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Statistical analysis for chemical compound based on several species of *Aquilaria* essential oil

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ABSTRACT

The paper examines the characterization of *Aquilaria* essential oils from different species, namely *Aquilaria malaccensis*, *Aquilaria beccariana*, *Aquilaria crassna*, and *Aquilaria subintegra*, renowned for agarwood production in Malaysia. Gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) were employed for extracting essential oil data, facilitating compound identification. Subsequently, a preliminary analysis focused on classifying significant chemical compounds in the samples. The study then utilized boxplot pre-processing for visualizing and interpreting data distribution. The statistical analyses were performed using MATLAB software version R2021b, considering two key parameters which are the peak area (%) of significant chemical compounds and the classification of *Aquilaria* species (*A. beccariana, A. malaccensis, A. crassna,* and *A. subintegra*) based on their chemical composition. The results, presented through boxplot analyses, demonstrated a clear representation of the parameters and their distribution in the data. This method not only confirmed the potential of boxplot analysis in statistical evaluation of significant compounds in Aquilaria essential oil but also suggested its applicability for further classification work.

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1. INTRODUCTION

Aquilaria species, famously known as agarwood are one of precious tree in the world especially in Asia due to its highest value of resin-impregnated heartwood. *Aquilaria* is a genus which belongs to the Thymelaeceae family [1]–[12]. Others name also are refer to *Aquilaria* such as oud, aloeswood, eaglewood, chén xiāng, and jinkoh [2], [8], [9], [13]. Agarwood plants can be easily found in East Asia, including Malaysia, Indonesia, Vietnam, and Thailand and also prevalent in South Asia, specifically in Bhutan and India [2], [4], [11], [12], [14]. Additionally, agarwood is present in the Middle East, with notable occurrences in Saudi Arabia, the United Arab Emirates, and Oman [2], [6], [11]. Agarwood plays an essential role in traditional medicine, cultural and religious practices in Buddhist, Hindu, Muslim, Jewish and Christian [2], [12]. It is also considered prestigious and is often associated with luxury and wealth. Owning and using agarwood-based products can be a status symbol in Middle Eastern societies [2], [6], [11]. This oil is extracted from *Aquilaria* species which are A. beccariana, A. malaccensis, A. crassna, and A. subintegra. There are more than eighty compounds are found

in *Aquilaria* essential oil, with the most significant ones being *allo*-aromadendrene, *β*-selinene, dihyro-*β*-agarofuran, *δ*-guaiene, *10*-epi-γ-eudesmol, and jinkoh-eremol [6], [7], [11], [15].

Traditionally, experts in identifying agarwood trees are solely responsible for classifying the species of agarwood oil based on physical characteristics, specifically examining its color concentration and evaluating the intensity of its aroma [9], [10], [16], [17]. These represent the primary criteria for determining the species of agarwood oil, discerning potential differences among them. Unfortunately, this approach has its drawbacks. The method can be subjective and unreliable due to significant variations within a single species, leading to a potential reduction in the sensory capabilities of the eyes and nose involved. This also requires an extended duration for the process and acquires high costs for the extensive classification procedure [16].

To enhance the classification process, this study leverages modern technology and scientific methods, specifically the analysis of chemical compounds in agarwood oil. Previous studies [18] have demonstrated the effectiveness of representing graphical data through boxplots, using quartiles for essential oils from various species, particularly for the classification of Citronella oils species. To further progress in the study of transforming the classification process of agarwood oil species, this research creates upon previous findings. Creating on prior research, artificial intelligence is incorporated to classify the four *Aquilaria* species of agarwood oil, assessing classification acceptability based on compound presence.

While previous studies used various graphical methods like histograms and normal Q-Q plots to evaluate data normality in agarwood oil classification [6], [19], this study opts for boxplot analysis. Despite its limitations in outlier labeling with skewed data, boxplots are widely used in research for summarizing data distribution and identifying outliers [20]–[23]. However, using boxplots for classifying the four *Aquilaria* species has constraints, including potential outliers obscuring patterns [21], [22]. It is recommended to employ complementary statistical analyses for a comprehensive understanding of the data [18]. This study introduces a new modification to boxplot analysis for the four *Aquilaria* species, summarizing data based on minimum and maximum range values, upper and lower quartiles, and the median. The study was conducted using MATLAB software version R2021b.

2. METHOD

The boxplot illustrates the five-number summary of a dataset, encompassing the minimum and maximum values of the range, the upper and lower quartiles, and the median, as shown in Figure 1. It serves as a valuable visual tool for effectively conveying the information inherent in a dataset through the boxplot [6], [7], [17], [18], [21]–[25] Based on Figure 1, each group forms 25% of the scores [6]. The line in the boxplot divides into three quartiles which are lower, median, and upper. These groups are arranged from the lowest to the highest point [6], [23]. The lower quartile (Q1) represents the 25^{th} percentile of the data, indicating that 25% of the data points fall below it, while 75% fall above. On the other hand, the upper quartile (Q3) represents the 75^{th} percentile, dividing the data into 75% lower values and 25% higher values. Q1 corresponds to the bottom edge of the box in the boxplot, and Q3 corresponds to the top edge. The distance between these lines is referred to as the interquartile range (IQR). The vertical line positioned at the center of the boxes signifies the 50^{th} percentile, commonly known as the median (Q2) [7], [18], [25]. However, if the median is closer to the upper or lower quartile, it indicates a skewed distribution towards that side [19].

$$\text{IQR} = \text{upper quartile (Q3)} - \text{lower quartile (Q1)} \quad (1)$$

$$\text{Minimum band} = \text{lower quartile (Q1)} - 1.5\ (\text{IQR}) \quad (2)$$

$$\text{Maximum band} = \text{upper quartile (Q3)} + 1.5\ (\text{IQR}) \quad (3)$$

In addition, the lower and upper adjacent values are the actual data points located outside the lower and upper whiskers of the boxplot, respectively. Their positions depend on the whisker length, calculated based on the interquartile range (IQR) as shown in (1)-(3). The lower adjacent value defines the edge of the "non-outlier" range in the lower half of the data. For '+' symbol, it represents an outlier, also known as an extreme value, positioned either above or below the whisker [20]–[22]. The minimum and maximum denote the range values within the sample data.

2.1. Sample acquisition

The *Aquilaria* essential oil samples from agarwood trees were prepared by the Bioaromatic Research Centre of Excellence (BARCE) at Universiti Malaysia Pahang (UMP). The oil extraction was carried out using gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization

detector (GC-FID) machines [13]. A total of 405 samples containing 82 chemical compounds were used. To ensure data significance, missing values were addressed, and only samples present in all species were considered. This process resulted in the retention of 14 chemical compounds as listed in Table 1, out of the initial 82. The compounds listed are include *allo*-aromadendrene; *β*-selinene; dihydro-*β*-agarofuran; *δ*-guaiene; α-calacorene; caryophyllene oxide; tetradecanal; 10-epi-*γ*-eudesmol; jinkoh-eremol; selina-3,11-dien-9-ol; 9,11-eremophiladien-8-one; selina-3,11-dien-14-oic acid; pentadecanoic acid and 2-hydroxyguaia-1(10),11-dien-15-oic acid, and coded by acronyms a, b, c, d, e, f, g, h, i, j, k, l, n, and m, respectively. The table also provides datasets for several *Aquilaria* species and samples, labeled as ABS1, ABS2, AMS1, AMS2, ACS1, ACS2, ASS1, and ASS2, representing *Aquilaria Beccariana* sample 1, *Aquilaria Beccariana* sample 2, *Aquilaria Malaccensis* sample 1, *Aquilaria Malaccensis* sample 2, *Aquilaria Crassna* sample 1, *Aquilaria Crassna* sample 2, *Aquilaria Subintegra* sample 1, and *Aquilaria Subintegra* sample 2, respectively. Sample 1 and sample 2 are from two datasets of GC-MS and GC-FID of the same species. The peak area measurements indicate the relative concentration of each compound in the respective *Aquilaria* species. This data was used for classifying significant chemical compounds in *Aquilaria* samples.

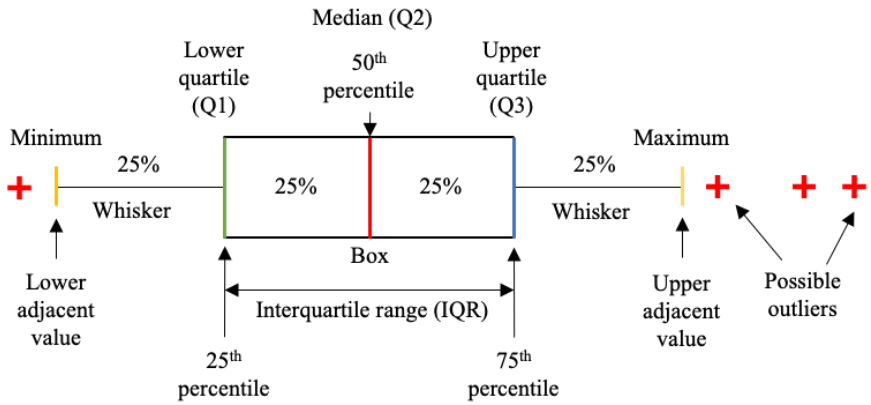

Figure 1. Boxplot structure

Table 1. Chemical compound datasets and peak area for each *Aquilaria* species

Code	Compound	Peak area/species							
		ABS1	ABS2	AMS1	AMS2	ACS1	ACS2	ASS1	ASS2
a	*allo*-aromadendrene	1.98	1.80	1.35	1.28	14.27	14.68	13.24	12.76
b	*β*-selinene	0.66	0.65	0.56	0.51	0.11	0.11	0.37	0.36
c	dihyro-*β*-agarofuran	1.25	1.24	0.55	0.54	0.48	0.49	0.44	0.41
d	*δ*-guaiene	0.74	0.68	2.02	2.04	0.21	0.21	0.35	0.33
e	*α*-calacorene	0.13	0.12	0.31	0.31	0.25	0.25	0.32	0.31
f	caryophyllene oxide	0.39	0.38	1.27	1.28	2.21	2.26	1.76	1.54
g	tetradecanal	0.13	0.12	1.56	1.10	0.34	0.32	0.42	0.36
h	10-epi-*γ*-eudesmol	0.34	0.32	6.73	6.43	2.54	2.49	2.16	2.09
i	jinkoh-eremol	0.16	0.16	1.30	1.19	8.23	8.37	0.17	0.57
j	selina-3,11-dien-9-ol	0.31	0.39	0.54	0.50	0.38	0.22	2.44	2.02
k	9,11-eremophiladien-8-one	0.28	0.30	1.86	1.87	1.90	1.99	0.81	0.75
l	selina-3,11-dien-14-oic acid	1.61	1.58	1.01	0.92	6.98	7.14	4.15	4.03
n	pentadecanoic acid	0.15	0.25	0.15	0.21	0.14	0.14	0.46	0.23
m	2-hydroxyguaia-1(10),11-dien-15-oic acid	0.50	0.67	3.72	3.71	0.54	0.55	0.94	0.92

2.2. Statistical analysis

To begin creating a boxplot, the data was first sorted into four groups representing different *Aquilaria* species, using GC-MS and GC-FID samples for 14 selected compounds. The analysis involved categorizing *Aquilaria* essential oil compounds along the x-axis, representing the peak area (%) of compounds as the dependent variable and the y-axis involved the 14 significant compounds across 112 samples, as independent variables. Ranges and nominal variables were set, and the boxplot's performance was assessed for data distribution. The experimental analysis flowchart in Figure 2 showed the distribution analysis on *Aquilaria* oil samples. The study aims to characterize *Aquilaria* essential oils from different species known for agarwood production in Malaysia. It introduces scientific methods, utilizing boxplot analysis and integrating artificial intelligence, to enhance *Aquilaria* species classification. Despite acknowledged limitations, the study justifies boxplot use by modifying the analysis for *Aquilaria* species,

aiming to improve the overall classification methodology and enhance data understanding. In summary, data selection involved comparing quartile values of peak area for chemical compounds within the four *Aquilaria* species, with a focus on boxplot analysis for agarwood oil classification.

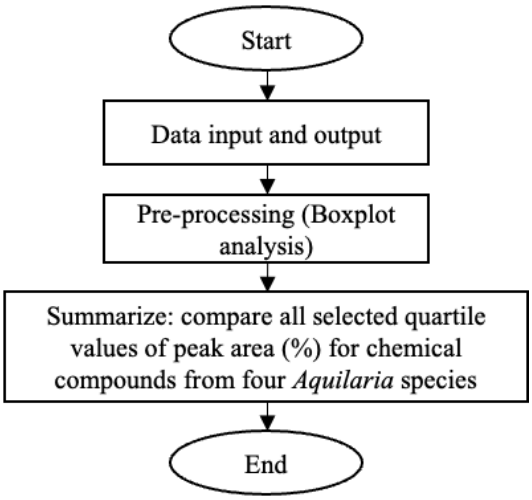

Figure 2. Flowchart of statistical analysis

3. RESULTS AND DISCUSSION

This section presents the boxplot analysis of *Aquilaria* essential oil from agarwood trees, focusing on four specific species which are *Aquilaria beccariana*, *Aquilaria malaccensis*, *Aquilaria crassna*, and *Aquilaria subintegra*. The dataset includes 405 samples from four *Aquilaria* species. Fourteen significant chemical compounds coded by acronyms a, b, c, d, e, f, g, h, i, j, k, l, n, and m stand for *allo*-aromadendrene, *β*-selinene, dihydro-*β*-agarofuran, *δ*-guaiene, *α*-calacorene, caryophyllene oxide, tetradecanal, 10-epi-*γ*-eudesmol, jinkoh-eremol, selina-3,11-dien-9-ol, 9,11-eremophiladien-8-one, selina-3,11-dien-14-oic acid, pentadecanoic acid, and 2-hydroxyguaia-1(10),11-dien-15-oic acid, respectively, were selected based on their peak area (%). These compounds served as input, while the output for the classification system was determined by the chemical composition of the four *Aquilaria* species. Two samples from each of the gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) datasets for the same species were used. MATLAB software version R2021b, was employed for the statistical analysis.

Figure 3 displays a boxplot for chemical compounds in *Aquilaria beccariana*. The figure illustrates varying median values for each compound. Compounds a, j, and m have lower adjacent values beyond the lower whiskers, indicating a lower distribution. In contrast, compounds e, h, and k have upper adjacent values beyond the upper whiskers, suggesting higher distribution. Compounds b, d, f, i, l, and n have an interquartile range (IQR) without whiskers, emphasizing a more concentrated distribution. Compound g's median value aligns with the 75th percentile, while compound c's median value aligns with both the 75th percentile and upper adjacent, indicating central and upper distribution tendencies. Compounds c and g accommodate outliers beyond the upper whiskers. The data can be classified into two groups where a, j, m, e, h, k, and g with whiskers ranges, and b, d, f, i, l, n, and c with no whisker ranges.

Figure 4 shows the lower quartile represents the 25th percentile (Q1) of the data while the upper quartile represents the 75th percentile (Q3). The distance between Q1 and Q3 is referred to as the interquartile range (IQR). The vertical line positioned at the center of the boxes signifies the 50th percentile, commonly known as the median (Q2). The range for the *Aquilaria beccariana* species is observed to be between 0.102 and 2.026. Notably, compound an exhibit the highest Q1 at 1.815, closely followed by compound l at 1.573. Similarly, compound a dominates Q2 with a value of 1.897, with compound l following at 1.595. Examining Q3, compound an again leads with 2.026, and compound l follows with 1.625.

Figure 5 demonstrates the significant chemical compounds identified in *Aquilaria malaccensis*. Remarkably, compounds a, d, e, g, h, i, j, l, and m illustrate the median value aligning with the 25th percentile and lower adjacent, whereas the remaining compounds feature the median value aligning with the 75th percentile and upper adjacent. As a result, the data pertaining to significant chemical compounds in agarwood oils from the *Aquilaria malaccensis* species can be separated into two different groups. One group involved

chemical compounds with median values aligned with the upper adjacent value, while the other group had median values aligned with the lower adjacent value. Notably, there are no outliers observed among the chemical compounds in this species. Figure 6 reveals that the range for the *Aquilaria malaccensis* species spans from 0.018 to 6.728. The highest Q1 is observed for compound h at 6.329, followed by compounds m, d, and k, listing values of 3.714, 1.968, and 1.769, respectively. Similarly, the maximum Q2 is associated with compound h at 6.329, followed by compound m at 3.714. Regarding Q3, the peak area is highest for compound h at 6.728.

Figure 7 displays the boxplot for significant chemical compounds found in *Aquilaria crassna*. Notably, compounds a and j exhibit lower adjacent values beyond the lower whiskers, while compounds h, i, k, and l display upper adjacent values outside the upper whiskers of the boxplot. However, it is noteworthy that compound l stands out with an outlier outside the lower whiskers of the boxplot, situated below the lower adjacent value. Concerning compound m, the median value aligns with the 25th percentile value, while compounds c, e, and g coincide with the 75th percentile. Compounds b, d, f, and n have an interquartile range (IQR) without whiskers. Consequently, the data on significant chemical compounds in *Aquilaria crassna* species of agarwood oils can be categorized into two groups which are b, d, f, and n, exhibiting no whiskers ranges, and the rest having whiskers range. Examining Figure 8, it is evident that the range for *Aquilaria crassna* species spans from 0.015 to 14.771. Compound a standout with the highest Q1 value at 14.539, followed by compounds i and l, listing values of 8.061 and 7.098, respectively. Notably, compounds b, d, and e exhibit the lowest quartile values of 0.110, 0.210, and 0.198, respectively, contributing to the median Q2 value. The maximum Q3 value is associated with compound a at 14.771.

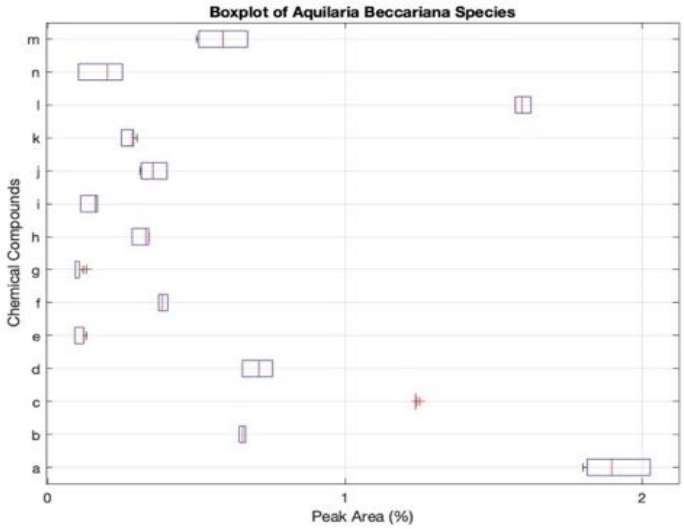

Figure 3. Boxplot of chemical compounds in *Aquilaria beccariana* species

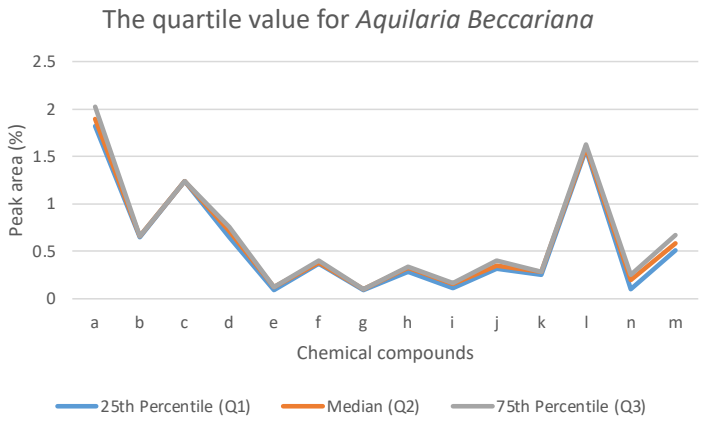

Figure 4. The quartile value for *Aquilaria beccariana*

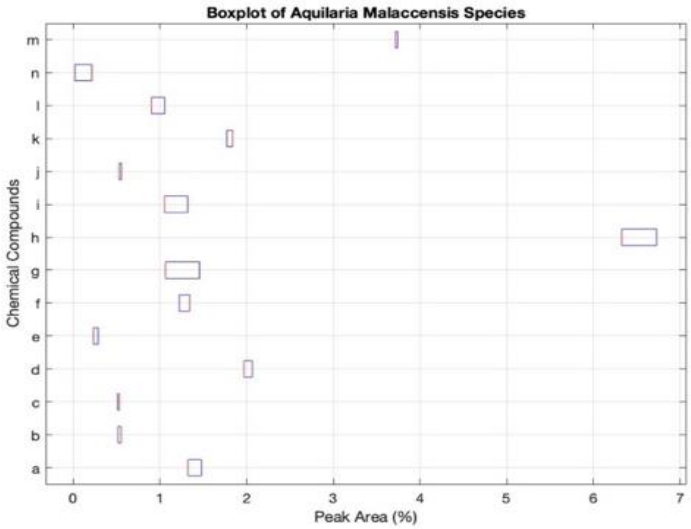

Figure 5. Boxplot of chemical compounds in *Aquilaria malaccensis* species

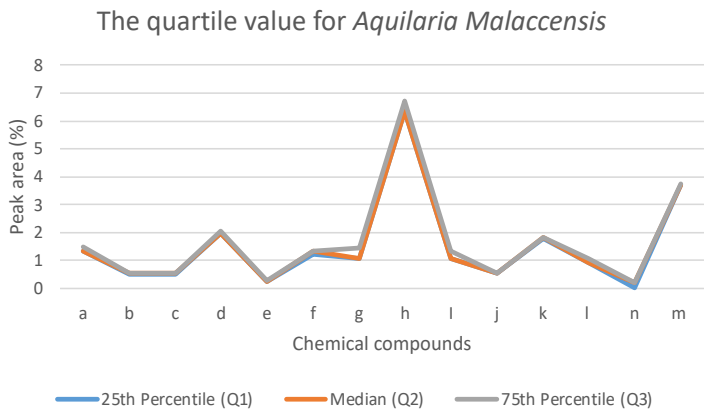

Figure 6. The quartile value for *Aquilaria malaccensis*

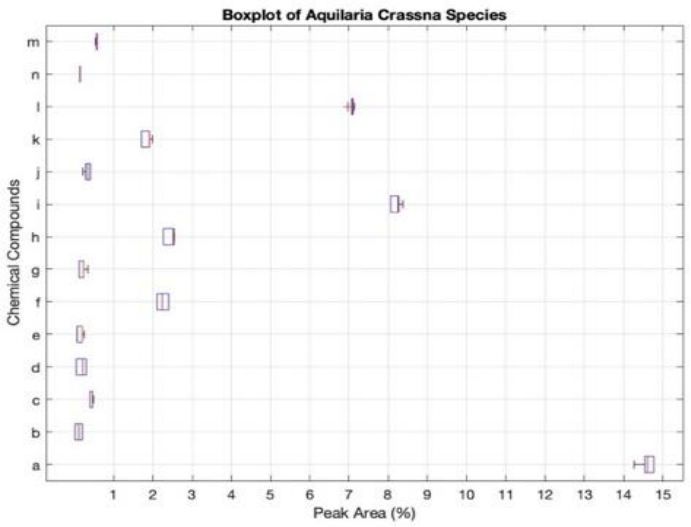

Figure 7. Boxplot of chemical compounds in *Aquilaria crassna* species

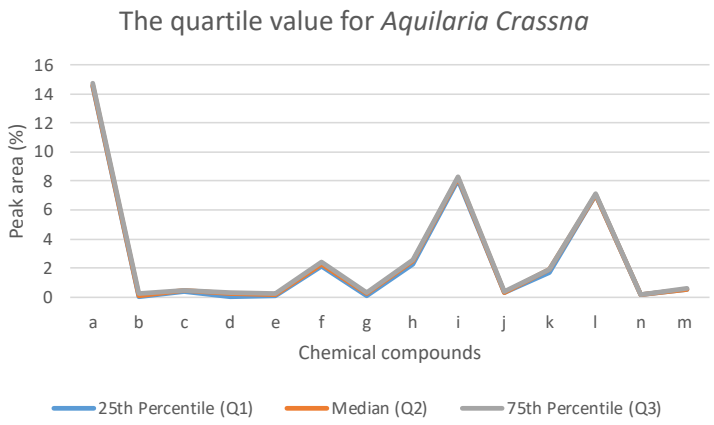

Figure 8. The quartile value for *Aquilaria crassna*

Figure 9 presents a boxplot illustrating significant chemical compounds identified in *Aquilaria subintegra*. Specifically, compounds a and n display lower adjacent values beyond the lower whiskers, while compounds f, h, j, and k exhibit upper adjacent values outside the upper whiskers of the boxplot. For compound d, the median value aligns with the 25th percentile, and for compound m, it aligns with the 75th percentile. Compounds b, c, e, g, i, and l show an interquartile range (IQR) without whiskers. Notably, there are no outliers among the chemical compounds in this species. Consequently, the data on significant chemical compounds in *Aquilaria subintegra* agarwood oils can be classified into two groups which are compounds b, c, e, g, i, and l exhibiting no whiskers ranges, and the remaining compounds having whiskers ranges. Figure 10 reveals that the range for *Aquilaria subintegra* species extends from 0.230 to 13.248. Compound a boast the highest Q1 value at 12.811, followed by compound l at 3.801. Notably, compound a lead in peak area with the highest median Q2 value, listing 13.026, while compound e records the lowest median Q2 value at 0.315. For Q3, the highest value is associated with compound a at 13.248, followed by compound l at 4.181.

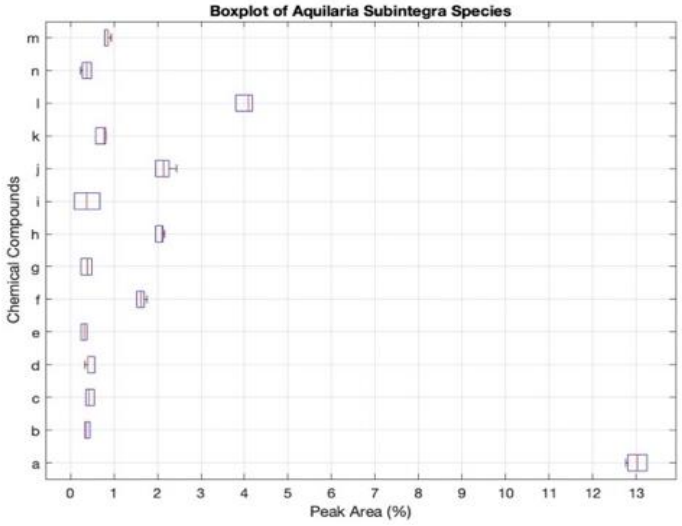

Figure 9. Boxplot of chemical compounds in *Aquilaria subintegra* species

To summarize, the boxplot analysis visually represented the performance of all samples, showcasing the behavior of each compound. Most boxplots fell into two categories which are, one with whiskers ranges for chemical compounds and another without whiskers ranges. *Aquilaria malaccensis* displayed unique behavior, falling into two groups which are one with median values aligned with the upper adjacent value and the other with median values aligned with the lower adjacent value. The graph provided insights into the samples' performance, and five compounds with the highest peak area values were selected for each species.

Subsequently, the selected compounds were sorted based on how frequently they were chosen within each species. As listed in Table 2, these compounds were coded as a, f, h, l, and m, representing *allo*-aromadendrene, caryophyllene oxide, 10-epi-γ-eudesmol, selina-3,11-dien-14-oic acid, and 2-hydroxyguaia-1(10),11-dien-15-oic acid, respectively. The quartile values include the 25th percentile (Q1), median (Q2), and 75th percentile (Q3) for four *Aquilaria* species, specifically *A. beccariana* (designated as AB1, AB2, and AB3), *A. malaccensis* (designated as AM1, AM2, and AM3), *A. crassna* (designated as AC1, AC2, and AC3), and *A. subintegra* (designated as AS1, AS2, and AS3). The classification of data using boxplots highlighted the distinct characteristics of each *Aquilaria* species, serving as reference points for their essential oil. The successful classification based on chemical composition yielded significant outcomes, with boxplots accurately representing unique characteristics of each species group. The study's findings revealed distinct patterns of chemical compounds for each agarwood oil species. The boxplot technique demonstrated effectiveness in classifying species, showcasing commendable performance in the analysis.

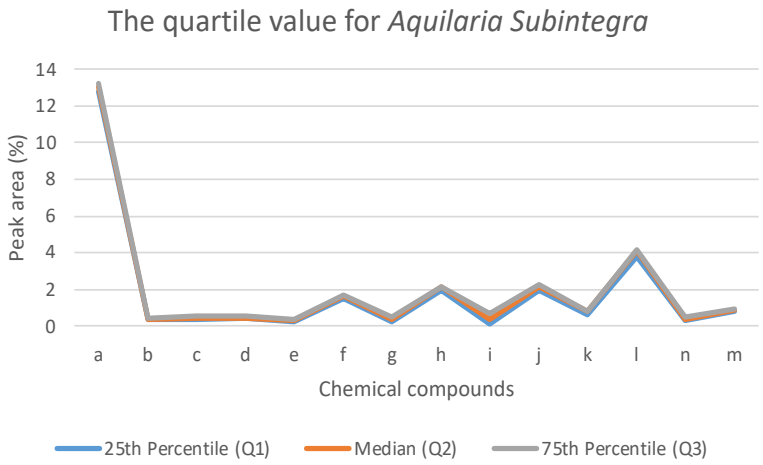

Figure 10. The quartile value for *Aquilaria subintegra*

Table 2. Highest quartile values in the four *Aquilaria* species

	25th Percentile (Q1)				Median (Q2)				75th Percentile (Q3)			
Code	AB1	AM1	AC1	AS1	AB2	AM2	AC2	AS2	AB3	AM3	AC3	AS3
a	1.815	1.321	14.539	12.811	1.897	1.897	14.610	13.026	2.026	1.478	14.771	13.248
f	0.372	1.221	2.107	1.522	0.385	1.344	2.235	1.618	0.403	1.344	2.402	1.697
h	0.283	6.329	2.264	1.957	0.330	0.330	2.513	2.105	0.339	6.728	2.536	2.119
l	1.573	0.901	7.069	3.801	1.595	1.595	7.083	4.090	1.625	1.052	7.098	4.181
m	0.507	3.714	0.560	0.790	0.589	0.589	0.589	0.865	0.672	3.740	0.582	0.940

4. CONCLUSION

The study presented in this paper has successfully achieved its objective by analyzing the *Aquilaria* species of agarwood oil through boxplot analysis. This chosen technique effectively presents the distribution shape, data variability, and significant values. Utilizing the boxplot method, which incorporates lower (Q1), median (Q2), and upper (Q3) quartiles, accurately differentiates between the types of samples from four *Aquilaria* oils species. The application of the boxplot technique for classifying *Aquilaria* oil samples not only contributes methodologically to essential oil analysis, but also holds practical significance for the community. Additionally, the findings suggest future extensions, including the integration of machine learning for automated classification. This interdisciplinary approach has broader implications for the research field by emphasizing the importance of visual tools in data analysis. In summary, the boxplot and table from these species yield results highlighting five compounds coded by a, f, h, l, and m, consist of *allo*-aromadendrene, caryophyllene oxide, 10-epi-γ-eudesmol, selina-3,11-dien-14-oic acid, and 2-hydroxyguaia-1(10),11-dien-15-oic acid, respectively, as crucial chemical substances for future analyses, indicating high peak area values across all *Aquilaria* species. The findings of this research are meaningful and offering noticeable benefits for both the research community and the well-being of the local community, particularly in the analysis of agarwood oils species in Malaysia. It has contributed to the agarwood oil industry as well as its research area.

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