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GC-MS olfactometry reveals sesquiterpenes α-humulene and δ-cadinene significantly influence the aroma of treated *Aquilaria malaccensis* essential oil

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Abstract

Aquilaria malaccensis is a tropical woody tree that produces agarwood, which is valued for its aromatic properties. Agarwood is widely used in traditional medicines and the cosmetics industry. Natural agarwood production in the wild, which occurs due to fungal infection, is scarce and cannot meet the market demand. Hence, many artificial techniques have been employed to stimulate the production of agarwood. Although many studies have determined the volatile compounds present in naturally produced agarwood, the characterisation of these compounds from differentially treated agarwood samples, particularly *A. malaccensis*, is still limited. This study was conducted to identify and differentiate the volatiles present in treated and healthy *A. malaccensis* wood and essential oil using the solid phase microextraction (SPME) and hydrodistillation techniques, respectively, coupled with gas chromatography-mass spectrometry (GC-MS) analysis. Subsequently, the olfactory active compounds in the treated agarwood essential oil were determined via gas chromatography-mass spectrometry, coupled with olfactometry analysis (GC-MS/O) with four panellists. The essential oil from the treated agarwood was found to be abundant in sesquiterpene and aromatic compounds (80.61%), which was a similar result to that obtained from the volatiles of the wood that yielded 86.94%. Among the major sesquiterpene constituents in the essential oil are α-cadinol (19.40%) and 10-epi-γ-eudesmol (13.39%), whereas α-humulene (13.33%), α-copaene (12.49%) and δ-guaiene (10.41%) predominated in the SPME extract. Meanwhile, GC-MS/O analysis revealed that the sweet, woody agarwood aroma is an orchestra of olfactory active compounds that are significantly influenced by sesquiterpenes such as α-humulene and δ-cadinene. This study highlights that although treated agarwood is rich in secondary metabolites, only a handful, particularly those belonging to sesquiterpenes, are actual aroma impact compounds.

Keywords: agarwood, SPME, hydrodistillation, essential oil, sesquiterpenes, GC-MS/O
Abbreviations: SPME_ solid phase microextraction; GC-MS_ gas chromatography-mass spectrometry; GC-MS/O_ gas chromatography-mass spectrometry/olfactometry; NIST_National Institute of Standards and Technology

Introduction

Aroma or fragrance has significant effects on human behaviours, cognitive functions and quality of life (Girona-Ruiz et al., 2021). It plays a vital psychophysiological role that helps to boost the memory and elevate the mood, and it is often used in physical, mental and spiritual healing (Sowndhararajan and Kim, 2016). Sowndhararajan and Kim (2016) further highlighted that in humans, almost 300 active olfactory receptor genes are devoted to determining thousands of diverse fragrance molecules, which consist of volatiles with a molecular weight of under 300 Da. Different plants emit an orchestra of volatile compounds that give each one its signature aroma.

Aquilaria malaccensis produces the precious aromatic oleoresin, agarwood, which is deposited at the stem area of this large evergreen tree. Agarwood is considered a pathological product produced by a fungal invasion of the host (Jalil et al., 2022). The fragrant agarwood is used as incense in Egypt, Arabia and the northeast of Bangladesh. Also known as *gaharu* by Malaysian locals and *jinkoh* in Japan, it serves as an oriental medicine that is used as a sedative, stimulant, cardiac tonic and carminative agent (Ye et al., 2016; Liu et al., 2017). Moreover, it is used in the cosmetics (perfumery) and pharmaceutical industries.

The unique aroma of naturally infected agarwood is believed to be a mixture of secondary metabolites, which mainly consist of chromones and sesquiterpenes of diverse classes. Some compounds that are exclusively present include jinkohol, agarospirol, α-agarofuran, aquillochin and oxoagarospirol (Nor Azah et al., 2008). From an economic viewpoint, these compounds are unique in nature and cannot be chemically synthesised. This outstanding abundance of structurally inimitable secondary metabolites makes agarwood an attractive subject for the pharmaceutical industry, while its flavour makes it important in the fragrance industry as a source material for new biological and olfactory active target compounds.

Natural agarwood production occurs via a fungal infection of the *Aquilaria* and *Gyrinops* species. However, many efforts have been made to induce agarwood production in healthy trees. Among the common methods used are the deliberate wounding of trees with large knives, hammering nails into tree trunks and chemical treatment using phytohormones, salts and minerals (Chen et al., 2011; Azren et al., 2018; Tan et al., 2019). Different artificial techniques have resulted in the production of different agarwood qualities. As such, many new techniques are being developed to form high-quality agarwood, which could fetch a high market price.

Although metabolites from various species of naturally infected agarwood have been extensively profiled, the profiling and subsequent determination of the olfactory active compounds, particularly from treated *Aquilaria malaccensis*, are still scarce. Hence, the uniqueness of this research is in its comparison of the volatiles from treated and healthy *A.malaccensis* and the subsequent identification of the significant olfactory active compounds in the treated sample. To achieve this, two extraction techniques - solid phase microextraction (SPME) and hydrodistillation - were employed, coupled with Gas Chromatography-Mass Spectrometry (GC-MS) analysis, to thoroughly profile the volatiles present in the treated and healthy agarwood samples. Following that, the significant olfactory active compounds in the treated agarwood essential oil were identified using gas chromatography-mass spectrometry and olfactometry (GC-MS/O) analysis.

Results & discussion

Volatile profiling of treated and healthy Aquilaria malaccensis

Volatile profiles of the woods extracted using the SPME technique

The major constituents in the treated agarwood were sesquiterpenes and aromatics (86.94%), and the remainder were fatty acids. On the other hand, an opposite trend was observed for the healthy wood, whose fatty acid content appeared to be dominant at about 58.45%, followed by the alkene/ aldehyde/ amide group compounds, which comprised 40.51%. Sesquiterpenes such as α-humulene (13.33%), α-copaene (12.49%) and δ-guaiene (10.41%) were the main volatiles found in the treated agarwood sample. These findings were similar to those obtained by Kumeta and Ito (2010) and Okudera and Ito (2009), who discovered three sesquiterpenes - α-guaiene, α-humulene and δ-guaiene - in a methyl jasmonate-induced callus culture of *Aquillaria crassna*. Although α-guaiene was detected in this work, the percentage was far lower (3.99%) than that of α-copaene. Nevertheless, oxidised sesquiterpenes were not reported in either study.

The findings of this research differ from those obtained by Ahmaed et al. (2017), who highlighted the presence of kessane, α-guaiene, β-dihydroagarofuran, β-selinene, caryophllene oxide, α-muurolene and epoxy bulnesene in the incense of *A.malaccensis* wood chips. Apparently, only α-guaiene and α-muurolene were detected in this study. One possible reason for this is the different extraction techniques used in the two studies. Ahmaed et al. (2017) utilised the headspace volatile technique, which directly absorbs volatiles from the area just above the sample. Meanwhile, in this study, the SPME technique involving fibre absorption was employed. It is notable that the extraction technique might also be a key factor in determining the volatiles identified for a given sample.

On the other hand, the healthy sample yielded mainly organic acids and alkene compounds, with no signs of sesquiterpenes. Chen et al. (2011) reported that healthy *Aquilaria sinensis* contained fatty acids and alkenes as its major components. However, they agreed that the presence of a small amount of sesquiterpenes (8.93%) was probably due to the stimulation of the sesquiterpene pathway upon cutting. The healthy sample in this study served as a good negative control, whereby no traces of sesquiterpenes were detected.

Comparing the treated and healthy tissues, both showed a distinct divergence in their volatile composition, whereby only one common compound (hexadecanoic acid) was detected from the overall set of analytes identified in both samples using the SPME technique. In healthy tissue, hexadecanoic acid (also known as palmitic acid) accounted for almost 20% but it was present to a lesser extent (only 13%) in the treated agarwood tissues. A similar trend of organic acid accumulation was observed in treated and healthy *A. sinensis*, as reported by Chen et al. (2011). One reason for this pattern might be the transition into the sesquiterpene synthesis pathway, which decreases the production of hexadecanoic acid and other organic acids in agarwood. Likewise, Nie et al. (2005) highlighted a reduction in the concentrations of free fatty acids such as palmitic, oleic, linoleic, linolenic and stearic acids, which has been associated with increased aroma production in apples. A complete profile of the volatiles detected in the healthy and treated agarwood using the SPME technique is presented in Table S1.

Volatile profiles of the essential oil extracted using the hydrodistillation technique

More compounds were detected in the essential oil than in the wood extract when using the SPME technique (Table S1). The essential oil from the treated agarwood was found to be rich in sesquiterpenes and aromatics (80.61%), which is similar to the SPME results reported in this study. Likewise, Abd Majid et al. (2018) revealed similar findings in their inoculated heartwood samples of *A. crassna, A. sinensis* and *A. subintegra*, in which sesquiterpenes and aromatics predominated in the volatiles extracted using Headspace-Solid Phase Microextraction (HS-SPME).

On the other hand, the essential oils of both the healthy and treated agarwood showed a marked difference in their compositions, with fatty acids being abundant in the former. Fatty acids such as oleic acid (54.16%) and hexadecanoic acid (26.26%) were found to be the major components in the healthy *A. malaccensis* essential oil, which explains its acidic smell. Since it lacks sesquiterpene and aromatics, the healthy tree is duly acknowledged as worth next to nothing.

The findings of this study are in total agreement with those of Fazila and Halim (2012), Hashim et al. (2014) and Jayachandran et al. (2014), in which the constituents of agarwood oil were shown to be primarily sesquiterpenoids. The presence of the *A. malaccensis* signature compound, 10- *epi*-γ- eudesmol, as the major sesquiterpenoid in the agarwood essential oil in this study (comprising about 13.3%) validates its source of origin. Aromadendrene was proposed as an effective chemical marker for agarwood grading since it is found in relatively higher percentages in high-quality agarwood (Pasaribu et al., 2015). Although slightly low in content (only about 4%), the occurrence of aromadendrene derivatives in the agarwood essential oil was reported in this study. Moreover, none of the 2(2-phenylethyl) chromone (PEC) derivatives (a compound that contributes to the agarwood aroma) were found in the agarwood essential oil, presumably due to the extraction technique used. These compounds can only be detected using the supercritical carbon dioxide and solvent extraction methods but not in the hydrodistillation-derived extract (Yoswathana, 2013; Jong et al., 2014).

Compared to the findings of a previous study by Nor Azah et al. (2008) on wild agarwood from several selected states in Peninsular Malaysia, the essential oil derived from the treated agarwood tree in this study showed some variation in the oil composition, with a lack of certain agarwood-specific compounds such as pheromones, α-agarofuran and aquillochin. Many factors may have influenced these differences, such as the geographical location, the season, the age of the tree, the starting material mass, the extraction technique including the distillation temperature (Ashaari et al., 2020) and, most importantly, the type of induction used to form the agarwood. The trees are either naturally infected by pathogens or treated using various artificial techniques.

The various artificial techniques used to stimulate agarwood formation have resulted in the production of different agarwood qualities (Chen et al., 2011). Essential oils of agarwood from fungal-inoculated and artificial screw injection showed similar compound distributions to those of healthy trees (Tamuli et al., 2005; Bhuiyan et al., 2009). Meanwhile, inserting a nail into and holing an agarwood tree for two years produced oil full of sesquiterpenes and aromatic compounds, whereas trunk-breaking for the same duration yielded oil with an abundant level of fatty acids (Lin et al., 2010). The metabolite profile of agarwood induced using the holing technique matched the outcome of this study, in which sesquiterpenes and aromatics were detected as the major compounds. Further investigation using principal component analysis (PCA) revealed distinct differences between the essential oils of treated and healthy agarwood samples, as depicted by the score plot in Fig 1a. PCA was performed to find the main contributions of variability and establish the relation between the volatile compounds. The first two PCs showed variations of 93.90 and 3.56%, respectively, with a total variation of 97.46%. The treated agarwood was clearly separated from the healthy samples by PC1, whereby the treated samples are clustered on the right of the plot and the healthy ones are on the left. Fig 1b presents the corresponding column loading plot that determined the relative importance of each sample, based on the identified compounds. Of these, 48 metabolites contributed to the separation of the treated samples from the healthy ones, and 31 metabolites were responsible for the distinctive clustering of the healthy samples (Fig 1b). The metabolites in the upper side (shown in red) of the column plot correspond to the treated ones, and the ones at the bottom side (shown in green) contributed to the separation of the healthy samples.

GC-MS/O analysis of the essential oil of treated agarwood

The identification results indicated that the agarwood fragrance is a mixture of varied aroma active compounds, which could be fractionated into four distinct phases (Fig 2). Phase 1 (6^{th}-15^{th} minutes) is described as a bitter to unpleasant smell; phase 2 (16^{th}-18^{th} minutes) is sweet and flowery; and phase 3 (19^{th}-29^{th} minutes) is when the actual agarwood aroma is emitted. It is described as spicy, sweet and woody. Lastly, phase 4 (30^{th}- 33^{rd} minutes) produces an oil-like odour. The aroma active compounds, based on the clusters, their intensity and their corresponding odour, are listed in Table 1.

Overall, 18 aroma active compounds emitted between nine and 33 minutes were successfully determined through the GC-MS/O analysis. At the initial stage, a bitter, unpleasant smell was reported, which was similar to the pattern observed in *Populus cathayana* Rehd. and *Hevea brasiliensis* wood by Liu et al. (2018) and in the packaging materials studied by Ezquerro et al. (2002). Despite the low occurrence percentage (lower than 0.2%), the aroma active compounds in phase 1 strongly imposed an unpleasant smell that remained for almost 10 minutes, after which a distinct sweet, floral scent with high intensity was produced by the benzylacetone. In this study, benzylacetone appears to have been detected as one of the major constituents of the agarwood essential oil. Between the retention times of 19 to 29 minutes, many odourants, among which sesquiterpenes and aromatics predominated, were detected. Of these, α-humulene and δ-cadinene appeared to be the key compounds. Notably, this was the phase in which the signature sweet, woody characteristic smell of agarwood oil was spotted. At the final testing stage from 30 to 33 minutes, two aroma active compounds, namely hexadecanoic acid and 3-pentanone, 1,5-diphenyl, emitted an odour described as oil-like.

The results obtained in this study differed from those of Pripdeevech et al. (2011), who identified β-agarofuran as the most intense aroma active component in the agarwood essential oils obtained from *A. malaccensis*, *A. crassna* and *A. subintegra*, but it was not detected in this study. Presumably, the source of the agarwood, together with the ecological conditions, might have influenced the outcome. Pripdeevech et al. (2011) utilised naturally infected agarwood obtained from the wild, whereas the agarwood in this study was collected from deliberately wounded trees at the experimental plot. Hence, the chemical constituents in both types of agarwood were probably different for this reason. Despite the difference, several other major agarwood odour

Table 1. Identified odor active compounds in agarwood essential oil.

Phases	Aroma active compounds[a,b]	Strength*	Corresponding odor**
Phase 1	Benzaldehyde	3	Burnt sugar
	Hexanoic acid	2	Sour, fatty, sweat, cheese
	1-Hexanol, 2-Ethyl-	5	coffee, bitter
	Octanoic acid	4	cheese-like
Phase 2	Benzylacetone	5	sweet, floral
Phase 3	α-copaene	3	wood, spice
	β-elemene	4	herb, wax
	β-guaiene	4	wood, spice
	α-cedrene	3	earth
	α-guaeine	4	wood, balsamic
	α-humulene	5	sweet, wood
	δ-cadinene	5	wood
	Isoaromadendrene epoxide	3	wood
	10-epi-γ-eudesmol	4	sweet, wood
	Ledene oxide-(II)	2	wood
	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester	3	wood
Phase 4	Hexadecanoic acid	3	oil-like
	Dibenzylacetone	2	sweet, oil-like

[a]Compounds identified by GC-MS Software using NIST and Wiley-Adams mass spectral library; [b]The results are the mean of three experiments; * Strength is measured in the scale of 1-5; with 1 being the weakest and 5 being the strongest odor; **Corresponding odor was as described by the sniff panelist and cross checked in the Flavornet database (Acree and Arn, 2004).

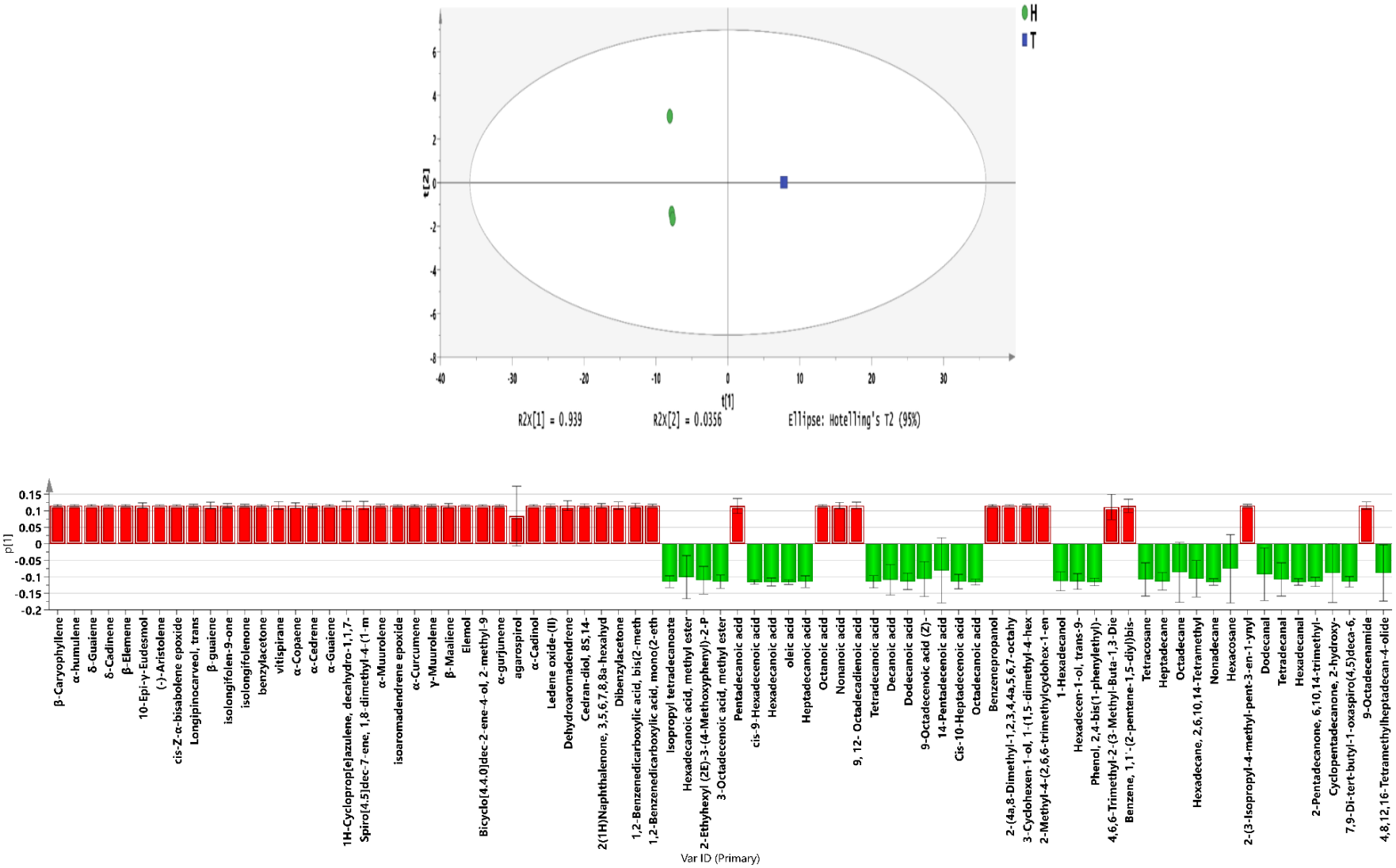

Fig. 1 Score plot (a) and loading plot (b) of volatile compounds in essential oil of treated agarwood (■) and healthy tree (●).

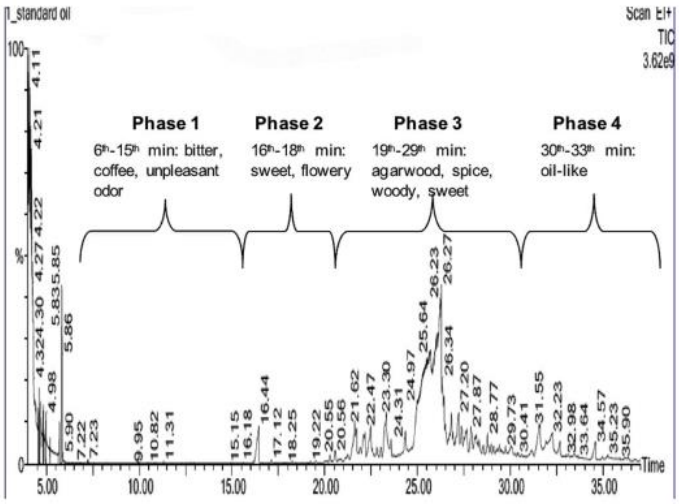

Fig. 2 Patterns of agarwood aroma fractionated into four distinct phases.The first phase is described as bitter to unpleasant and the second as sweet, flowery. The third phase is when the actual agarwood aroma described as sweet, woody is emitted. The last phase produces an oil-like odor.

components such as benzaldehyde and benzylacetone were recognised in all the agarwood samples. Likewise, the presence of the odourants α-guaiene and β-elemene in this study matched those detected in both *A. crassna* and *A. subintegra*, whereas α-humulene was only traced in the former. Apparently, not all the volatile compounds identified earlier in the agarwood essential oil contributed to its fragrance, a finding that is in good agreement with Liu et al. (2018) and Brattoli et al. (2014), who mentioned that the concentration of the volatiles should be higher than the human threshold odour concentration in order to meet the necessary conditions. In this study, it was reported that only a handful of compounds from the sesquiterpene, aldehyde, ketone, carboxylic acid and ester groups were attributed to the aroma emission. Of these, sesquiterpenes and their derivatives are the character-impact odourants that impart the sweet, woody smell of agarwood.The highest odour-emission strength (scoring 5 on a scale of 1 to 5) was displayed by sesquiterpenes such as α-humulene and δ-cadinene. α-humulene is a naturally occurring monocyclic sesquiterpene that is characteristically found in hops, *Humulus lupulus* and other plants such as sage, cannabis and ginseng (Hartsel et al., 2016). Besides often being used in flavouring and the perfumery industry, it displays several pharmacological properties such as anti-inflammatory activity and serves as an effective analgesic when taken topically, orally or by aerosol (Rogerio et al., 2009). On the other hand, δ-cadinene is a bicyclic sesquiterpene that is widely distributed in the essential oil of plants such as grapes (Li et al., 2019) and possesses anti-proliferative and anti-cancer effects (Hui et al., 2015).

Materials and methods

Plant materials

Plant materials consisting of agarwood from a treated five-year-old *Aquilaria malaccensis* tree and healthy stem tissue from a non-treated *A.malaccensis* of the same age were collected from an experimental plot at the Malaysia Nuclear Agency, Hulu Langat, Selangor (2.911567, 101.770096). The identification of *A. malaccensis* Lam. (IPNI, 2020) was performed by A. Damanhuri (Curator for Universiti Kebangsaan Malaysia Herbarium; UKMB) for a voucher specimen labelled M. H. Azhari 1 (Abdul Kadir, 2021). The one-year-old treated sample was authenticated by Dr Chong Saw Peng, a researcher at the Malaysia Nuclear Agency. Holing the tree trunk using a nail followed by inoculation with a mixture containing honey is the type of treatment given to induce agarwood formation in *A.malaccensis* trees (Abdul Kadir, 2020). A total of three samples were collected from three individual trees to represent biological replicates for both categories of tissue types. The plant materials were immediately frozen in liquid nitrogen and transferred to the Metabolomics Lab, Institute of Systems Biology (INBIOSIS), Universiti Kebangsaan Malaysia (UKM). There, the samples were stored in a freezer at -80 ^{o}C for further analysis. Prior to analysis, the wood samples were cut into small pieces and dry-ground separately using a blender to obtain a fine fibre, which was again stored at -80 ^{o}C before the extraction.

Extraction and analysis of volatile compounds

Volatile compounds were extracted using the SPME and hydrodistillation techniques and analysed using GC-MS with certain parameters, as proposed by Kumeta and Ito (2010) and Bhuiyan et al. (2009), respectively. A detailed explanation of the procedures is as follows.

SPME

Approximately 0.5 g of dry-ground wood samples were weighed, inserted separately into a 20 mL clear vial with a polypropylene cap and heated at 45 ^{o}C for 25 minutes. The volatiles emitted were extracted using an SPME fibre (100 mm bonded polydimethylsiloxane coating; Supelco, Bellefonte, PA, USA) for about 40 seconds before being injected into the injecting port of the GC-MS machine (Perkin Elmer Clarus 600 chromatograph).

Hydrodistillation

About 100 g of ground wood tissues were soaked overnight in 1.5 L of water in a hydrodistillation flask. The following day, hydrodistillation was performed and the product was eluted using acetone. The same procedure was repeated for about three days to obtain a sufficient amount of elute. The product was dried under nitrogen gas and concentrated with 500 µl of acetone before being stored at -80 ^{o}C for further analysis.

GC-MS parameter for SPME analysis

For the SPME analysis, the polydimethylsiloxane-coated fibre, which was used to absorb the volatiles from the wood samples, was inserted directly into the injection port of a GC-MS system (Perkin Elmer Clarus 600 chromatograph). The GC was equipped with a capillary column (Elite-5 30 m x 0.25 mm, film thickness 0.25 µm) and the initial oven temperature was set to 250 ^{o}C. The program was immediately started, and the fibre was removed after 10 minutes. The helium flow rate was 1 mL min^{-1} at a constant pressure of 90 Kpa. The program had an initial oven temperature of 80 ^{o}C, a ramp of 5 ^{o}C min^{-1} until 220 ^{o}C with a 10-minute hold and then a further ramp of 10 ^{o}C min^{-1} until 240 ^{o}C followed by a three-minute final hold (Kumeta and Ito, 2010). The acquisition parameters full scan included a scan range of 40 to 350 amu. The identification of the volatile compounds was based on hits in the NIST (version 2.0) and Wiley Registry 8th Edition database of chemical library software.

GC-MS parameter for essential oil analysis

The two types of essential oil from the treated agarwood and healthy trees were analysed using the GC-MS electron impact ionisation (EI) mode in a Clarus 600 GC-MS system (Perkin Elmer, Shelton, CT, USA), with a fused silica capillary column (30 m x 2.5mm x 0.25 mm film thickness) coated with DB-5 ms. The column temperature was set at 100 ^{o}C for two minutes and increased to 250 ^{o}C at a rate of 3 ^{o}C/min $^{-1}$, with the carrier gas helium flowing at 1 mL min^{-1} at a constant pressure of 90 Kpa (Bhuiyan et al., 2009). The acquisition parameters full scan included a scan range of 40 to 350 amu. The identification of the metabolites was based on hits in the NIST (version 2.0) and Wiley Registry 8th Edition database of chemical library software.

Data Processing of GC-MS

All the peaks exceeding a signal to noise ratio (S/N) of 100 were detected. A library search was carried out for peak identification using the National Institute of Standards and Technology (NIST, version 2.0, Gaithersburg, MD, USA) database, and all the peaks were combined into a single peak table before being transferred into Microsoft Excel. The volatile information was extracted based on the name of the compounds, with the match and reverse match values below 800 filtered. New peak tables were formed following the filtering step. The percentage areas relative to the total percentage area of all the compounds were calculated to normalise the data, as suggested by Hai-Peng et al. (2012): Relative content (% = single constituent area ÷ total area × 100%).

Principal Component Analysis (PCA)

Multivariate statistical analysis via PCA was conducted as described by Rafidah et al. *(*2014). Briefly, all the peaks and spectra from each sample were combined into a single peak table and transferred into Microsoft Excel. After the filtering step, the table containing the total counts was imported into SIMCA-P+ 14.0 software (Umetrics, Sweden) for multivariate data analysis (MVDA). Scaling using the square root of the standard deviation (UV scaling) was performed during the analysis. The PCA output comprised score plots in order to visualise the contrast between the two different samples and the loading plots to explain the cluster separation.

Olfactory active fingerprinting of agarwood essential oil using GC-MS/O

The GC-MS/O analysis was carried out using a Perkin Elmer Clarus 600 chromatograph instrument equipped with a Clarus 600T mass spectrometer and an ODO II sniffing port (Olfactory Detector, SGE Analytical Science, Melbourne, Australia). The program parameters were those suggested by Misnawi and Ariza (2011), with four panellists chosen to sniff individually the volatiles emitted by the agarwood at the sniffing port. The initial oven temperature was programmed to change from 60 to 220 °C at a rate of 5 °C/min and it was then held at 220 °C for five minutes. The injections of an ion source EI were adjusted to 270 °C and 280 °C, respectively. The helium carrier gas was set to a flow rate of 1 mL min^{-1} at a constant pressure of 90 Kpa. The acquisition parameters full scan included a scan range of 50 to 350 amu.

For the odour profiling, the method recommended by Rusdi et al. (2016) was followed. A total of 2 µL of the extract was auto-injected into the column where the column outlet split into two lines in a ratio of approximately 1:1 leading to the detector and the sniffing port (the olfactory detector outlet). In the sniffing port, the effluent was mixed with humidified air. The GC-MS/O separated the complex mixtures into individual components, which were subsequently split and simultaneously directed to a mass spectrometer featuring an olfactory port (the sniffer). The sniffer port allowed the panellists to assess the components through their perceptions of the emitted odour (Rusdi et al., 2016). The four panellists repeated the analysis thrice for each sample. Simultaneous MS analysis enabled the odourous molecules to be identified using the hits on the chemical library software of NIST (version 2.0) and the Wiley Registry 8th Edition database.

Conclusion

The results of this research imply that agarwood consists of a complex mixture of volatile compounds, particularly those belonging to the sesquiterpene and aromatics families. On the other hand, the healthy tree was found to be abundant in organic acids, with no traces of sesquiterpenes. The oil composition varied markedly, depending on the ways used to induce agarwood formation in the healthy trees. The treatment method used to stimulate the agarwood in this study is an efficient way of inducing resin formation in *A.malaccensis* since the oil and wood content were identified as being rich in sesquiterpenes and aromatics.

Moreover, it was also revealed that despite the agarwood containing a vast mixture of volatiles, only a handful are actually responsible for the odour property. Sesquiterpenes such as α-humulene and δ-cadinene were determined to be among the significant key compounds to which the unique sweet woody aroma of agarwood was attributed in this study.

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