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Chemical Profiling of Smoky Compounds in Agarwood Woodchip and Oils using HSGC-MS Analysis

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Aquilaria malaccensis, renowned for its distinctive aroma, is highly valued in perfumery production. This study investigates the volatile compounds contributing to the smoky scent of agarwood by applying comparative analyses of three different agarwood forms: woodchip, oil extract, and industrial essential oils using the advanced analytical technique of headspace gas chromatography-mass spectrometry (HSGC-MS). The results revealed a complex mixture of sesquiterpenes and aromatic compounds, indicators for evaluating agarwood quality. Woodchip samples exhibited key volatile compounds, such as β-agarofuran (0.66% and 0.24%), agarospirol (0.70% and 2.48%), jinkoh-eremol (6.81%), and 4-phenyl-2-butanone (0.47% and 0.41%). Meanwhile, oil extracts showed the following results: β-agarofuran (35.70%), agarospirol (0.40%), and 4-phenyl-2-butanone (22.70%). The analysis performed on industrial essential oil exhibited the key volatile compounds, such as β-agarofuran (28.34% and 9.42%) and 4-phenyl-2-butanone (3.16% and 3.97%). This research provides a deeper understanding of the chemical composition of agarwood aromatic profile using the HSGC-MS technique, which minimizes the potential degradation of compounds during the extraction process, thereby providing a more accurate representation of the actual aroma profile. The analysis also effectively captured the appropriate heating conditions of non-volatile or semi-volatile compounds, thus providing significant implications for quality control, product development, and the standardization of agarwood-based products in the fragrance industry.

Keywords: Agarwood, smoky, volatile compound, HSGC-MS, agarwood aroma

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Agarwood, a fragrant resinous wood of the *Aquilaria* genus trees, is one of the valued aromatic materials used in perfumery, traditional medicine, and spiritual practices. The smoky scent of agarwood is particularly favored for its complexity, richness, and long-lasting woody and resinous notes [1, 2]. This distinct aroma is particularly attributed to volatile compounds of sesquiterpenes and aromatic molecules produced by naturally infected or artificially induced agarwood trees [3, 4]. Compounds such as β-agarofuran, agarospirol, and 4-phenyl-2-butanone have been identified as major contributors to this characteristic scent [5, 6, 7].

Other benefits of these smoky compounds include their anti-inflammatory and antimicrobial properties, which are utilized in traditional medicine systems across Asia. Modern analytical techniques such as gas chromatography-mass spectrometry (GC-MS) and headspace solid-phase microextraction (HS-SPME) enable researchers to identify the key volatile compounds correlating to its aroma quality [1, 2, 6, 8]. Such research contributes to important implications for the grading, authentication, and standardization of agarwood products in both the perfumery and pharmaceutical industries [7, 5]. None of the previous research provided the aroma descriptors of the specific smoky compounds of agarwood. Comparative data across different forms of agarwood (woodchip, oil extract, and industrial essential oils) are also limited. Moreover, the lack of standardized chemical markers for smoky aroma evaluation in previous research was addressed using industrial essential oils as a comparison in this study. Therefore, this study aims to review and compare the volatile compounds identified in *Aquilaria malaccensis* agarwood and highlight their role in defining the unique olfactory profile of this valuable material.

The primary objective of this study is to identify and compare the key volatile compounds responsible for the smoky aroma of *A. malaccensis* agarwood by synthesizing the chemical profiles obtained from analytical techniques such as

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headspace gas chromatography-mass spectrometry (HS-GC-MS) and solid-phase microextraction (SPME). The study aims to determine the major smoky volatile compounds consistently present across agarwood woodchips, oil extracts, and industrial essential oils. These compounds were then evaluated for their aroma characteristics, as described in previous studies, thus presenting a comprehensive comparison of chemical profiles in the different types of agarwood samples.

EXPERIMENTAL

Chemicals and Materials

The woodchip samples were prepared by collecting the resin from a 12-month artificial inoculation period of *A. malaccensis* trees harvested from the plantation. Resin and non-resinous wood portions were chopped into woodchips after being dried for a week at room temperature for the HS-SPME analysis. Meanwhile, the oil extract samples were prepared by drying at 60°C, grinding agarwood samples into powder, and sieving using a 1.00-mm particle size sieve for plastic packaging. Approximately 500 g of ground agarwood sample was added to the round-bottom flask, followed by the addition of distilled water at a ratio of 1:2 (sample to water) for the hydrodistillation process. As the water boiled, the volatile compounds from the agarwood vaporized and were carried by steam through the condenser, where they were condensed and collected in a receiving flask. During distillation, the heat was monitored at 100°C for 24 hours using a thermometer to control the process and to ensure effective collection of essential oil. Once the distillation was completed, the distillate was cooled and separated into the aqueous and essential oil layers. The essential oil, which contained the agarwood oil, was carefully separated. To ensure purity, the extracted oil was passed through anhydrous sodium sulfate to remove any residual water. The yield of the extracted oil was calculated using the following equation:

$$\text{Yield (\%)} = \left(\frac{\text{Weight of oil produced (g)}}{\text{Weight of sample (g)}}\right) \text{x } 100$$

Then, the oil was weighed and stored in an appropriate container to protect it from light. Lastly, the industrial essential oils were prepared by placing 1 mL of agarwood oil into the vial for HS-SPME extraction.

Characterization Methods

HSGC-MS analysis was used to determine the chemical composition of the samples. GC-MS is a very useful technique used for the identification of mixture compounds (volatile and semivolatile), and it can be used to determine the structure of unknown compounds by matching the spectra with reference spectra [9]. Approximately 0.11 g of each woodchip sample was weighed and placed in 4 mL vials for analysis. Prior to extraction, the 50/30 µm (DVB/CAR/PDMS) Supelco Gray Fiber was preconditioned in an oven at 250°C–270°C for 15 min to remove the contaminants before being used for the HS-SPME volatile compound extraction.

Each woodchip sample was extracted for 30 min at 60°C, while 1 µL of oil extract and industrial essential oil samples were diluted in hexane to be injected into the GC-MS system. The compounds were then further analyzed using GC-MS. The blank control was analyzed to rule out contamination, and triplicate analyses were conducted. SPME was performed at 250°C for 10 min at the GC injection port of Agilent 7890B GC system coupled with MS 5977A instrument equipped with DB-1ms capillary column (0.25 µm film thickness; 30 m × 0.25 mm ID). Carrier gas helium with a 1.0 mL/min constant flow rate was used. MS ionization mode (EI) 70 Ev, with a scan range of 20–500 and an ion source temperature of 230°C was used. The analysis of woodchip started at 70°C in the oven for 3 min, which was ramped up at 3°C/min to a final temperature of 250°C and held for 5 min. Meanwhile, in the analysis of oil extract and industrial essential oil, the initial temperature started at 80°C and was held for 2 min to allow complete vaporization and focused on the analytes. The temperature was then raised at the rate of 3°C per min until it reached 250°C, where it was held for 5 min. Volatile compounds with matches of above 80% were identified by comparing the results with the National Institute of Standards and Technology (NIST) library.

RESULTS AND DISCUSSION

Volatile compounds for the agarwood samples are listed in Table 1. The chemical components of the five samples are found to differ noticeably from one another. The aromatic terpene compound group, including monoterpenes and sesquiterpenes, contributes significantly to the fragrance profile of agarwood oil [5, 10]. In this analysis, eight compounds were chosen for comparison purposes. The compounds were 4-phenyl-2-butanone, α-copaene, δ-guaiene, δ-cadinene, β-agarofuran, dihydro-β-agarofuran, agarospirol, and jinkoh-eremol. The compound 4-phenyl-2-butanone was reported as one of the major compounds, which contributed to the odor of agarwood oil, including laboratory agarwood sample extraction by the hydrodistillation method [1,11]. This is shown by the high relative peak area of 22.70% of the oil extract (HD) compared to other agarwood samples. 4-phenyl-2-butanone, described as having a sweet and floral note, was identified in multiple studies and listed among the key aroma-active compounds [1, 3, 7]. α-copaene is present in industrial essential oils and one of the woodchip samples. This compound contributes to the woody and spicy odor of agarwood [2]. In this analysis, α-copaene was detected in woodchip **2**, industrial essential oil **1**, and industrial essential oil **2** with relative peak area (%) of 0.14, 0.65, and 1.16, respectively. δ-guaiene, detected mainly in SPME extracts, contributes to the complex woody and spicy odor of treated agarwood [2]. This compound was detected in all agarwood samples. Sesquiterpenes such as δ-cadinene have also been associated with woody, earthy notes, particularly in treated samples analyzed via GC-MS/O techniques [2, 5].

Among all identified compounds, β-agarofuran was the most frequently reported across oil, smoke, and headspace samples [1, 4, 5, 6]. This compound is commonly associated with smoky, resinous notes and serves as a chemical marker for high-quality agarwood. Dihydro-β-agarofuran was found in all samples except woodchip samples and identified as a sweet and woody impact compound, particularly in Qi-Nan agarwood samples [6, 7]. Agarospirol, another sesquiterpene, was detected in woodchip and oil extract samples. It is widely recognized for its woody and balsamic aroma and was consistently found in studies using GC-MS, SPME, and olfactometry [3, 6, 8]. Jinkoh-eremol was only detected in one of the woodchip samples. It was identified primarily in high-grade agarwood oils and is believed to enhance the complexity and richness of the aroma [1, 8]. Olfactometric analyses in some studies confirmed that these specific compounds were among the few that had a significant sensory impact despite the presence of many volatiles [2, 6].

Table 1. Chemical composition (percentage relative peak area) of agarwood samples.

Compounds	Aroma description	Relative peak area (%)				
		Woodchip 1	Woodchip 2	Oil extract (HD)	Industrial Essential Oil 1	Industrial Essential Oil 2
Ketone						
4-phenyl-2-butanone	Odor of high-quality agarwood	0.47	0.41	22.70	3.16	3.97
Sesquiterpene						
α-copaene	Woody, slightly spicy; present in volatile extracts, especially in SPME profiles	-	0.14	-	0.65	1.16
δ-guaiene	Woody, spicy; found in treated wood and oils using SPME	1.67	1.90	0.24	1.10	0.89
δ-cadinene	Woody scent; a marker of aroma impact in treated agarwood	0.57	0.75	0.24	0.83	0.92
β-agarofuran	Characteristic aroma compound found in oil and SPME samples	0.66	0.24	35.70	28.34	9.42
dihydro-β-agarofuran	Sweet, woody; major aroma-active in cultivated Qi-Nan agarwood samples	-	-	1.96	1.73	1.45

agarospirol	Woody, balsamic, a dominant aroma-active sesquiterpene in essential oil	0.70	2.48	0.40	-	-
jinkoh-eremol	Warm, woody aroma; one of the distinguishing compounds of high-grade agarwood	-	6.81	-	-	-

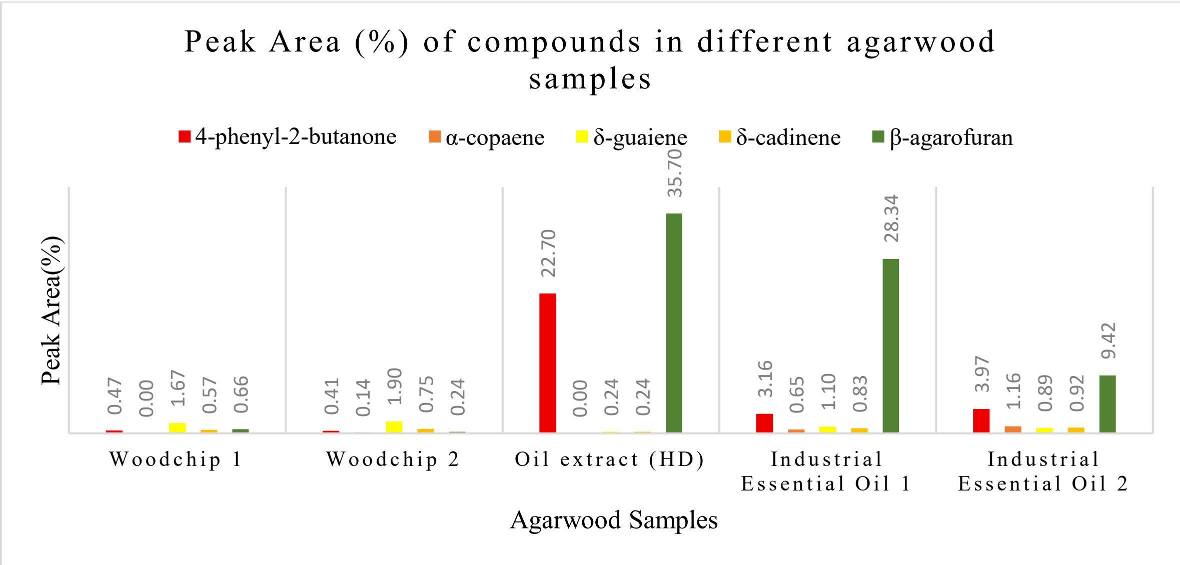

Figure 1. Peak area (%) of compounds in different agarwood samples.

Figure 1 shows a comparison of the compound peak area (%)in five agarwood samples. Industrial essential oil 1 and industrial essential oil 2 are chosen as the reference for high-quality agarwood samples for comparison purposes. In this study, β-agarofuran is known as the compound found in high-quality agarwood; the presence of this compound in oil extract (HD) can be considered important to determine the overall aroma of agarwood. The higher amount of sesquiterpenes in the samples indicates the higher quality of agarwood oil [11]. Hydrodistillation is known as an effective method for extracting various compounds from plant materials, yielding higher concentrations of sesquiterpenes such as β-agarofuran, as shown in Figure 1.

Figure 2 presents the chromatogram of each agarwood sample analyzed in this study. Woodchip **1** and woodchip **2**, which were samples of inoculated trees, were compared against wild agarwood trees. Both samples offer a wider variety of compounds, indicating that the inoculation process stimulated the production of a more diverse chemical profile. A wide range of secondary metabolites is produced in response to stressors, indicating that pathogen infection from the inoculation process led to increased metabolic diversity [12]. Interestingly, 40 compounds were identified in the oil extract (HD) sample, i.e., the hydrodistillation extracts, indicating a rich profile of volatile components. The chromatogram of industrial essential oil **1** provides a distinctive profile with 43 identified compounds. Significant components such as 4-phenyl-2-butanone contribute to its intense aroma. The presence of β-agarofuran aligns with its strong and lasting fragrance, supporting its suitability for high-end perfumery. The chemical profile of industrial essential oil **2** suggests a balanced composition of sesquiterpenes and sesquiterpenoids, offering a milder yet complex aroma. Oud is also known for its smoky undertones from resinous extraction in high-end perfumery production. Therefore, the abundance of sesquiterpenes such as 4-phenyl-2-butanone and β-agarofuran indicates their high aroma quality, which may contribute to the smoky notes of agarwood.

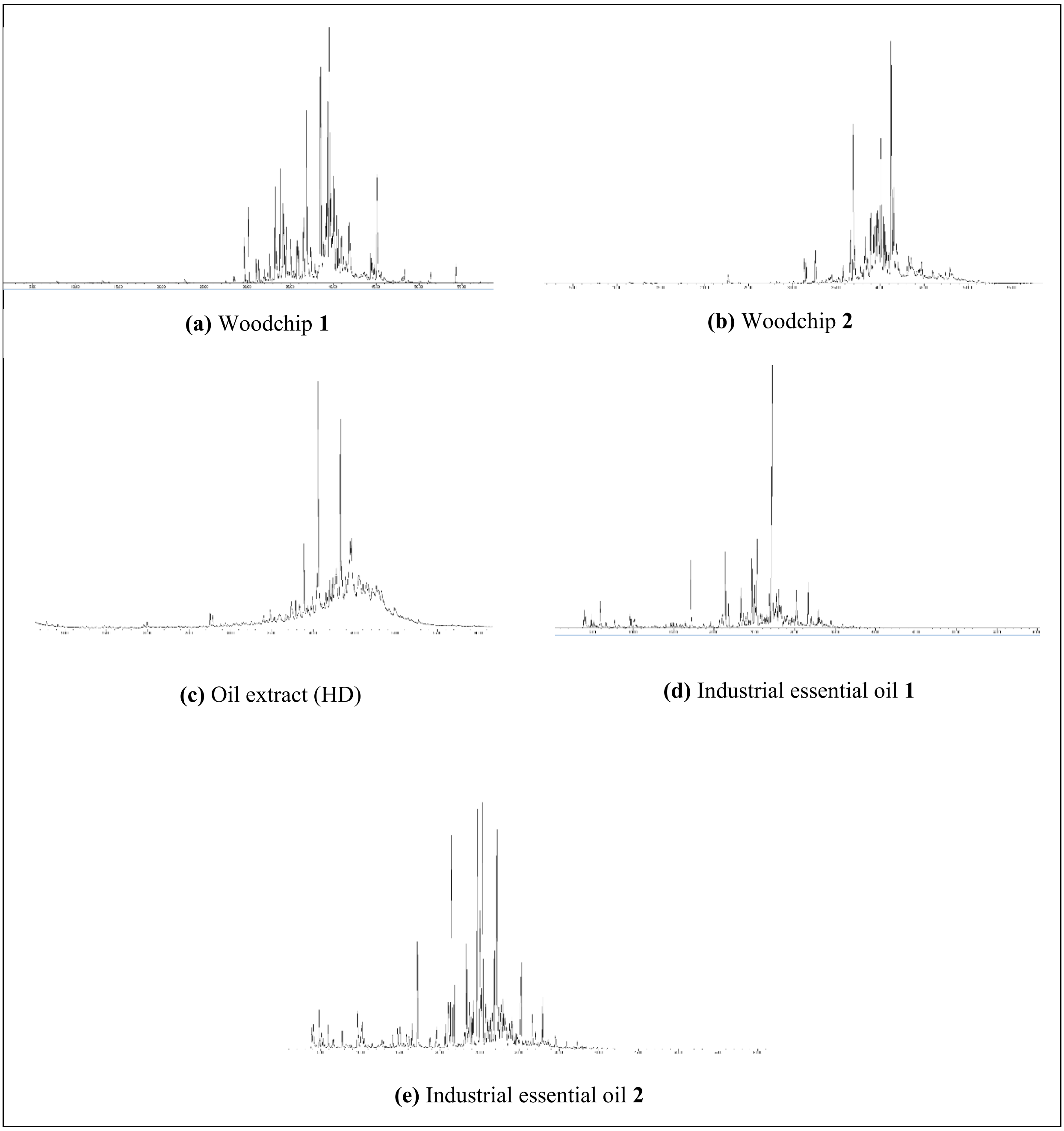

Figure 2. Chromatogram of agarwood samples: (a) woodchip **1** (b) woodchip **2** (c) oil extract (HD) (d) industrial essential oil **1** (e) industrial essential oil **2**.

CONCLUSION

The present study successfully characterized the major volatile compounds present in various *A. malaccensis* agarwood samples that contribute to its distinctive high-quality aroma using the headspace gas chromatography-mass spectrometry (HSGC-MS) technique. The analysis revealed that the dominant constituents belong primarily to the monoterpene and sesquiterpene classes. Key aroma-active compounds, including 4-phenyl-2-butanone, α-copaene, δ-guaiene, δ-cadinene, β-agarofuran, dihydro-β-agarofuran, agarospirol, and jinkoh-eremol, were identified and compared across all sample types. The comparative findings provide valuable insights into the chemical composition of agarwood's aromatic profile, offering significant implications for quality control, product formulation, and the standardization of agarwood-based products within the fragrance and essential oil industries.

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