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Identification of chemical markers for species differentiation in *Aquilaria* essential oils using self-organizing maps

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ABSTRACT

This study analyzes the chemical diversity of essential oils from four *Aquilaria* species, *A. beccariana, A. malaccensis, A. crassna*, and *A. subintegra*, which are important sources of agarwood used in perfumery and traditional medicine. Despite their economic and ecological value, the chemical profiles of these species remain insufficiently characterized, hindering accurate species differentiation and resource management. This research aims to identify distinctive chemical patterns to improve species classification. Self-organizing maps (SOMs) were employed to analyze complex chemical composition data and to identify significant compounds responsible for species separation. The analysis revealed several compounds with strong discriminatory power and species-specific distribution patterns, with compounds C, D, and E identified as the most significant markers. These findings demonstrate substantial biochemical diversity among *Aquilaria* species and confirm the effectiveness of SOM for essential oil profiling. The results support improved species identification and have important implications for ecological conservation, sustainable agarwood management, and pharmacological development.

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1. INTRODUCTION

The *Aquilaria* genus, which includes species such as *A. beccariana, A. malaccensis, A. crassna, and A. subintegra*, is widely known for its production of agarwood. Agarwood is a resinous material highly valued in perfumery, traditional medicine, and cultural practices [1]–[3]. It forms through a complex chemical process caused by environmental and biological stress, leading to the concentration of compounds like natural oils, chromones (a type of organic compound), and phenolic substances [2], [3]. These compounds are not only responsible for the unique aroma of agarwood but also serve as chemical markers that assist in species identification [3]. Accurate identification is critical for protecting the environment and ensuring the sustainable use of this resource [3]. Given the diverse and intricate chemical profiles of *Aquilaria* species, advanced analytical methods are required to capture this complexity, as traditional techniques often prove insufficient [3]–[5].

Among the natural compounds found in agarwood, certain plant-based oils play an important role in determining its chemical composition. Studies have shown that the amount of these oils in agarwood essential oil differs between species and is influenced by the methods used to extract the oil [3], [6].

Specific oils, such as aromadendrene, have been identified as markers for assessing the quality of agarwood [7], [8]. These findings underline the importance of identifying significant compounds for distinguishing between species while also enhancing the understanding of their roles in nature and their potential pharmaceutical applications [4], [9].

Understanding the chemical composition of *Aquilaria* species is critical for species identification, sustainable harvesting, and the development of pharmacological applications [3], [9]. Despite numerous studies exploring agarwood's chemical properties, the challenge of species differentiation based on chemical markers remains [3], [10]. Additionally, the chemical composition of agarwood is strongly influenced by the environmental conditions in which it forms, highlighting the importance of a systematic approach to studying *Aquilaria* species, particularly in relation to conservation and sustainable use [3], [11], [12].

Self-organizing maps (SOMs), an unsupervised machine learning method, offer a powerful approach for analyzing high-dimensional biological data [13]. SOMs are effective in clustering and visualizing complex datasets, enabling researchers to uncover patterns and relationships that might not be easily detected using traditional statistical methods [13], [14]. By converting multi-dimensional data onto a two-dimensional grid, SOMs provide an accessible visual representation of the chemical and biological diversity within *Aquilaria* species [13], [15]. Although SOMs have been widely used in areas like chemotaxonomy and metabolomics, their application to agarwood analysis has been limited. When combined with quantitative methods, such as boxplots, SOMs create a thorough framework for understanding the specific variations in compounds and their biological importance [16].

Compared with traditional clustering techniques such as principal component analysis (PCA) and hierarchical clustering, SOM excels at preserving the topological structure of high-dimensional data, providing more intuitive and accurate insights into complex datasets [13], [14], [17]. This makes SOM particularly effective for identifying subtle chemical patterns and relationships, which are critical for species-specific classification and ecological studies [13], [15]. The use of SOM in this study allows for the detailed mapping of chemical diversity and the identification of significant markers that differentiate *Aquilaria* species, contributing to both scientific understanding and practical conservation efforts.

This study uses SOMs to explore the chemical diversity among *Aquilaria* species and identify significant chemical compounds for species classification. It also helps reduce the number of input variables by focusing on relevant data and removing unnecessary information [16]. The findings aim to provide insights into the evolutionary adaptations of these species and their responses to environmental stress [2], [3]. These insights are crucial for sustainable agarwood management, considering the growing global demand for agarwood and the increasing risks of overexploitation [3], [13], [18]. Therefore, accurate chemical characterization is essential for conservation planning and ensuring the long-term survival of *Aquilaria* populations [3], [11], [18]. Overall, this research provides valuable insights to support efforts in protecting these species and enhancing the ecological and medicinal potential of agarwood.

2. METHOD

This section describes the methodology used to analyze the essential oils from various *Aquilaria* species, with a focus on sample extraction, compound identification, and advanced data analysis techniques. The approach ensures accurate chemical profiling, aiding in the differentiation of species based on their chemical composition. The section is divided into two subsections: 2.1 data collection and experimental setup, detailing the sample preparation, extraction methods, and the use of gas chromatography-mass spectrometry (GC-MS) coupled with gas chromatography-flame ionization detector (GC-FID) for compound detection; and 2.2 SOM analysis, which outlines the use of machine learning techniques to identify underlying patterns in the data. This combined approach enhances the precision of chemical analysis and species classification. The overall experimental and analytical workflow of the study, from sample preparation to species differentiation, is summarized in Figure 1.

2.1. Data collection and experimental setup

The essential oil samples evaluated in this study were derived from various *Aquilaria* species and prepared by the BioAromatic Research Centre of Excellence (BARCE) at Universiti Malaysia Pahang Al-Sultan Abdullah (UMPSA). The chemical profiling of these samples was conducted using both GC-MS and GC-FID. A total of 720 oil samples were analyzed, focusing on six principal chemical constituents: β-Selinene, Dihydro-β-agarofuran, δ-Guaiene, 10-epi-γ-Eudesmol, γ-Eudesmol, and Pentadecanoic acid, labelled as compounds A through F, respectively. These compounds were identified in oils extracted from four *Aquilaria* species: *A. beccariana* (AB), *A. malaccensis* (AM), *A. crassna* (AC), and *A. subintegra* (AS). The relative abundance of each compound, expressed as peak area (%) from GC-MS analysis, was used to compare the chemical profiles across species.

The extraction of essential oils was performed using a method that involved soaking agarwood chips in water for several days to soften the oil-bearing structures. This was followed by hydrodistillation, which lasted three to five days. After extraction, the oils were diluted with analytical-grade dichloromethane (DCM) to prepare them for analysis. For GC-MS, an Agilent 7890B GC system coupled with an Agilent 5977A mass spectrometer detector (MSD) was utilized. The analysis employed a DB-1ms column (30 m×250 μm×0.25 μm) with helium as the carrier gas at a flow rate of 1.0 mL/min. The oven was initially set to 80 °C and ramped up by 3 °C/min until it reached 250 °C, where it was held for three minutes. The mass spectrometer operated in electron ionization (EI) mode at 70 eV. Identification of compounds was based on spectral comparison with entries in the NIST library, using a minimum match threshold of 80%. For the GC-FID analysis, the same GC system was employed, but with a flame ionization detector set at 250 °C to quantify the target compounds. The combined results from GC-MS and GC-FID provided quantitative peak area data for each compound, facilitating a comprehensive comparison of chemical constituents across the four *Aquilaria* species.

Table 1 provides a summary of the dataset, comprising 720 samples representing the four *Aquilaria* species. The six selected Compounds (A, B, C, D, E, and F) were consistently present across all samples, as evidenced by their prominent peak areas (%) in the chromatographic analyses [19]. These compounds are recognized as important indicators of agarwood quality, influencing both the fragrance profile and resin composition of the oils [4], [20]. Among them, Compounds A, C, and E are particularly associated with the distinctive aroma and resinous properties of agarwood. Compounds B and D are notable for their biological relevance, while F, although less prominent, serves as a secondary marker for species differentiation. The reliable presence and significant representation of these compounds in GC-MS and GC-FID analyses made them ideal for further investigation and classification. For data analysis and classification, the chemical composition data were processed using MATLAB (version R2024a). The selected compounds served as inputs, while the species classification served as the output, forming the basis for further computational and statistical analyses.

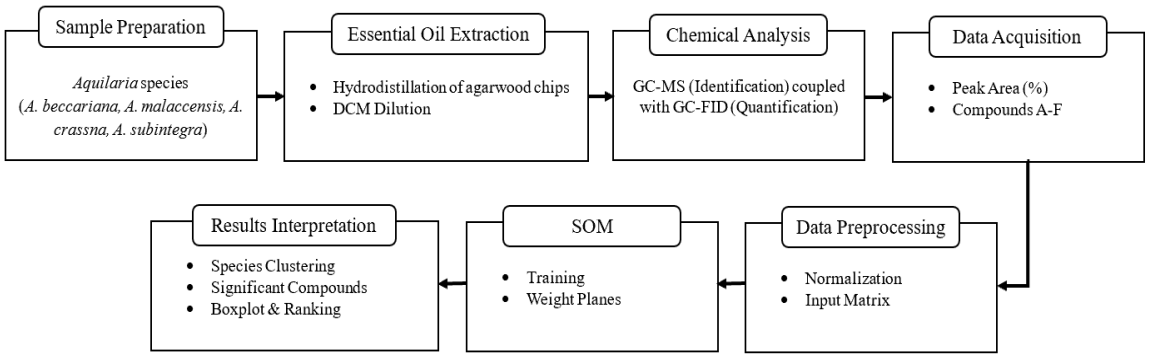

Figure 1. SOM workflow for chemical marker identification

Table 1. Distribution of chemical compounds and their peak areas % among *Aquilaria* species

Code	Compounds	Indent, mode	Peak area (%)			
			AB	AM	AC	AS
A	β-Selinene	MS, FID	0.66	0.56	0.11	0.37
B	Dihyro-β-agarofuran	MS, FID	1.25	0.55	0.48	0.44
C	δ-Guaiene	MS, FID	0.74	2.02	0.21	0.35
D	10-epi-γ-Eudesmol	MS, FID	0.34	6.73	2.54	2.16
E	γ-Eudesmol	MS, FID	0.26	2.17	0.95	1.85
F	Pentadecanoic acid	MS, FID	0.15	0.15	0.14	0.46

Before the SOM analysis, the chemical composition data were preprocessed to ensure consistency and comparability across samples. The peak area (%) of compounds A through F were normalized to reduce scale differences and minimize bias arising from variation in compound abundance. The normalized values were then arranged into an input matrix, with each row representing an individual oil sample and each column corresponding to a selected chemical compound. Species labels were assigned as output variables. This input matrix served as the basis for subsequent SOM training and analysis.

2.2. Self-organizing map analysis

SOM is a powerful unsupervised machine learning technique for analyzing and visualizing high-dimensional datasets. By mapping multi-dimensional input data onto a two-dimensional grid, SOM reveals hidden patterns, clusters, and relationships, making it particularly useful in biological and chemical research involving complex, multi-variable data [13], [17], [21]. A key advantage of SOM lies in its capacity to preserve the topological relationships of the input data while grouping similar observations and emphasizing differences among them [22]. Component or weight plane illustrate the distribution of individual variables across the SOM grid through color gradients, where variations in color intensity correspond to relative weight values for each variable at the neuron level [15].

Another important visualization tool for SOMs is the unified distance matrix (U-matrix), which depicts the distances between neighboring neurons using a color scale. In this representation, darker colors typically indicate larger inter-neuron distances corresponding to cluster boundaries, whereas lighter colors denote smaller distances within clusters, thereby facilitating the identification of cluster structure and relationships [15], [23]–[26]. The U-matrix is often paired with sample distribution maps and component planes for deeper insights into data structures [26], [27]. In the context of *Aquilaria* species, SOM has proven valuable in distinguishing chemical markers that are specific to certain species or environmental conditions, such as certain aromatic chemicals (e.g., plant-based oils and resins), chromones (a type of organic molecule) and other secondary metabolites that contribute to the unique characteristics of agarwood [13], [15]. Furthermore, SOM exhibits strong robustness when applied to large and noisy datasets, particularly in situations where high data complexity presents significant analytical challenges [28].

Numerous studies highlight the effectiveness of SOM across various fields. For instance, SOM has been used to classify plant species by their chemical composition, distinguishing medicinal plants based on volatile organic compounds [28]. In *Aquilaria* research, SOM has analyzed the chemical diversity of agarwood oils, uncovered species-specific chemical profiles and provided insights into their ecological and pharmacological properties [13], [15]. By visualizing complex data, SOM identifies chemical markers crucial for species differentiation, conservation, and sustainable resource management. When combined with methods like boxplots or hierarchical clustering, SOM forms a robust framework for interpreting species-specific biochemical characteristics.

Recent studies have investigated chemical diversity among *Aquilaria* species using SOMs, leading to the identification of chemical markers that distinguish species and to improved understanding of their ecological roles, evolutionary adaptation, and potential applications in pharmacology and conservation [13]. Owing to its capacity to handle complex, high-dimensional datasets, SOM has emerged as an essential analytical tool in chemical ecology and sustainable natural resource management. In this context, its application to agarwood research is particularly significant. Analysis of chemical profiles across different *Aquilaria* species using SOM enables the identification of key discriminative compounds and provides insight into species-specific ecological interactions and evolutionary history.

3. RESULTS AND DISCUSSION

This section presents the findings of the study, focusing on the identification and analysis of significant chemical markers for species differentiation in the *Aquilaria* genus. The results provide valuable insights into the chemical composition of AB, AM, AC, and AS, highlighting the importance of specific chemical compounds in distinguishing these species. The discussion includes an analysis of clustering patterns based on the SOM approach, the ranking of compound abundance, and the identification of signature compounds. These findings shed light on the potential ecological, metabolic, and pharmacological implications of the chemical diversity within these species.

3.1. Identification of key chemical markers for species differentiation

The SOM approach was applied to analyze the distribution and relative importance of chemical compounds across four *Aquilaria* species. Figure 2 illustrate the SOM weight planes, which depict clustering patterns based on compounds 1 to 6. In these visualizations, lighter colors (e.g., yellow and bright red) represent higher weight values, indicating stronger similarities between input data and corresponding clusters, while darker colors (e.g., deep red and black) denote weaker associations. The clustering in Figure 2 reveals four distinct regions corresponding to the four *Aquilaria* species. Clusters 1 (AB) and 2 (AM) show lighter colors, particularly in inputs 1 (A) and 2 (B), indicating a strong affinity for these compounds. Conversely, clusters 3 (AC) and 4 (AS) display darker gradients in these compounds, suggesting weaker associations. These results demonstrate that the SOM effectively differentiates species by emphasizing the importance of specific features, providing a high-resolution view of species-compound relationships.

Further insights are provided in Table 2, which summarizes the SOM color gradient representations for each cluster across all input compounds. Lighter colors indicate larger or higher weight values, signifying a closer similarity to the input data or sample. These lighter shades reflect an effective mapping of the input's characteristics, suggesting that the input data point plays a significant role in the overall pattern or clustering. In contrast, darker colors represent lower weight values, indicating less similarity to the input data.

For example, looking at the 'cluster/species' rows, it can be observed that for cluster '1 (AB)', the color gradients across the 'Inputs/compounds' columns range from yellow to red. This indicates that the input data in columns 1(A) and 2(B) have a stronger similarity or higher weight value to cluster 1 (AB). The yellow coloring suggests that the input in those columns is more effectively mapped to that cluster.

In contrast, for cluster '3 (AC)', the colors are predominantly darker, ranging from orange to black across the columns. This signifies that the input data in those columns have a smaller or lower weight value and are less similar to cluster 3 (AC), meaning the mapping is not as effective compared to cluster 1 (AB). By reading the color representations, one can quickly identify which input data or compounds are most closely associated with each cluster or species.

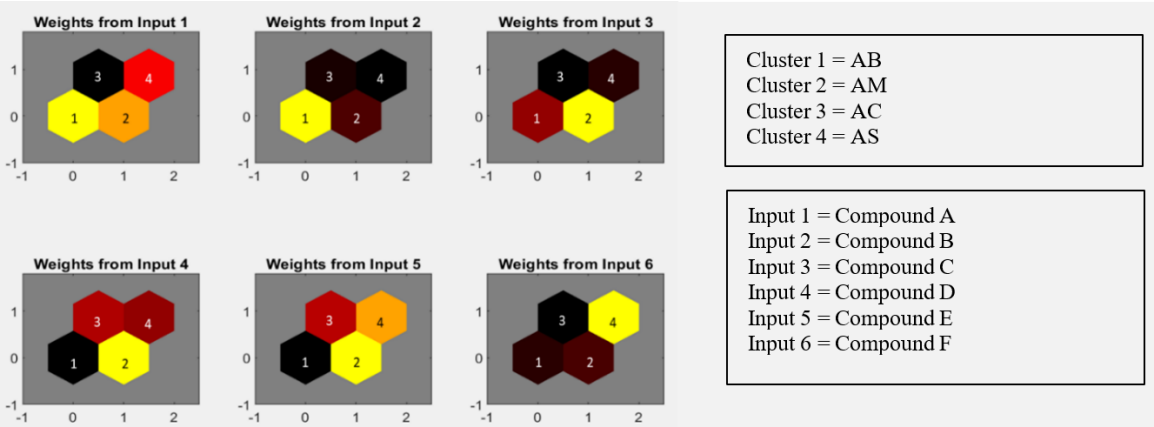

Figure 2. SOM weight plane

Table 2. Summary SOM color representations

Cluster/species	Inputs/compounds					
	1 (A)	2 (B)	3 (C)	4 (D)	5 (E)	6 (F)
1 (AB)						
2 (AM)						
3 (AC)						
4 (AS)						

The significance of these clustering patterns is further explored in Figure 3, which presents the ranking abundance of each chemical compound across all species. This visualization offers a quantitative perspective, showing the relative concentration of compounds a through F for each species. Compounds B, C, D, and E emerge as the most abundant and consistent across all four species, highlighting their potential role as significant markers in species differentiation. The consistent high concentration of these compounds, particularly in species AB and AM for B and C, and in species AC and AS for D and E, suggests that they contribute significantly to the overall chemical composition and may play important biological or ecological roles. This stability across species points to their relevance in determining species distribution and differentiation.

Notably, compound A appears more prominent in species AB and AM, while species AC and AS show reduced levels, further supporting the distinct chemical profiles of these species. Similarly, compound F shows limited abundance across all clusters, suggesting that it may have a less significant role in differentiating species compared to the other compounds. This hierarchical representation of compound abundance allows for the identification of potential “signature” compounds that can be linked to specific species or groups of species, providing valuable insights for future applications in ecological monitoring or pharmacological exploration.

The implications of these findings are substantial. The SOM has demonstrated its effectiveness as a powerful tool for clustering complex chemical datasets, enabling the identification of key patterns and

relationships between species and compounds. By mapping the feature space of the input data, SOM highlights both similarities and differences in the chemical composition of the four species. This capability is particularly important for species like *Aquilaria*, where understanding chemical variation can have ecological, economic, and pharmacological relevance.

The identification of key compounds, particularly B, C, D, and E, as consistent and significant contributors across all species highlights their stable impact on species differentiation. These compounds likely play critical roles in defining the unique characteristics of each species, whether through ecological interactions, metabolic processes, or their potential as bioactive agents. Their high concentrations make them priority targets for further chemical characterization and functional studies. On the other hand, compounds like A and F, which show species-specific patterns of abundance, may provide additional insights into interspecies variations and adaptations, warranting further investigation.

The boxplot distributions of the compounds in Figure 4 provide additional evidence for the clustering patterns and compound significance observed in the SOM analysis. Specifically, compound B demonstrates its highest values in cluster 1, making it a distinguishing feature of this cluster, while cluster 4 displays the lowest values. The presence of outliers in clusters 2 and 3 suggests some degree of variability, indicating potential overlap or shared chemical properties between species associated with these clusters. However, its role appears less definitive compared to other compounds.

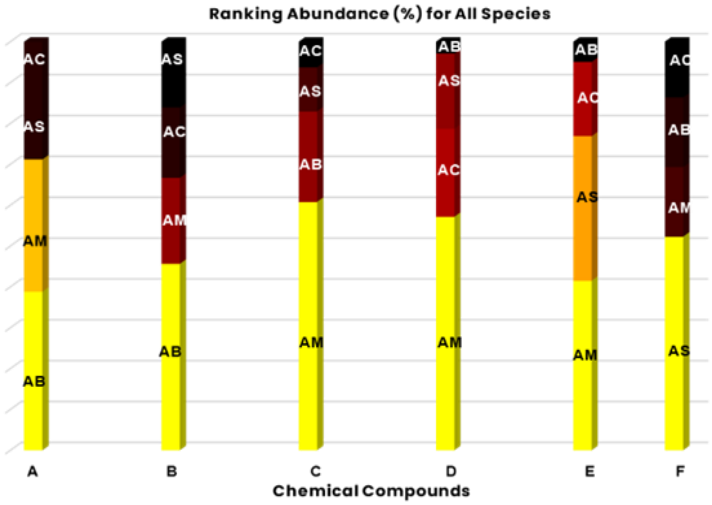

Figure 3. Ranking abundance (%) for all species

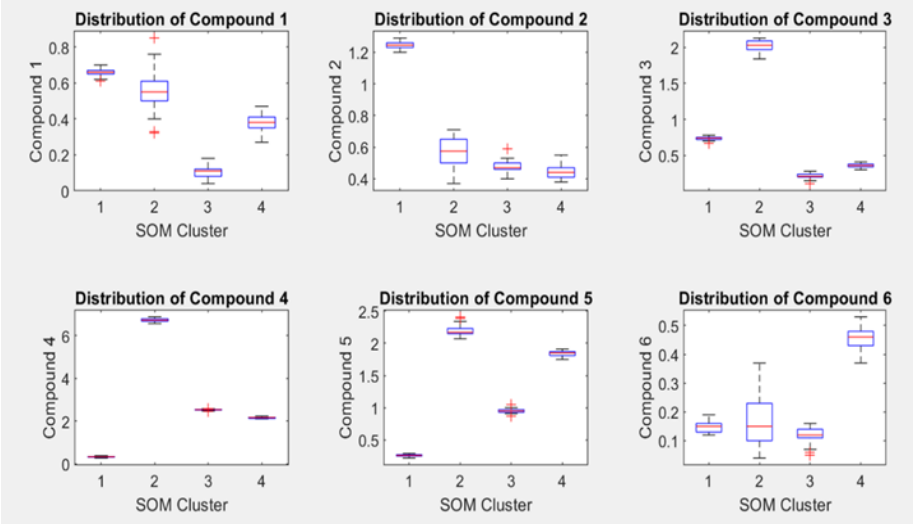

Figure 4. Boxplot distributions of the compounds for all species

Compound C exhibits a clear trend, with cluster 2 displaying significantly higher values, whereas clusters 1, 3, and 4 maintain consistently low concentrations. This pattern highlights the strong association of Compound C with cluster 2, emphasizing its specificity and importance in distinguishing this cluster from others. Similarly, compound D shows its peak values in cluster 2, with minimal to no overlap with other clusters. Its consistently low values in cluster 1 underscore its strong representation in cluster 2, making it a pivotal marker for this cluster.

Compound E also follows a pattern similar to compound D, with its highest values concentrated in cluster 2, moderate concentrations in cluster 3, and lower levels in cluster 4. This distribution indicates its shared yet specific role across certain clusters, particularly in differentiating cluster 2 from clusters 3 and 4. The distinct concentration gradients of compounds C, D, and E collectively underscore their stronger roles in defining the chemical profiles of species associated with cluster 2, compared to compound B. Therefore, compounds C, D, and E are more significant than compound B, owing to their greater variation, cluster specificity, consistent distributions, and stronger capacity to distinguish all *Aquilaria* species. These findings, supported by the SOM visualization and boxplot analysis, provide critical insights into the differential roles of chemical compounds in species differentiation, emphasizing their potential as biological markers in ecological and pharmacological contexts.

The identified compounds, particularly C, D, and E, play pivotal roles in defining the chemical profiles of the studied *Aquilaria* species. Compounds C and D, both sesquiterpenoids, are known for their contributions to the distinctive aroma and resin quality of agarwood [29]. Their high abundance in species AM and AC suggests a strong ecological adaptation to environmental stressors. Furthermore, compound E, another sesquiterpenoid, has been linked to the defense mechanisms of plants, indicating its significance in protecting *Aquilaria* species against pathogenic threats [4], [10], [30]. These findings are consistent with prior studies emphasizing the role of sesquiterpenoids in plant resilience and their utility in pharmacological applications [8]. By highlighting these compounds' ecological and biological relevance, the study underscores their importance in species differentiation and potential industrial use.

These findings are consistent with recent studies that have applied SOM to explore the chemical diversity among *Aquilaria* species, emphasizing the relationships between chemical compounds and their biological significance. The SOM approach has proven instrumental in identifying chemical markers that differentiate species, providing valuable insights into their ecological roles, evolutionary adaptations, and potential applications in fields such as pharmacology and conservation [13], [14], [26], [27], [31]. The versatility of SOM in handling complex datasets underscores its importance as a powerful tool for advancing research in chemical ecology and sustainable natural resource management. By combining SOM visualizations with quantitative analyses, such as boxplots, researchers can achieve a comprehensive understanding of compound-specific variations and their biological implications. As a whole, these insights not only confirm the effectiveness of the SOM methodology in identifying chemical markers but also emphasize its potential for future studies aimed at enhancing our understanding of species differentiation and its wider implications in ecology, pharmacology, and conservation.

4. CONCLUSION

This study investigated the chemical diversity of essential oils from four *Aquilaria* species (AB, AM, AC, AS) using SOM to identify significant compounds for species differentiation. The analysis revealed that specific compounds were highly significant in distinguishing between species, with some showing species-specific patterns, while others had limited relevance. Among the identified markers, compounds C, D, and E were the most significant for distinguishing species, with compound A showing a species-specific abundance in AB and AM, while compound F demonstrated limited significance across all species. These findings provide valuable insights into the biochemical diversity of *Aquilaria*, offering robust support for ecological conservation, sustainable agarwood management, and the development of pharmacological applications. By demonstrating SOM's effectiveness in analyzing complex chemical datasets, this research establishes a robust framework for chemotaxonomy and highlights its potential for broader applications. Future studies should examine how environmental factors influence chemical marker distribution and expand SOM analysis to other plant species with intricate chemical profiles. This approach not only advances the sustainable use and conservation of agarwood but also unlocks its broader ecological and medicinal potential.

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AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

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C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

Authors state no conflict of interest.

INFORMED CONSENT

We have obtained informed consent from all individuals included in this study.

DATA AVAILABILITY

Data availability is not applicable to this paper as no new data were created or analyzed in this study.

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