* have been documented (Naef 2011), not many studies have recorded the wood extractive or resin content of gaharu wood. Only recently did Zhang et al. (2012) reported the production of high quality gaharu by whole-tree-induction technology and compared levels of ethanol-soluble extractives with samples from the wild. The average resin levels of induced gaharu were 19–22%, which surpassed the 10% requirement for traditional Chinese medicine.

Wood extractives are a heterogeneous group of compounds that can dissolve in organic solvent or water (Fengel & Wegener 1984). Examples of wood extractives are triglycerides, fatty acids, resin acids, steryl esters, sterols and lignans (Kallioinen et al. 2003). In the herbal industry, ethanol-soluble extractive contents are used to determine the amount of active constituents in the herbal materials (WHO 1998, Liu et al. 2012). This protocol is now being used to measure the amount of resin in the agarwood of *A. crassna* by companies in Vietnam and China, which helps verify the quality of their products for medicinal uses (Zhang et al. 2012).

In this research, Z-score standard scale was used to cluster the data and distinguish a sample data population to an explicit

Table 1 Physical appearance of various grades of gaharu

Grade	Characteristic
A	Dark, dense, concentrated, heavy
B	Dark purple, less dense, small hole
C	Dark yellow stripes, dark yellow
D	Whitish yellow

Lim et al. (2011)

grouping (Brown & Daniel 1990). Many researchers have adopted Z-score methods to define a specific grouping, including Distefano et al. (2009) who reported the use of Z-score as a common method to standardise information and create a factor score. Z-score scaling technique can also be used for clustering an IQ (intelligence quotient) index for three groups by correlating the IQ scores with the electroencephalogram power spectrum density (Sahrim et al. 2010).

Boxplot is used in this research as it is the simplest statistical method used for data visualisation and exploration. The summary statistics is easily displayed in order to have better understanding of the data being analysed. Boxplot displays the first, second and third quartile as well as the interquartile data range. It is also a method to recognise data location, spread, skewness as well as the tails of the data. Moreover, it does not use extreme potential outliers in computing a data spread. The information displayed by boxplot and most of its variations is based on the median of the data (Schwertman et al. 2004, Hubert & Vandervieren 2008, Carter et al. 2009, Marmolejo-Ramos & Tian 2010).

This study was undertaken to determine the resin content of various grades of gaharu wood samples and classify them using the Z-score transformation technique. The aim was to establish a scientific approach to using resin content as a rapid method for grading gaharu in addition to E-nose method and the current physical grading practised by the industry and government authorities.

MATERIALS AND METHODS

Plant materials

Thirty-four samples of gaharu wood from various grades were obtained from the market, supplier, trader or collector in Malaysia. Thirty-two samples were natural gaharu and were graded as Super, A, B and C. Two other samples were induced gaharu, which were obtained through different inoculation techniques.

Sample preparation

Gaharu wood chip samples were initially dried overnight using an oven at 45 °C or until constant weight. They were finely ground using basic analytical mill.

Resin content analysis

This analysis followed closely the protocol developed by WHO (1998). Ground sample (4 g) was accurately weighed in a flask and 100 mL of 95% ethanol were added. The mixture was shaken and allowed to stand for 1 hour. It was then refluxed for 1 hour and filtered rapidly using filter paper. A 25-mL subsample of filtrate was then transferred to a ceramic evaporating dish and evaporated to dryness in a water bath. The evaporated product was dried at 105 °C for 6 hours and cooled in a desiccator for 30 min before weighing. The resin content of the extractable matter from each of the 34 gaharu wood samples was calculated in % (w/w).

Statistical analysis

The variation in resin content was analysed using general linear model procedure by one-way analysis of variance followed by posthoc analysis (SPSS version 12). Analyses were performed in three replicates. All data were expressed as means ± standard deviations (SDs) and divided into four groups. Significant differences between means within and between groups were analysed. The statistical probability was considered to be significantly different at $p < 0.05$. Boxplot was used to display the mean differences between the proposed four groups and also to distinguish location of each data group, spread and skewness. Additionally, boxplot was used to detect outlier/s from each group.

This study applied Z-score standard scaling method from equation 1 (Thatcher et al. 2004), where Z = value of Z-score transformation, x_i = k^{th} data point and $\overline{X}$ = mean of the data population from raw resin content containing 34 samples with three replicates each. Y from equation 2 is the Gaussian distribution for the transformation scale ranging from less than -SD to larger than +SD with mean equals to zero between them. Here, we decided to reconstruct the Y scale into four groups and named it alphabetically ascending to suit the currently available gaharu trading grades. This study used boxplot interpretation to compare means.

$$Z = \frac{x_i - \overline{X}}{SD} \qquad (1)$$

$$Y = \frac{1}{\sqrt{2\pi}} e^{-\frac{Z^2}{2}} \qquad (2)$$

RESULTS AND DISCUSSION

Tables 2 and 3 indicate that there are marked differences between the different grades of gaharu wood. The grades could be classified according to the presence of extractive or resin content in wood chips.

From the Z-score transformation technique results in Table 2, the raw data were transformed and clustered into four groups, which contained eight to nine samples each. The highest resin contents (Table 3) were found in group A (33.40–42.96%), followed by groups B (22.06–27.58%), C (9.84–18.43%) and D (2.01–7.14%).

The majority of samples in Table 3 fell into the same groups that were provided by traders to researchers during sample collection. The exceptions were C1 (Melaka), C (Gua Musang), C8 (Malaysia) and C bungkus (Malaysia) which fell into group D; C+ (Kelantan) and Duga grade A which fell into group B; and grade B (Kelantan) which fell into group A.

Most of the samples in group A were traded as Super and grade A in the market except for grade B (Kelantan). In group B, the samples were originally traded as B, Super, Double super and C+, which were considered as high grade due to the shape of the gaharu wood structure that was suitable for wood carving and aesthetic purposes. Samples classified under group C were mainly from grade A5, C and other grade codes such as OUD1 and OUD2 as provided by the supplier. Lastly, samples in group D were those from grade C, inoculated wood and gaharu wood chips, which were commonly used for oil distillation. Based on the findings, the higher proposed market grade of gaharu were found to have higher resin content values.

Figure 1 shows that there are significantly different mean values between each of the proposed resin content groups. Group A had the highest mean resin content. There was an extreme outlier and two acceptable outliers which came from group A and all data from the other three groups fell into the 95% confidence interval. The outliers in group A were from Super (Pagoh). From the boxplot pattern, it could be deduced that as the resin content increased, the proposed gaharu grading group significantly increased and the correlation was a linear relationship.

Based on statistical analyses in this study, gaharu wood is recommended to be classified into four separate grades based on the resin content. Using generated Z-score actual scale in Table 2, a new scale was proposed to cover four different groups of resin content data: group A (> 30%), group B (20–30%), group C (10%–20%) and group D (< 9%) (Table 4).

CONCLUSIONS

Quality grading of gaharu has been a major concern in the gaharu business. In assisting gaharu industries, FRIM is providing services in resin content determination of gaharu wood and essential oil analysis. Apart from fingerprint profiling via chromatographic techniques, it is hoped that resin content

Table 2 Z-score standard scale and resin content values (%) after transformation

Z-score grouping	D (x < - SD)	C (-SD ≤ x ≤ mean)	B (mean ≤ x ≤ +SD)	A (x > +SD)
Resin real value (%)	< 7.14	9.84–18.43	22.06–27.58	> 33.3

x = Z-score value; SD = standard deviation

Table 3 Percentage of resin content in gaharu wood chips from natural sources and inoculated samples

Original name	Resin content (%)
Super (Pagoh)	42.96 ± 3.22 a
A (Ulu Tembeling)	37.19 ± 1.47 a
B (Kelantan)	36.22 ± 0.64 a
A (Malaysia)	35.74 ± 0.42 a
A1 (Gua Musang)	35.41 ± 1.56 a
Raub	35.02 ± 0.37 a
Super (Cambodia)	33.43 ± 1.71 a
A (Pagoh)	33.40 ± 0.73 a
Sufi (Malaysia)	27.58 ± 0.92 b
Duga Grade A	29.51 ± 0.92 b
C+ (Kelantan)	28.50 ± 0.68 b
Super (Laos)	27.35 ± 0.32 b
Double Super (Kedah)	26.20 ± 0.07 b
B (Melaka)	25.24 ± 0.01 b
Super (Terengganu)	22.96 ± 1.02 b
B (Gua Musang)	22.08 ± 0.41 b
B (Terengganu)	22.06 ± 0.42 b
A5 (Malaysia)	18.43 ± 1.50 c
OUD 1 (Malaysia)	18.29 ± 0.62 c
OUD 2 (Malaysia)	16.61 ± 0.88 c
OUD 1 (Cambodia)	16.26 ± 0.37 c
OUD 1 (Indonesia)	16.24 ± 0.89 c
C (Terengganu)	15.27 ± 0.13 c
Kepala kayu 1 (Kelantan)	10.00 ± 0.15 c
C1 (Merapoh)	9.84 ± 0.41 c
C1 (Melaka)	7.14 ± 0.07 d
Gaharu P.S	6.74 ± 0.23 d
C (Gua Musang)	6.23 ± 0.43 d
KC inoculated wood (Malaysia)	6.13 ± 1.31 d
Gaharu (Indonesia)	6.01 ± 0.22 d
C8 (Malaysia)	3.63 ± 0.26 d
Gaharu wood for distillation (Malaysia)	3.41 ± 0.13 d
C bungkus (Malaysia)	2.47 ± 1.14 d
Gaharu wood for distillation (Kelantan)	2.01 ± 0.21 d

Values are means ± standard deviations of three replicates; different letters indicate significant difference ($p < 0.05$)

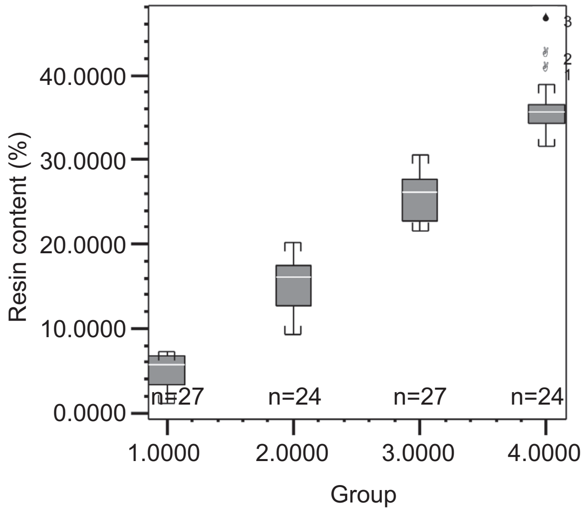

Figure 1 Boxplot for the proposed resin content of gaharu grading groups; from right groups A, B, C and D

analysis will be able to support the grading and quality assessment of gaharu in Malaysia and South-East Asia.

Table 4 Recommendation of classification for gaharu wood from natural sources and inoculated material based on resin content

Grade	Percentage of resin content
A	30% and above
B	20–29.99%
C	9–19.99%
D	< 9%

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