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Statistical analysis of agarwood oil chemical compound exists in four species of *Aquilaria*

Amir Hussairi Zaidi[1], Anis Hazirah 'Izzati Hasnu Al-Hadi[1], Siti Mariatul Hazwa Mohd Huzir Hassan[2], Zakiah Mohd Yusoff[2], Nurlaila Ismail[1], Ali Abd Almisreb[4], Saiful Nizam Tajuddin[3], Mohd Nasir Taib[1]
[1]School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA, Selangor, Malaysia
[2]School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA, Johor, Malaysia
[3]BioAromatic Research Centre of Excellence, Universiti Malaysia Pahang, Gambang, Malaysia
[4]Faculty of Computer Science and Engineering, International University of Sarajevo, Sarajevo, Bosnia and Herzegovina

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ABSTRACT

Aquilaria, renowned for its agarwood, and valued for its aromatic wood and rich resin, finds use in cosmetics, fragrances, incense, and medicine. Identifying the agarwood-producing species among 21 species of *Aquilaria* is challenging. This study analyzes chemical compounds in agarwood oil from 4 *Aquilaria* species: *Aquilaria beccariana*, *Aquilaria crassna*, *Aquilaria malaccensis*, and *Aquilaria subintegra* using gas chromatography-flame ionization detector (GC-FID). Statistical analysis explores compound abundance, employing methods like mean and Z-score tests. This analysis summarizes those 14 compounds that are consistently present based on zero and non-zero observations, and the Z-score test highlights five chemical compounds, with three compounds appearing in both analyses. These compounds can serve as a reference for future studies on *Aquilaria* species and agarwood oil, enhancing classification efforts.

Corresponding Author:

Nurlaila Ismail
School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA
40450 Shah Alam, Selangor, Malaysia
Email: nurlaila0583@uitm.edu.my

1. INTRODUCTION

Aquilaria, part of the Thymelaeaceae family, is best known for producing gaharu or agarwood, from its tree trunks, valued for its distinctive aroma and high-quality resin [1], [2]. Among the 21 known species, only 13 to 15 can produce agarwood, with *Aquilaria malaccensis* being the primary source [3]-[5]. The *Aquilaria* tree is notable not for its branches or bark but for the unique resin produced by its trunks, which appeals to consumers [6], [7]. The resin, which forms in response to natural or artificial injuries, is used in cosmetics, fragrances, incense, and pharmaceuticals [2], [8].

Traditional methods for classifying and grading *Aquilaria* species rely heavily on human expertise, assessing morphological features like leaf shape and size [9], [10]. However, the reliance on human evaluation poses challenges, as prolonged and repetitive tasks can lead to fatigue, reduced accuracy, and a labor-intensive, time-consuming process [11]. To improve accuracy and efficiency, chemical compound analysis through gas chromatography (GC) is used. Techniques like gas chromatography-mass spectrometry (GC-MS) or gas chromatography-flame ionization detector (GC-FID) help identify chemical profiles, aiding in species classification based on quality and grade [12]-[14].

This paper aims to use data from GC analysis to statistically analyze the chemical compound profiles of agarwood oil from various *Aquilaria* species. GC-FID data from four Malaysian *Aquilaria* species: *Aquilaria beccariana*, *Aquilaria crassna*, *Aquilaria malaccensis*, and *Aquilaria subintegra* provided

by BioAromatic Research Centre of Excellence at Universiti Malaysia Pahang [15], is used. Statistical analysis, including descriptive observations and Z-score tests, will identify significant chemical compounds to serve as markers for *Aquilaria* species classification [16], [17], [18], [19], [20].

2. LITERATURE REVIEW

This literature review examines the role of statistical analysis and the Z-score test in analyzing chemical compounds and classifying *Aquilaria* species based on their agarwood oil. It emphasizes the importance of statistical methods in understanding data distribution and patterns [21], [22] and highlights the utility of the Z-score test in standardizing data and assessing deviations [23], [24]. Identifying significant chemical compounds is crucial for classifying *Aquilaria* species and enhancing their overall understanding. The (1) calculates the Z-score, where Z represents the Z-score value, x is the sample value, μ is the population mean and σ is the population standard deviation.

$$Z = \frac{(x - \mu)}{\sigma} \quad (1)$$

Figure 1 shows Z-score values in a normal distribution curve, as referenced in [25] and [26]. Specifically, as Z-scores increase from 2.0 to 3.0, percentiles rise from 97.7 to 99.9%, while as they decrease from -2.9 to -3.0, percentiles drop from 2.3 to 0.1%. Preferred Z-scores fall above the 90th percentile or below the 10th percentile, aligning with p-values less than 0.10, 0.05, or 0.01 [27], [28]. Table 1 summarizes these Z-scores with corresponding p-values and percentiles.

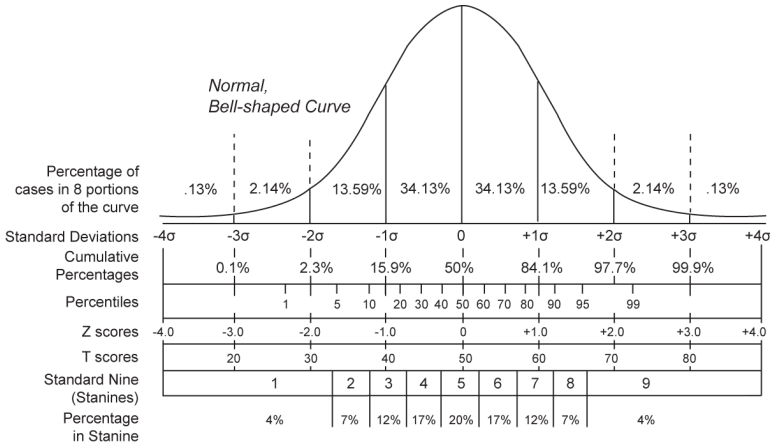

Figure 1. Tabulation of Z-score values in normal distribution curve

Table 1. Summary of Z-score value and p-value with percentiles

Z-score range	p-value	Percentile (confidence level) (%)
Z<-1.65 or Z>1.65	<0.10	90
Z<-1.96 or Z>1.96	<0.05	95
Z<-2.58 or Z>2.58	<0.01	99

3. METHODOLOGY

3.1. Data extraction of GC analysis

Agarwood oil from 4 Malaysian *Aquilaria* species: *Aquilaria beccariana*, *Aquilaria crassna*, *Aquilaria malaccensis*, and *Aquilaria subintegra*-is extracted by BioAromatic Research Centre of Excellence at Universiti Malaysia Pahang. The process involves soaking ground samples in alcohol and oils for a week, followed by hydrodistillation for 3 to 5 days. GC analysis is then conducted using GC-FID and GC-MS systems. The GC-FID uses the Agilent 7890A network system, while the GC-MS utilizes the Agilent 5975C series MSD. The analysis runs for 60 minutes, starting at 60 °C and ramping up to 250 °C. A 1 μL sample is injected at a 1:5 split ratio. The retention times in the chromatogram identifies the chemical compounds. This process yields a dataset of 203 samples and 82 chemical compounds, with data analysis focusing on GC-FID results.

3.2. Data pre-processing

The dataset comprises 203 samples of agarwood oil, containing a total of 82 chemical compounds, resulting in a data dimension of 82 by 203. Specifically, there are 40 samples from *Aquilaria beccariana*, 47 from *Aquilaria crassna*, 61 from *Aquilaria malaccensis*, and 55 from *Aquilaria subintegra*. The extraction procedure is conducted by the BioAromatic Research Centre of Excellence following standard operating procedures (SOP). Next, data from the GC-FID analysis undergo preprocessing to handle missing values for certain compounds in specific *Aquilaria* species, substituting them with zero (0.0) to indicate their absence. Figure 2 shows the flowchart of the experimental set-up and Figure 3 illustrates the algorithm for this missing values substitution.

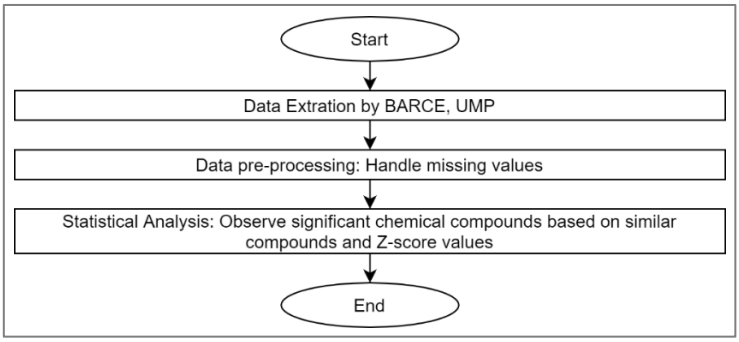

Figure 2. Flowchart of the experimental setup

Handling Missing Value (Subsitution of Zero Values) Algorithm

Input: Data with missing values
Output: Data without missing values

```
load Data
set x = Data
for all samples x do
    find x = Nan
    sub x(Nan) = 0
return Data = x
```

Figure 3. Algorithm for substituting missing values

3.3. Statistical analysis

After handling missing values, the process proceeds to statistical analysis. This analysis involves descriptive observations, focusing on parameters like minimum, maximum, mean, zero, and non-zero values, along with Z-score values, aiming to identify significant chemical compounds. Two approaches are employed: first, identify similar compounds across all four *Aquilaria* species based on non-zero values, and second utilize Z-score tests to validate these compounds. For the first approach, non-zero values indicate compound absence across all species. The second approach selects significant compounds based on Z-score values higher than 1.645, implying p-values smaller than 0.10. This indicates high-grade compounds.

4. RESULTS AND DISCUSSION

4.1. Analysis of chemical compounds that have non-zero value in every species (similar compounds)

Table 2 presents extraction data for *Aquilaria* species, obtained through GC-FID analysis of agarwood oil after replacing missing values with zeros (0.0), and Figures 4-7 illustrate those compound abundances for each species based on the table, zero values indicate the absence of chemical compounds in respective species. There, *Aquilaria malaccensis* exhibits the highest extraction, with 61 samples, followed by *Aquilaria subintegra* (55), *Aquilaria crassna* (47) and *Aquilaria beccariana* (44). Abundance ranges vary among species, with *Aquilaria beccariana* having the lowest (0 to 10.99) while *Aquilaria malaccensis* having the highest (0 to 14.56). From the range of abundance, it can be assumed that all the species have a minimum abundance of zero values (0.0) but difference maximum abundance. *Aquilaria beccariana* has the maximum

abundance of 10.99, *Aquilaria crassna* is 14.48, *Aquilaria malaccensis* is 14.56 and *Aquilaria subintegra* is 13.00. In Figure 4, the top highest abundance in *Aquilaria beccariana* are benzyl benzoate with values of 10.99. In *Aquilaria Crassna* (Figure 5), allo-Aromadendrene exhibits the highest abundances (14.48). In *Aquilaria malaccensis* (Figure 6), 4-phenyl-2-butanone has the highest abundance (14.56) while *Aquilaria subintegra* (Figure 7), allo-Aromadendrene again dominates as the highest abundance (13.00). In summary, *Aquilaria* species has a distinct highest abundance of certain chemical compounds, but not all compounds are present in every species as shown in Table 3. 14 specific compounds (highlighted in yellow in Table 2) are consistently found across every species. These highlighted compounds are presented in Table 4.

Table 2. GC-FID data extraction from 4 species of *Aquilaria*

No	Chemical compound	Abundances (%)				No	Chemical compound	Abundances (%)			
		AB	*AC*	*AM*	*AS*			*AB*	*AC*	*AM*	*AS*
1	β-Gurjunene	0.1	0	0	0	42	β-Selinene	0.66	0.11	0.54	0.37
2	α-Gurjunene	0.13	0	0	0	43	n-Hexadecanoic acid	0	0	0.24	1.53
3	γ-Yetivenene	0.18	0	0	0	44	Heptanoic acid	0	0	0.86	0.91
4	Ethylbenzene	0	0	0.19	0	45	α-Costol	0	0.42	1.26	0.18
5	Benzaldehyde	0	0	0.19	0	46	Tetradecanal	0.13	0.33	1.33	0.39
6	β-Elemene	0.11	0	0.13	0	47	nor-Ketoagarofuran	0	0.75	0.64	0.96
7	β-Vetispirene	0	0.25	0	0	48	1, 5-Diphenyl-3-pentanone	0	0.41	0.76	1.3
8	γ-Selinene	0	0	0	0.3	49	Guaia-1(10), 11-dien-15-oic acid	2.58	0	0	0
9	Hydrocinnamic acid	0	0	0.14	0.21	50	α-Bisabolol	0	1.77	0.72	0.13
10	γ-Cadinene	0.36	0	0	0	51	Hexadecanal	1.65	0	0.79	0.19
11	α-Caryophyllene	0	0	0.38	0	52	Dihyro-β-Agarofuran	1.25	0.49	0.55	0.43
12	Dihydrocolumellarin	0	0.39	0	0	53	Dehydrojinkoh-eremol	0.58	0	0.57	1.93
13	cis-Calamenene	0.17	0.27	0	0	54	Hexanoic acid	0	0	1.75	1.34
14	lsocaryophyllene	0.3	0	0.16	0	55	Nootkatone	1.01	0	2.18	0
15	Valencene	0.33	0.14	0	0	56	Sinenofuranol	1.55	0.19	1.53	0
16	Dehydro-aromadendrene	0	0.25	0.29	0	57	δ-Guaiene	0.71	0.21	2.03	0.34
17	Octanoic acid	0	0	0.54	0	58	Selina-3, 11-dien-9-ol	0.35	0.3	0.52	2.23
18	Methyl palmitate	0.62	0	0	0	59	epi-α-Cadinol	0	0	2.88	1.07
19	γ-Gurjunene	0.2	0	0.18	0.24	60	Nonanoic acid	0	0	0	4.11
20	Kessane	0	0.24	0.42	0	61	Guaiol	0	1.93	0.72	1.47
21	γ-Muurolene	0	0.05	0.29	0.33	62	n-Decanoic acid	0.23	0	0	4.49
22	δ-Cadinene	0.53	0.15	0	0	63	γ-Eudesmol	0.26	0	2.13	1.87
23	Epoxybulnesene	0	0.44	0	0.35	64	cis-Nerolidol	0	3.39	0.93	0.49
24	Dihydrokaranone	0	0	0	0.81	65	9, 11-Eremophiladien-8-one	0.29	1.95	1.87	0.78
25	Elemol	0	0.48	0.17	0.22	66	epi-α-Bisabolol	0	1.37	2.62	0.94
26	Pentadecanoic acid	0.2	0.14	0.18	0.35	67	Caryophyllene oxide	0.39	2.24	1.28	1.65
27	α-Calacorene	0.13	0.25	0.31	0.32	68	2-hydroxyguaia-1(10), 11-dien-15-oic acid	0.59	0.55	3.72	0.93
28	τ-Muurolol	0	0	0.63	0.46	69	Cyperotundone	0.16	5.72	0	0
29	Selina-3, 11-dien-9-one	0	0.32	0.64	0.24	70	Rotundone	0	0	6.57	1.02
30	Cyperene	1.11	0.21	0	0	71	Selina-4, 11-dien-14-oic acid	0	5.73	1.22	1.03
31	n-Dodecanoic acid	0	0.49	0.27	0.56	72	Kusunol	1.39	0	3.26	4.46
32	α-Guaiene	0.71	0	0.68	0	73	α-Eudesmol	0	0	2.93	6.4
33	Selina-4, 11-dien-14-al	0	0.26	1.15	0	74	Bulnesol	0	7.69	1.17	0.93
34	β-Patchoulene	1.48	0	0	0	75	Jinkoh-eremol	0.16	8.3	1.25	0.37
35	α-Agarofuran	0	0.39	0.85	0.3	76	Agarospirol	0	5.46	1.72	3.54
36	Aromadendrene	1.33	0	0	0.21	77	Benzyl benzoate	10.99	0	0	0
37	Guaia-1(10), 11-dien-9-one	0.57	0	0.5	0.48	78	10-epi-γ-Eudesmol	0.33	2.52	6.58	2.13
38	Dehydrofukinone	0	0.17	1.11	0.31	79	β-Eudesmol	0	6.02	2.38	4.62
39	α-Selinene	0	0.25	0.79	0.55	80	Selina-3, 11-dien-14-oic acid	1.6	7.06	0.97	4.09
40	Tetradecanoic acid	0	0.81	0.45	0.35	81	4-phenyl-2-butanone	0	1.19	14.56	1.38
41	Karanone	0	0.79	0.44	0.43	82	allo-Aromadendrene	1.89	14.48	1.32	13

*Note: AB=*Aquilaria beccariana*, AC=*Aquilaria crassna*, AM=*Aquilaria malaccensis*, AS=*Aquilaria subintegra*

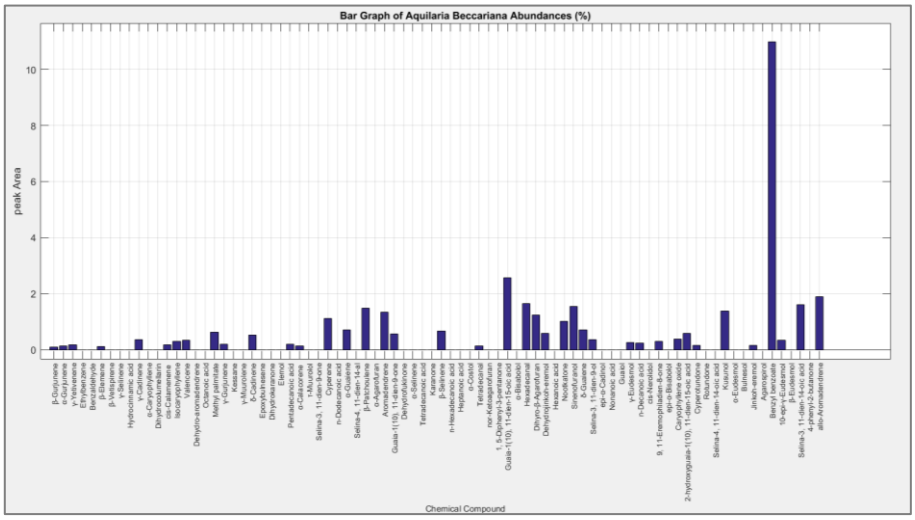

Figure 4. Bar graph of *Aquilaria beccariana* abundances (%)

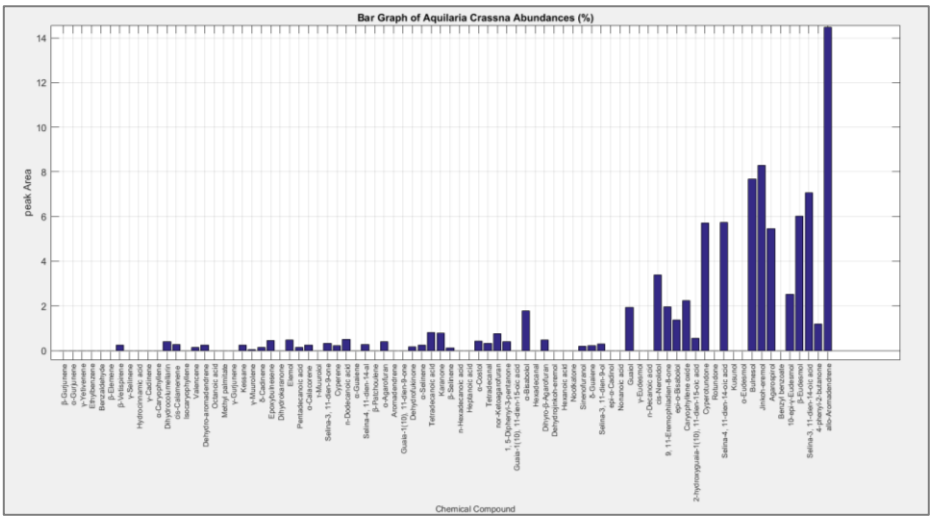

Figure 5. Bar graph of *Aquilaria crassna* abundances (%)

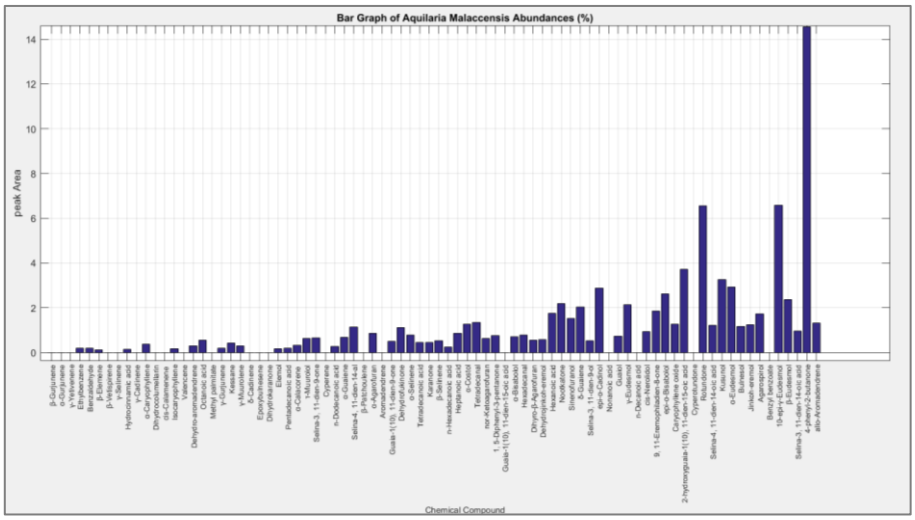

Figure 6. Bar graph of *Aquilaria malaccensis* abundances (%)

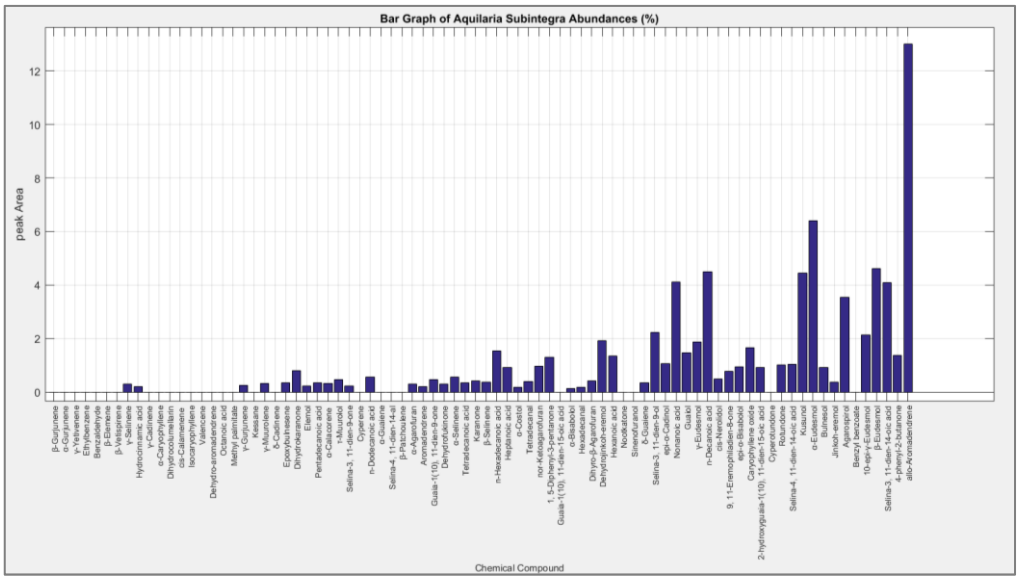

Figure 7. Bar graph of *Aquilaria subintegra* abundances (%)

Table 3. Summary of descriptive observation for each species of *Aquilaria*

Descriptive observation	*Aquilaria beccariana*	*Aquilaria crassna*	*Aquilaria malaccensis*	*Aquilaria subintegra*
Minimum value	0.00	0.00	0.00	0.00
Maximum value	10.99	14.48	14.56	13.00
Range	0.00 to 10.99	0.00 to 14.48	0.00 to 14.56	0.00 to 13.00

Table 4. Summarize chemical compounds that exist across every 4 species of *Aquilaria*

No	Chemical Compound	Abundances (%)			
		Aquilaria beccariana	*Aquilaria crassna*	*Aquilaria malaccensis*	*Aquilaria subintegra*
26	Pentadecanoic acid	0.20	0.14	0.18	0.35
27	α-Calacorene	0.13	0.25	0.31	0.32
42	β-Selinene	0.66	0.11	0.54	0.37
46	Tetradecanal	0.13	0.33	1.33	0.39
52	Dihyro-β-Agarofuran	1.25	0.49	0.55	0.43
57	δ-Guaiene	0.71	0.21	2.03	0.34
58	Selina-3, 11-dien-9-ol	0.35	0.30	0.52	2.23
65	9, 11-Eremophiladien-8-one	0.29	1.95	1.87	0.78
67	Caryophyllene oxide	0.39	2.24	1.28	1.65
68	2-hydroxyguaia-1(10), 11-dien-15-oic acid	0.59	0.55	3.72	0.93
75	Jinkoh-eremol	0.16	8.30	1.25	0.37
78	10-epi-γ-Eudesmol	0.33	2.52	6.58	2.13
80	Selina-3, 11-dien-14-oic acid	1.60	7.06	0.97	4.09
82	allo-Aromadendrene	1.89	14.48	1.32	13.00

4.2. Analysis of chemical compounds using Z-score test

Table 5 shows the mean values and Z-score test results for each chemical compound across all *Aquilaria* species, and Figure 8 shows these Z-scores in a bar graph. The test identifies five compounds with Z-scores exceeding the threshold of 1.645: 10-epi-γ-Eudesmol (1.68), β-Eudesmol (1.99), Selina-3,11-dien-14-oic acid (2.14), 4-phenyl-2-butanone (2.86) and allo-Aromadendrene (5.71).

From all 5 highlighted chemical compounds, 3 compounds-10-epi-γ-Eudesmol, Selina-3,11-dien-14-oic acid, and allo-Aromadendrene-appearing in both analyses. Notably, 10-epi-γ-Eudesmol and allo-Aromadendrene have been previously mentioned by Ismail *et al.* [29] and Al-Hadi *et al.* [30] as significant compounds for high-grade agarwood oil in *Aquilaria malaccensis*. This suggests that these 3 compounds can serve as markers in measuring agarwood oil across different *Aquilaria* species. Statistical analysis is more robust than traditional methods as it provides a broader range of information through graphical distribution patterns.

Table 5. Mean values and Z-score test across 4 species of *Aquilaria*

No	Chemical compound	Sample mean (x̄)	Z-Score (Z)	Z<-1.65 or Z>1.65	No	Chemical compound	Sample mean (x̄)	Z-score (Z)	Z<-1.65 or Z >1.65
1	β-Gurjunene	0.024	-0.73	No	42	β-Selinene	0.416	-0.4	No
2	α-Gurjunene	0.031	-0.72	No	43	n-Hexadecanoic acid	0.441	-0.38	No
3	γ-Yetivenene	0.044	-0.71	No	44	Heptanoic acid	0.443	-0.38	No
4	Ethylbenzene	0.046	-0.71	No	45	α-Costol	0.465	-0.36	No
5	Benzaldehyde	0.046	-0.71	No	46	Tetradecanal	0.544	-0.29	No
6	β-Elemene	0.058	-0.7	No	47	nor-Ketoagarofuran	0.585	-0.26	No
7	β-Vetispirene	0.063	-0.7	No	48	1, 5-Diphenyl-3-pentanone	0.615	-0.23	No
8	γ-Selinene	0.075	-0.69	No	49	Guaia-1(10), 11-dien-15-oic acid	0.644	-0.21	No
9	Hydrocinnamic acid	0.086	-0.68	No	50	α-Bisabolol	0.653	-0.2	No
10	γ-Cadinene	0.089	-0.67	No	51	Hexadecanal	0.655	-0.2	No
11	α-Caryophyllene	0.094	-0.67	No	52	Dihyro-β-Agarofuran	0.675	-0.18	No
12	Dihydrocolumellarin	0.098	-0.67	No	53	Dehydrojinkoh-eremol	0.768	-0.1	No
13	cis-Calamenene	0.108	-0.66	No	54	Hexanoic acid	0.771	-0.1	No
14	lsocaryophyllene	0.114	-0.65	No	55	Nootkatone	0.798	-0.08	No
15	Valencene	0.118	-0.65	No	56	Sinenofuranol	0.815	-0.06	No
16	Dehydro-aromadendrene	0.133	-0.64	No	57	δ-Guaiene	0.823	-0.06	No
17	Octanoic acid	0.135	-0.63	No	58	Selina-3, 11-dien-9-ol	0.85	-0.03	No
18	Methyl palmitate	0.154	-0.62	No	59	epi-α-Cadinol	0.986	0.08	No
19	γ-Gurjunene	0.155	-0.62	No	60	Nonanoic acid	1.028	0.12	No
20	Kessane	0.164	-0.61	No	61	Guaiol	1.03	0.12	No
21	γ-Muurolene	0.165	-0.61	No	62	n-Decanoic acid	1.063	0.15	No
22	δ-Cadinene	0.17	-0.61	No	63	γ-Eudesmol	1.178	0.24	No
23	Epoxybulnesene	0.196	-0.58	No	64	cis-Nerolidol	1.2	0.26	No
24	Dihydrokaranone	0.203	-0.58	No	65	9, 11-Eremophiladien-8-one	1.22	0.28	No
25	Elemol	0.215	-0.57	No	66	epi-α-Bisabolol	1.231	0.29	No
26	Pentadecanoic acid	0.216	-0.57	No	67	Caryophyllene oxide	1.386	0.42	No
27	α-Calacorene	0.25	-0.54	No	68	2-hydroxyguaia-1(10), 11-dien-15-oic acid	1.444	0.47	No
28	τ-Muurolol	0.27	-0.52	No	69	Cyperotundone	1.469	0.49	No
29	Selina-3, 11-dien-9-one	0.299	-0.5	No	70	Rotundone	1.895	0.85	No
30	Cyperene	0.328	-0.47	No	71	Selina-4, 11-dien-14-oic acid	1.993	0.93	No
31	n-Dodecanoic acid	0.329	-0.47	No	72	Kusunol	2.275	1.17	No
32	α-Guaiene	0.345	-0.46	No	73	α-Eudesmol	2.333	1.22	No
33	Selina-4, 11-dien-14-al	0.351	-0.45	No	74	Bulnesol	2.445	1.31	No
34	β-Patchoulene	0.37	-0.44	No	75	Jinkoh-eremol	2.519	1.37	No
35	α-Agarofuran	0.383	-0.43	No	76	Agarospirol	2.679	1.51	No
36	Aromadendrene	0.385	-0.42	No	77	Benzyl benzoate	2.748	1.57	No
37	Guaia-1(10), 11-dien-9-one	0.385	-0.42	No	78	10-epi-γ-Eudesmol	2.888	1.68	Yes
38	Dehydrofukinone	0.396	-0.41	No	79	β-Eudesmol	3.254	1.99	Yes
39	α-Selinene	0.396	-0.41	No	80	Selina-3, 11-dien-14-oic acid	3.428	2.14	Yes
40	Tetradecanoic acid	0.401	-0.41	No	81	4-phenyl-2-butanone	4.281	2.86	Yes
41	Karanone	0.414	-0.4	No	82	allo-Aromadendrene	7.67	5.71	Yes

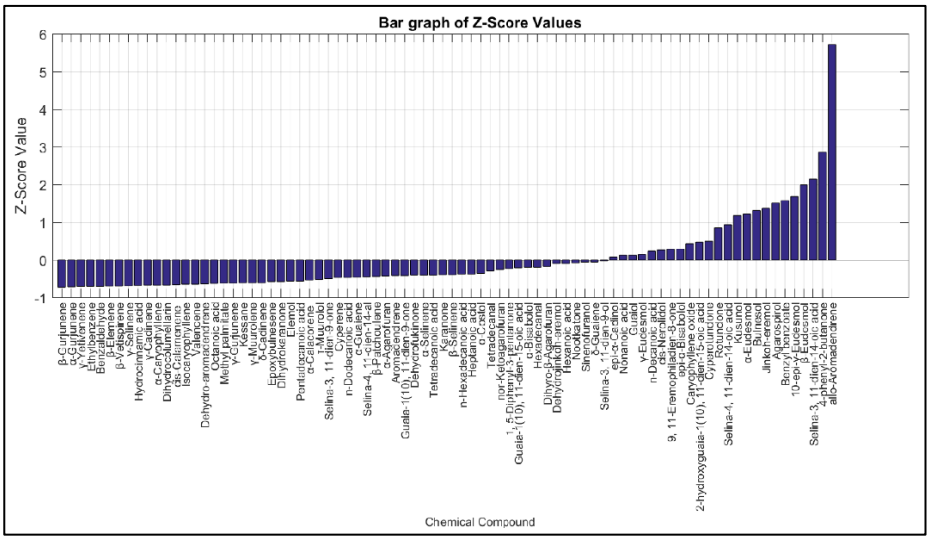

Figure 8. Bar graph of Z-score values

5. CONCLUSION

The study demonstrates that statistical analysis is a reliable method for examining the abundance of chemical compounds in all *Aquilaria* species. It identifies 14 consistently present compounds and highlights 5 compounds using the Z-score test. 3 compounds-10-epi-γ-Eudesmol, Selina-3,11-dien-14-oic acid, and allo-Aromadendrene-are significant in both analyses. These 3 compounds are important in agarwood oil across all *Aquilaria* species since they can serve as markers for future classification studies. The study shows that transitioning from traditional sensory evaluation to chemical compound identification allows for a more objective precise assessment of agarwood oil and *Aquilaria* species classification. This approach validates traditional assessments and offers an accurate method for evaluating agarwood oil based on its chemical properties.

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BIOGRAPHIES OF AUTHORS

Amir Hussairi Zaidi was born in Malaysia, in May 1998. He received his B. Eng. (Hons) in Electronic Engineering from Universiti Teknologi MARA (UiTM) Shah Alam, Selangor, Malaysia. He currently pursuing his studies as a postgraduate student at the School of Electrical Engineering, College of Engineering, at Universiti Teknologi MARA (UiTM) Shah Alam, Selangor, Malaysia. He can be contacted at email: amirxhussairi@gmail.com.

Anis Hazirah 'Izzati Hasnu Al-Hadi was born in Malaysia, in July 1998. She received her B. Eng (Hons) in Electronics Engineering from Universiti Teknologi MARA (UiTM) Shah Alam, Selangor, Malaysia. She is currently an ASIC Design Engineer at Microchip Operations (Malaysia) Sdn Bhd and at the same time, she is a full-time postgraduate student at the School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA (UiTM) Shah Alam, Selangor, Malaysia. She can be contacted at email: anshzrh@gmail.com.

Siti Mariatul Hazwa Mohd Huzir Hassan was born in Malaysia, in May 1997. She is currently pursuing her studies as a postgraduate student majoring in [2]School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA, Cawangan Johor, Kampus Pasir Gudang, Johor, Malaysia. She received her B. Eng (Hons) in Electronics Engineering from Universiti Teknologi MARA (UiTM) Shah Alam, Selangor, Malaysia. She can be contacted at email: mariatulhazwa97@gmail.com.

Assoc. Prof. Ts. Dr. Zakiah Mohd Yusoff received her Bachelor's Degree in Electrical Engineering and PhD in Electrical Engineering from Universiti Teknologi MARA Shah Alam, in 2009 and 2014, respectively. She is a senior lecturer who is currently working at the School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA Kampus Pasir Gudang, Malaysia. In Mei 2014, she joined Universiti Teknologi MARA as a teaching staff. Her major interests include process control, system identification, and essential oil extraction systems. She can be contacted at email: zakiah9018@uitm.edu.my.

Assoc. Prof. Ir. Ts. Dr. Nurlaila Ismail received her Ph.D. in Electrical Engineering from Universiti Teknologi MARA, Malaysia. She is currently a senior lecturer at the School of Electrical Engineering, College of Engineering, Universiti Teknologi MARA Shah Alam, Malaysia. Her research interests include advanced signal processing and artificial intelligence. She can be contacted at email: nurlaila0583@uitm.edu.my.

Assoc. Prof. Ali Abd Almisreb received a Master's Degree in Computer Science and a Doctorate Doctor's Degree in Electrical Engineering/Computer Engineering from Universiti Teknologi MARA, Malaysia. He is currently an associate professor at the Faculty of Computer Sciences and Engineering, Director of the Graduate Council, and Editor in Chief at the International University of Sarajevo. His major interests include deep learning, machine learning, computer vision voice recognition, and quantum computing. He can be contacted at email: alimes96@yahoo.com.

Prof. Dr. Saiful Nizam Tajuddin received his Ph.D. from Universiti Malaysia Pahang (UMP). He is a professor and director of the Bioaromatic Research Center of Excellence (BARCE) at Universiti Malaysia Pahang. He is a director and researcher at Synbion Sdn Bhd, Kuantan, Pahang, Malaysia. He has been a very active researcher and over the years has authored and/or co-authored many papers published in refereed journals and conferences. He can be contacted at email: saifulnizam@ump.edu.my.

Prof. Ir. Ts. Dr. Haji Mohd Nasir Taib received his Ph.D. from the University of Manchester Institute of Science and Technology (UMIST), UK. He is a Senior Professor at Universiti Teknologi MARA (UiTM). He heads the Advanced Signal Processing Research Group at the School of Electrical Engineering, College of Engineering, UiTM. He has been a very active researcher and over the years has authored and/or co-authored many papers published in refereed journals and conferences. He can be contacted at email: dr.nasir@uitm.edu.my.