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AGARWOOD ESSENTIAL OIL QUALITY AND ANTIOXIDANT PROPERTIES OF *AQUILARIA MALACCENSIS* AND *AQUILARIA SINENSIS*

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Agarwood is produced by reacting to physical damage, chemical injection, and microbial and entomological activity on *Aquilaria* spp. (Thymelaeceae). Agarwood oil is popular as one of the most precious and most expensive oils being traded. As a precious oil, the quality of the oil is an essential issue. The quality of *A. malaccensis* and *A. sinensis* oils were analysed using electronic nose, gas chromatography (GC), and gas chromatography/mass spectrometry (GC/MS). The electronic nose signals categorised both oils into the Agarwood Pure Oil Cluster with 100% accuracy via FRIM's Agarwood Identification System (AIS) database. Chemical analyses of *A. malaccensis* oil revealed the presence of 4-phenyl-2-butanone (3.27%), β-agarofuran (4.51%), and kusunol (11.30%) as the major compounds. Valerianol (15.78%) was the most abundant compound identified in *A. sinensis*, followed by β-agarofuran, and γ-eudesmol with a percentage of 7.45 and 6.75, respectively. Unfortunately, the detection of monoethylhexyl phthalate (5.24%) showed that the *A. sinensis* oil was contaminated with a plasticiser. Simultaneously, as part of our bioprospecting study, exploring the potential of selected *Aquilaria* spp. oils as antioxidant alternatives were conducted. The total phenolic content of *A. malaccensis* and *A. sinensis* was 10.8 and 8.5 mg GAE/g, respectively. *A. sinensis* essential oil exhibited higher scavenging DPPH radical activity with IC_{50} value of 8.46 mg/mL compared to *A. malaccensis* with IC_{50} value of 11.49 mg/mL. The ferric-reducing power activity of *A. malaccensis* showed absorbance slightly higher than *A. sinensis* at 0.165 and 0.104, respectively. The β-carotene/linoleic acid bleaching assay revealed an IC_{50} of 6.25 mg/mL for *A. sinensis* and an IC_{50} value of 3.66 mg/mL for *A. malaccensis*. *A. malaccensis* oil revealed higher antioxidant activities in TPC, FRAP, and β-carotene/linoleic acid bleaching assays compared to *A. sinensis* oil. This study suggests that pure *A. malaccensis* oil may potentially act an as antioxidant agent in product development.

Keywords: *Aquilaria* sp., volatile composition, electronic nose, quality, antioxidant

INTRODUCTION

Aquilaria malaccensis and *A. sinensis* are tropical trees belonging to the family Thymelaeaceae. These species are among the sources of agarwood. Agarwood is produced due to a stress reaction process triggered by physical, microbial, chemical, and/or entomological activity damage. Agarwood has been used to treat postpartum nausea, rheumatism, body aches, and smallpox (Nor Azah et al. 2016). In pharmacological studies, agarwood has been shown to possess bacteriostatic, antiasthmatic, anticancer, anti-inflammatory, antidiabetic, and antidepressant effects (Wang et al. 2021, Mokhtar et al. 2021). In *Aquilaria* spp. plantations, inoculation techniques are the best approach to promote the rapid production of agarwood. The quality of agarwood depends on the species, duration of inoculation, pre-processing, and extraction techniques. High-quality agarwood contains two important compounds known as sesquiterpenes and 2-(2-phenylethyl) chromone (Naef 2011).

Agarwood oils were mainly used as perfume and cosmetic ingredients. In perfumery, agarwood oil is categorised as a base note. These notes give a perfume depth and a long-lasting aroma with the most minor volatile compounds, ensuring it lasts up to several hours (Irshad et al. 2020). Low-grade agarwood woodchips are usually used to produce essential oils. Local agarwood players most commonly use the technique of water distillation. The

quality of the oils often differs due to sensory evaluation and individual preferences. The quality of agarwood oils is vital in determining their commercial value. Globally, the agarwood essential oil market is valued at USD 140.9 million (2024) and is forecasted to reach USD 224.6 million by the end of 2034 (FACT9206MR 2023). The quality of agarwood oils was determined by chemical analyses (Nurlaila et al. 2024, Zhou et al. 2022, Sun et al. 2022, Yao et al. 2022, Tajuddin et al. 2016, Nor Azah et al. 2014).

There is some research on the determination of oil quality by sensory analysis in combination with chemometric approaches (Saiful Nizam 2024, Sahrim et al. 2022, Sahrim et al. 2016, Sahrim et al. 2015, Hidayat et al. 2010). FRIM has succeeded in developing a system and database for classifying agarwood oil quality based on electronic noses, namely FRIM's Agarwood Identification System (AIS). This system consists of a combination of commercially available equipment, a self-developed method, and a database. The system can classify the sample as pure or mixed oil with percentage accuracy and is currently benefited by the local agarwood industry through technical services offered (Abd Majid et al. 2024, Mailina et al. 2020). This paper reports the purity and quality of *A. malaccensis* and *A. sinensis* oil by electronic nose and chemical analysis and evaluates their antioxidant properties by various assays. The results could be useful for selecting agarwood oil to develop cosmetic and/or personal care products.

MATERIALS AND METHODS

Essential oil

The agarwood oils of *A. malaccensis* (OUD037) and *A. sinensis* (OUDOI14) were purchased from Oud Agarwood Enterprise in Kelantan and Mega Agarwood Trading (M) Sdn. Bhd. in Melaka, respectively. Both traders have their own plantations and processing facilities for essential oils. The oils were stored securely in the fridge before further work. The oil samples were diluted in diethyl ether before GC and GC/MS analysis. While, for antioxidant assays, 1 mg/mL of oil samples were diluted in dimethyl sulfoxide (DMSO) and distilled water.

Electronic nose experiment

Each sample was replicated in 10 vials for 20 sample vials available for the E-nose experiments. A commercial FOX4000 E-Nose (EN) from Alpha-MOS (Toulouse, France) was used together with the data analysis software AlphaSOFT version 12, which consists of 18 chemical sensors with metal oxide semiconductors (MOSs) that can detect combustible gases, organic compounds, toxic gases, and oxidising gases. The carrier gas used is purified air (P=5 psi). Each EN experiment begins with sample preparation, heating the sample using a heating block, manually injecting the generated headspace into the EN using a gas-tight syringe, and recording the reaction data from the sensors in the main database.

Electronic nose data analysis (Features extraction and Discriminant Factorial Analysis (DFA) model)

Each vial produces 120 continuous data corresponding to 120 seconds of acquisition duration with a 1 s sampling rate. The "Delta R/R0" option displays the sensor values as relative resistance variation whereby R is the sensor response corresponding to the sample injected and RO is the initial resistance value as a baseline (no sample injected). The "Maximum (automatic selection of signal extrema)" option criteria is selected for the main EN database. Therefore, only the maximum peak data (positive and negative peak) from each sensor with a total of 18 data per vial, were extracted. These databases comprised of 20 ×18 data size were then used for the development of the DFA model.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

Chemical analysis of the agarwood oils samples was conducted using Shimadzu GC-2010 Plus capillary chromatograph, which was equipped with a flame ionization detector (FID) and using split/splitless mode injection technique, under the following conditions: carrier gas helium; similar temperature for injector and detector at 250°C. This chromatograph uses a non-polar

capillary column HP-5MS (30m by 0.25mm, film thickness 0.25µm). Operating conditions are as follows; initial oven temperature, 60 °C for 10 min, up to 230 °C at 3 °C/min, and then 230 °C for 10 min. Gas Chromatography/ Mass Spectrometry (GC/MS) analyses were conducted on Agilent Technologies GCMS 7890A/5975C Series MSD with similar columns, dimensions, and conditions described in GC programmes. The chemical constituents were identified by comparison of retention times and calculated Kovat's Indices (KI) with literature values and matching their mass spectra with the database library (HPCH2205.L; Wiley7Nist05.L; NIST05a.L).

Folin-Ciocalteu assay

The total phenolic content was determined calorimetrically using the Folin-Ciocalteu method with some modifications (Ariffin et al. 2017). Briefly, 200 µl of crude extract (1 mg/mL) was made up to 3 mL with distilled water thoroughly mixed with 0.5 mL of Folin-Ciocalteu reagent for 10 minutes. 2 mL of 20 % (w/v) sodium carbonate was added. The mixture was allowed to stand for 60 minutes in the dark, and absorbance was measured at 650 nm against blank. The total phenolic content was calculated from the calibration curve, and the results were expressed as mg of gallic acid equivalent per g dry weight. The phenol reagent of Folin-Ciocalteu was tightly capped at room temperature and deionised water was diluted. All experiments were conducted in triplicates.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Firstly, 3750 µL of DPPH solution was mixed with 750 µL of sample. Then, the mixture was incubated in a dark room at 30 °C for 30 min. The decolourisation of DPPH was observed using a spectrophotometer at 517 nm. The control was prepared using the DPPH solution without essential oil, while methanol was used as a blank. The percentage of the scavenging effect was calculated using the equation:

$$I\ \% = [1 - (A^1 - A^2)] \times 100\ \% \qquad (1)$$

where A^1 is the absorbance of the control, and A^2 is the absorbance of the sample (Adebiyi et al. 2017). The DPPH reagent and Trolox standard were prepared before use and all experiments were conducted in triplicates (Shimamura et al. 2014).

Ferric Reducing Antioxidant Power (FRAP) Assay

The determination of ferric reducing/ antioxidant power FRAP was a simple direct test for measuring antioxidant capacity (Kamaruddin et al. 2016). The 25 mL acetate buffer (3.1 g sodium acetate and 16 mL concentrated acetic acid per 1 L of buffer solution) was mixed accurately with 2.5 mL 2,4,6-tripyridyl-s-triazine (TPTZ) solution (10 Mm) and 2.5 mL iron (III) chloride hexahydrate solution (20 mM) as the FRAP working solution. A 150 µL sample or standard was mixed with 2850 µL FRAP reagent. The mixture was then incubated at room temperature for 4 minutes. Absorbance readings at 593 nm were recorded using a spectrophotometer. All experiments were conducted in triplicates.

β-carotene/Linoleic Acid Bleaching Assay

The β-carotene bleaching assay was performed according to an optimally modified procedure (Ahmed et al. 2022). β-carotene emulsion was prepared by combining β-carotene in chloroform (2 mg/10 mL) with Tween 20 (200 mg) and linoleic acid (20 mg). The mixture underwent chloroform removal using the Buchi R-210 Rotavapor System. After the chloroform was removed, 50 mL of distilled water was immediately added, and the mixture was vigorously shaken. A volume of 2500 µL of emulsion was mixed with 300 µL of essential oil. The solution was thoroughly mixed and heated at 45 °C. Absorbance readings were taken at 470 nm using a spectrophotometer, and the tested sample's absorbance was recorded at 0 min and 60 mins. The β-carotene inhibitory activity was calculated using the formula:

$$\%\ \text{Activity} = A_t / A_0 \times 100\ \% \qquad (2)$$

where, A_t is the absorbance of the tested sample at 60 mins and A_0 is the absorbance of the tested sample at 0 min. Ascorbic acid was the positive

control. All experiments were conducted in triplicates.

Statistical analysis

The experimental data from the antioxidant activity assay were presented as MEAN±SD. An independent t-test was used to determine the significance of the assay's difference ($p<0.05$).

RESULTS AND DISCUSSION

Electronic nose analysis

An electronic nose was used to capture the uniqueness of the odour of the agarwood oil samples and convert the information into sensor response data. Each essential oil data set was compared to the FRIM's AIS database for quality determination. In Figure 1a, both species were classified into two distinct clusters in blue and red, respectively, with a 100% cross-validation score.

Result obtained shows that both samples had completely different scent and EN data. Figure 1b shows average EN data from 18 sensors in radar form that were tabulated in different blue and red colours with different data patterns. Figure 2 shows the results after both samples were projected as unknown samples to the AIS database with pre-defined pure and mixture clusters. Results showed that both *A. malaccensis* and *A. sinensis* samples were classified as Agarwood Pure Oil clusters with 100% accuracy.

Volatile constituents of agarwood essential oils

The chemical constituents and their abundances (%) in *A. malaccensis* and *A. sinensis* essential oils are listed in Table 1. Figures 3 and 4 show the total ion chromatogram of *A. malaccensis* and *A. sinensis*. Generally, agarwood oils consist of sesquiterpenoids. Sesquiterpene provides various health benefits, including anti-inflammatory, antioxidant, and anti-cancer properties, contributing to overall well-being. Additionally, sesquiterpene has been found to have antibacterial and antifungal properties (Li et al. 2022). GC and GC/MS analyses of *A. malaccensis* oil revealed the presence of 4-phenyl-2-butanone or benzylacetone (3.27%), β-agarofuran (4.51%), α-bulnesene (1.92%), kusunol (11.30%), and γ-eudesmol (3.35%) as the major compounds. Benzylacetone-like compounds have been proven to have a sedative effect depending on the functional group on the carbon chain, the substituent on the benzene ring, and their combinations (Miyoshi et al. 2013). The other appreciable compounds detected in *A. malaccensis* oil include 10-*epi*-γ-eudesmol (2.42%), eremoligenol (2.39%), bulnesol (2.39%), silphiperfol-6-en-5-one (1.91%) and α-guaiene (0.56%). Nasution et al. (2000) reported the chemical composition of *A. malaccensis* that has been found to consist of sesquiterpenoid group such as *cis*-jasmone and aromadendrene epoxide, chromone derivatives; 8-methoxy-2-(2-phenylethyl) chromen-4-one and 7-(benzyloxy)-5-hydroxy-2-methylchromone, benzylacetone, guaiacol, palmitic acid and oleic acid.

On the other hand, valerianol (15.78%) was the most abundant compound identified in *A. sinensis* oil, followed by β-agarofuran, γ-eudesmol and agarospirol with a percentage of 7.45, 6.75, and 4.14, respectively. Pripdeevech et al. (2011) reported that some of the appreciable constituents such as agarospirol, α-agarofuran, jinkoh-eremol, β-agarofuran, α-bulnesene, and γ-eudesmol contributed to the blend of aromas such as woody, sweet, balsam, peppery, nutty, warm, burnt and spicy.

10-*epi*-γ-Eudesmol (3.81%), 4-phenyl-2-butanone (1.45%), *epi*-cyclocolorenone (6.69%) and cyclocolorenone (4.41%) are the main compounds presented in *A. sinensis* oil. The monoethylhexyl phthalate (MEHP) (5.24%) detection showed that the *A. sinensis* oil was contaminated with a plasticiser. MEHP is a clear, colourless to cloudy white viscous liquid (NTP, 1992). It may be present due to the usage of plastic-based materials or tools during sample handling, extraction, packaging, and storage of the agarwood essential oils. Inhalation is a significant exposure route for MEHP and effects on the lungs include inflammation, cell differentiation changes, and alteration of postnatal maturation (Rafael-Vazquez et al. 2018).

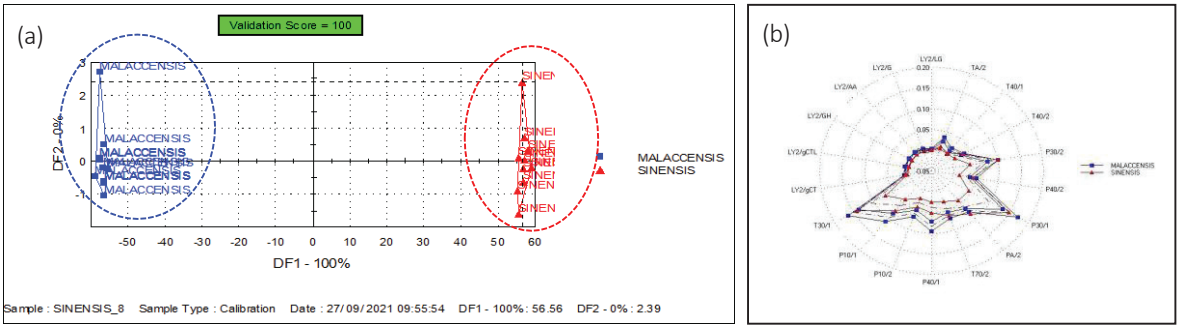

Figure 1 DFA results for *A. malaccensis* and *A. sinensis* species: (a) EN data were classified into two clusters of blue and red colours respectively with 100% cross-validation scores and (b) EN data in radar form were tabulated in different blue- and red-colour

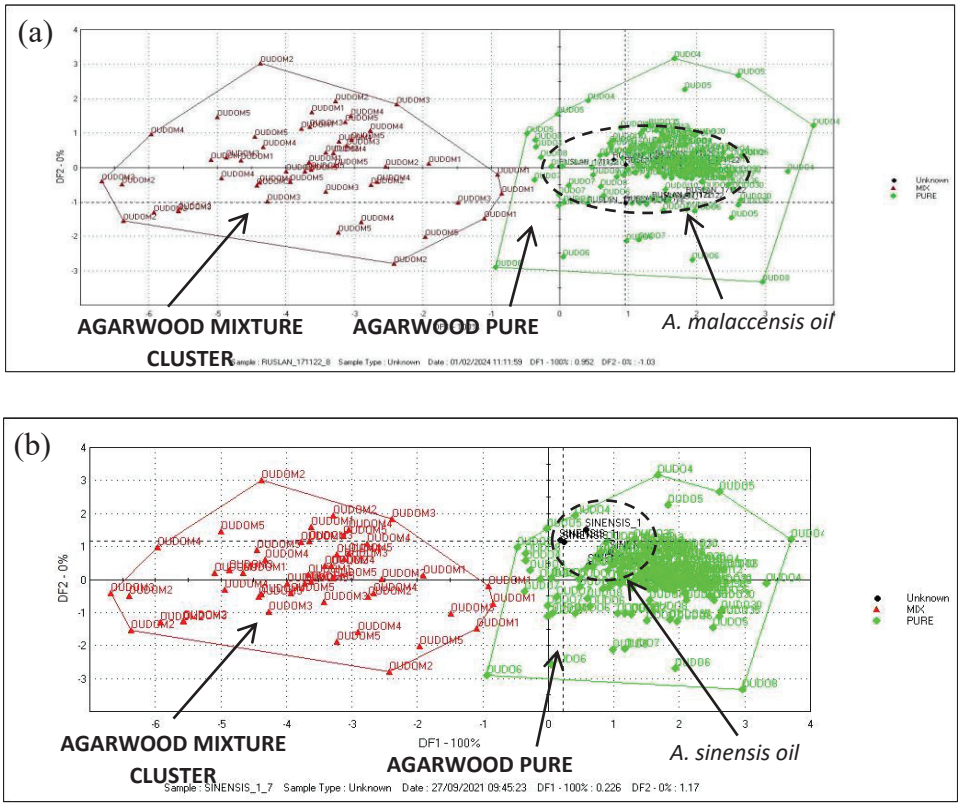

Figure 2 Classification of (a) *A. malaccensis* and (b) *A. sinensis* oils samples

Table 1 The major chemical constituents of *A. malaccensis* (AM) and *A. sinensis* (AS) essential oil

Peak No	Chemical name	Formula	KI	%	
				AM	AS
1	Benzaldehyde	C_7H_6O	952	0.22	-
2	3,5-Dimethylphenol	$C_8H_{10}O$	1103	0.64	-
3	Acetophenone	C_8H_8O	1106	0.75	-
4	Neoalloocimene	$C_{10}H_{16}$	1140	1.29	-
5	Eucarvone	$C_{10}H_{14}O$	1146	0.95	-
6	4-Phenyl-2-butanone	$C_{10}H_{12}O$	1218	**3.27**	1.45
7	Silphiperfol-4,7(14)-diene	$C_{15}H_{22}$	1358	0.90	-
8	(*E*)-Jasmone	$C_{11}H_{16}O$	1390	0.35	-
9	α-Guaiene	$C_{15}H_{24}$	1437	0.56	-
10	α-Humulene	$C_{15}H_{24}$	1452	0.28	-
11	β-Agarofuran	$C_{15}H_{24}O$	1474	**4.51**	**7.45**
12	β-Selinene	$C_{15}H_{24}$	1489	0.31	-
13	4-*epi*-*cis*-Dihydroagarofuran	$C_{15}H_{26}O$	1490	0.55	-
14	α-Muurolene	$C_{15}H_{24}$	1496	0.55	-
15	β-Dihydroagarofuran	$C_{15}H_{26}O$	1503	-	1.35
16	α-Bulnesene	$C_{15}H_{24}$	1509	**1.92**	-
17	*cis*-Muurola-3,5-diene	$C_{15}H_{24}$	1510	0.62	-
18	δ-Amorphene	$C_{15}H_{24}$	1513	0.20	-
19	Nootkatene	$C_{15}H_{22}$	1517	0.14	-
20	Elemol	$C_{15}H_{26}O$	1529	0.16	-
21	(*E*)-Nerolidol	$C_{15}H_{26}O$	1531	0.18	-
22	α-Agarofuran	$C_{15}H_{24}O$	1548	-	0.58
23	Germacrene B	$C_{15}H_{24}$	1559	-	2.11
24	10-*epi*-γ-Eudesmol	$C_{15}H_{26}O$	1622	2.42	3.81
25	Silphiperfol-6-en-5-one	$C_{15}H_{22}O$	1624	1.91	-
26	Eremoligenol	$C_{15}H_{26}O$	1629	2.39	-
27	γ-Eudesmol	$C_{15}H_{26}O$	1630	**3.35**	**6.75**
28	Hinesol	$C_{15}H_{26}O$	1640	1.84	2.43
29	Agarospirol	$C_{15}H_{26}O$	1646	**-**	4.14
30	Kusunol	$C_{15}H_{26}O$	1651	**11.30**	-
31	Valerianol	$C_{15}H_{26}O$	1656	-	**15.78**
32	Bulnesol	$C_{15}H_{26}O$	1670	2.39	-
33	*epi*-α-Bisabolol	$C_{15}H_{26}O$	1678	-	0.82
34	α-Bisabolol	$C_{15}H_{26}O$	1683	0.23	-
35	Germacra-4(15),5,10(14)-trien-1-α-ol	$C_{15}H_{24}O$	1685	-	1.90
36	Selina-3,11-dien-9-one	$C_{15}H_{22}O$	1687	0.78	1.57
37	Cyperotundone	$C_{15}H_{22}O$	1695	0.54	1.82
38	*cis*-Thujopsenal	$C_{15}H_{22}O$	1708	0.76	-
39	Curcuphenol	$C_{15}H_{22}O$	1717	0.58	-

continued

Table 1 Continued

Peak No	Chemical name	Formula	KI	%	
				AM	AS
41	Cyclocolorenone	$C_{15}H_{22}O$	1759	-	4.41
42	Aristolone	$C_{15}H_{22}O$	1763	1.76	-
43	β-Costol	$C_{15}H_{24}O$	1766	1.19	-
44	Drimenol	$C_{15}H_{26}O$	1767	3.25	-
45	*epi*-Cyclocolorenone	$C_{15}H_{22}O$	1775	-	6.69
46	Guaia-1(10),11-dien-15-al	$C_{15}H_{22}O$	1806	6.67	-
47	Dihydrocolumellarin	$C_{15}H_{22}O_2$	1900	4.28	0.78
48	Drimenin	$C_{15}H_{22}O_2$	1941	-	2.19
49	Columellarin	$C_{15}H_{22}O_2$	1952	1.26	1.19
50	Monoethylhexyl phthalate	$C_{16}H_{22}O_4$	2152	-	5.24

KI = Kovat index. Components are listed in order of their KI value and recorded for >0.1% relative content

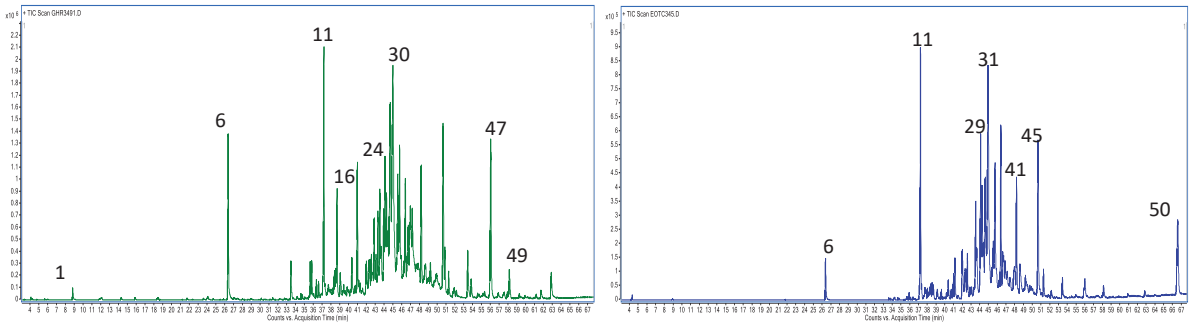

Figure 3 Total ion chromatogram (TIC) of volatiles from (a) *A. malaccensis* and (b) *A. sinensis* essential oil

Total phenolic content

The total phenolic content of *A. malaccensis* and *A. sinensis* was determined via Folin-Ciocalteu (FC) method (Everette et al. 2010). The mechanism of the Folin-Ciocalteu method is the formation of a blue colour complex which is detectable at a wavelength of 650 nm. The colour changes from yellow to green in the Folin-Ciocalteu reaction upon introduction of ascorbic acid due to the reduction of the molybdenum-tungsten complex present in the Folin-Ciocalteu reagent (FCR). FCR will oxidise phenol or phenolic-hydroxy groups into the molybdenum-tungsten complex by hetero-poly-phosphotungstate-molybdate reduction in FCR. The phenolic compound reacts with FCR only under basic conditions and forms a blue colour complex. The basic condition is provided by sodium carbonate after 60 minutes of incubation in the dark at room temperature. The intensity of the blue colour complex is positively correlated with the total phenol concentration. Various concentrations of gallic acid (5 mg/mL) were prepared to plot the standard calibration curve ranging from 50–250 mg/mL. Gallic acid was used as standard. The samples' TPC values were calculated from the calibration linear regression, y = 0.0033×-0.009 with a correlation coefficient, of R2 = 0.9908. Student's t-test showed the significant value of $p<0.05$. The total phenolic content was expressed as Gallic Acid Equivalent (GAE), in milligrams per gram (mg/g) weight. Total phenolic content of selected *Aquilaria* spp. essential oils show that *A. malaccensis* consists

higher phenols group than *A. sinensis,* with a TPC amount of 10.8 mg GAE/g and 8.5 mg GAE/g respectively (Table 2). The result is similar to Rashid et al. (2020), who found that *A. malaccensis* contained higher TPC than *A. sinensis*. Previous studies also show that different *Aquilaria* species extracts, drying methods, and solvents contribute to different TPC, which might be responsible for antioxidant activities (Wan-Nadilah et al. 2020).

Table 2 Total phenolics content in selected *Aquilaria* spp. oils

Sample	Absorbance (650 nm)	Total Phenolics Content (mg GAE/g) (2 mg/mL)
A. malaccensis	0.3160 ± 0.000	10.8
A. sinensis	0.2460 ± 0.000	8.5
Gallic acid (100µg/mL)	0.2513 ± 0.000	8.7

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH free radical method is an antioxidant assay based on electron transfer. It involves the reduction of stable DPPH free radicals in ethanol by antioxidant molecules, resulting in a colour change from violet to colourless (Garcia et al. 2012, Gulcin et al. 2023). As it transforms from the radical to the neutralised DPPH form through electron captures, the colour changes to colourless or pale yellow. Higher antioxidant levels correlate with increased discolouration; more significant discolouration indicates higher reducing ability. Lobo et al. (2010) noted that maintaining a balance between free radicals and antioxidants is essential for maintaining proper physiological function. When free radicals exceed the body's regulatory capacity, oxidative stress occurs, resulting in damage to lipids, proteins, and DNA, and contributing to various diseases. External antioxidants can help mitigate oxidative stress. In essence, a delicate equilibrium is necessary to prevent the adverse effects of free radicals on cellular components and overall health. Various concentrations of standard ascorbic acid (0.1 mg/mL) were prepared to plot the standard calibration curve ranging from 0.0500–0.0031 mg/mL. Percentage inhibition was calculated using the recorded absorbance, and standard calibration curves were plotted. The samples' antioxidant capacity was calculated from the calibration linear regression, y = 1275.7x + 0.5578 with correlation coefficient, R2=0.9984. The student's t-test showed a significant value of $p<0.05$. The IC_{50} represents the concentration of an antioxidant-containing substance needed to neutralize 50 % of initial DPPH radicals. The IC_{50} value is inversely related to a sample's free radical activity and antioxidant properties. A lower IC_{50} value indicates greater potency in scavenging DPPH, suggesting higher antioxidant activity; hence, the sample requires less quantity to scavenge free radicals (Olugbami et al. 2014). The IC_{50} value and percentage radical scavenging activity of selected *Aquilaria* spp. oils were calculated and recorded in Table 3.

Table 3 Radical scavenging activity (% RSA) and IC_{50} of selected *Aquilaria* spp. oils

Sample	Absorbance (650 nm)	RSA %	IC_{50} (mg/mL) (2 mg/mL)
A. malaccensis	0.625 ± 0.000	11.221	11.490
A. sinensis	0.658 ± 0.000	6.534	8.463
Ascorbic acid	-	-	0.039

Based on the colourimetric result, *A. malaccensis* showed a higher reducing ability than *A. sinensis*, which has a pale purple colour and slight discolouration. The IC_{50} result, however, revealed *A. sinensis* to possess a higher antioxidant activity than *A. malaccensis*, with 8.463 mg/mL and 11.490 mg/mL, respectively. This is due to the lower IC_{50}, which indicates higher antioxidant activity. The results were partially consistent with Rashid et al. (2020), who reported that *A. malaccensis* has a higher DPPH antiradical capacity than *A. sinensis*. The IC_{50} value is inversely related to the potential for free radical reduction, a higher IC_{50} indicates a smaller potential for antioxidant activity, DPPH radical scavenging activity, and vice versa (Batubara et al. 2020).

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay, as described by Halim et al. (2022), measures the reduction of a colourless ferric (Fe^{3+})-TPTZ complex to a blue-coloured ferrous (Fe^{2+})-TPTZ complex, driven by electrons donated by antioxidants. This reaction is observed at 593 nm and quantified using a Trolox standard curve. Additionally, Njoya (2021) explained that the brown colour of ferric ion (Fe^{3+}) transforms into an intense blue colour representing ferrous ion (Fe^{2+}) after complete reduction by a potential antioxidant. The 2,4,6-Tripyridyl-S-triazine (TPTZ) reagent, utilized for iron detection, reacts with Fe (II) to evaluate phenolic compounds. The sample's higher levels of antioxidant compounds are associated with increased total reducing activity. The preparation of FRAP reagent is crucial in this assay, to obtain FRAP value, the FRAP reagent must be in yellow-orange. A reduction of iron compounds during the preparation will result in a purple-blue-coloured FRAP reagent. Note that even if there is a little hint of blue/purple colour in the reagent, the reagent must be prepared again. Various concentrations of Trolox were prepared to plot the standard calibration curve ranging from 500–2500 µM. Trolox was used as standard. The sample values were calculated from the calibration linear regression, $y = 0.0001x + 0.0095$ with correlation coefficient, $R2 = 0.9942$. The student's t-test showed a significant value of $p<0.05$.

Comparison of FRAP value from previous studies is limited. Marliani (2021) reported no FRAP value from *A. malaccensis* and *A. sinensis* in their research on the pharmacological aspect. Wang et al. (2018a) however reported the FRAP value of *A. sinensis* in their research which as concentration increased, the ferric reducing power also increased. Hashim et al. (2016) also only reported the FRAP value *A.* crassna. The findings from Halim et al. (2022) align with Triyasmono et al. (2017) research, where the ethanol extract of agarwood leaves from *A. malaccensis* exhibited the highest antioxidant activity. Various extracts (ethanol, ethyl acetate, and n-hexane) from *A. malaccensis* demonstrated antioxidant activity across DPPH, ABTS, and FRAP assays. The ethanol extract consistently displayed the highest antioxidant activity in all assays, attributed to its elevated phenolic content, as evidenced by the UV/Vis scanning spectrogram. *A. malaccensis* has a higher ferric-reducing ability based on the colourimetric and spectrometric results (Table 4). *A. sinensis* has a pale blue/purple colour than *A. malaccensis* and thus possesses a lower ferric-reducing ability. An amount of 4 mg/mL of selected essential oils was used due to the low ferric-reducing ability shown by 1, 2, and 3 mg/mL. Therefore, 4 mg/mL diluted essential oils were selected for the assay as they demonstrated antioxidant activity.

Table 4 Ferric reducing antioxidant activity of selected *Aquilaria* spp. oils

Sample	Absorbance (593 nm)
A. malaccensis	0.1650 ± 0.000
A. sinensis	0.1040 ± 0.000
Ascorbic acid	0.1578 ± 0.001

β-Carotene/Linoleic acid bleaching assay

The β-carotene/linoleic acid bleaching assay assesses antioxidant activity by measuring a substance's ability to inhibit the oxidation of β-carotene in the presence of linoleic acid and free radicals (Musa et al. 2019). In this method, the orange colour of β-carotene is lost due to interruption by free radicals produced during linoleic acid oxidation. Antioxidants in the reaction medium slow the bleaching process by scavenging the free radicals attacking the β-carotene molecule (Ahmed et al. 2022, Lu et al. 2014). Various ascorbic acid concentrations were prepared to plot the standard calibration curve ranging from 0.025–0.800 mg/mL. Ascorbic acid was used as standard. The sample values were calculated from the calibration linear regression, $y = 42.449x + 2.9611$ with correlation coefficient, $R2 = 0.9747$. Student's t-test showed the significant value of $p<0.05$. *A. malaccensis* has higher antioxidant activity than *A. sinensis*. It can be seen that *A. sinensis* is unable to retain the orange colour of β-carotene. This indicates low antioxidant activity. On the contrary, the pale yellow of *A. malaccensis* showed that the essential oil has

higher antioxidant activity than *A. sinensis*. The IC_{50} result revealed *A. malaccensis* to possess a higher antioxidant activity than *A. sinensis* with 3.66 mg/mL and 6.25 mg/mL, respectively. A lower IC_{50} indicates a higher antioxidant activity. Despite the notable differences in colourimetric and IC_{50} values, essential oils are classified under low antioxidant activity. The lack of previous studies on lipid peroxidation by β-carotene/linoleic acid bleaching assay, a comparison of *Aquilaria* spp. essential oils' antioxidant activity cannot be done. Despite that, Ibrahim et al. 2017 reported *A. malaccensis* demonstrated higher efficacy in counteracting enzyme activity, suggesting a potentially higher concentration of active compounds. This observation is consistent with previous studies highlighting the highest antioxidant properties of methanol extracts from *A. malaccensis* leaves, indicating its ability to protect against the free radical formation and delay lipid peroxidation (Huda et al. 2009). Lipid peroxidation properties were found on *A. sinensis*' leaves with IC_{50} of 0.49 ug/mL (Hashim et al. 2016). IC_{50} value of selected *Aquilaria* spp. essential oils and standard ascorbic acid were calculated and recorded in Table 5.

CONCLUSION

Electronic nose in combination with DFA was successfully classified and distinguished the *A. malaccensis* and *A. sinensis* oil as two different essential oil samples with 100% cross-validation results. E-Nose with AIS successfully evaluated the quality of both samples with the pure recognition group. The agarwood essential oil analysis results suggest that the sample falls into the pure Agarwood oil group with 100% accuracy similarity in the AIS database. In the GC and GC/MS analysis, the volatile compounds commonly found in agarwood oil were detected. Both samples are pure and of good quality without adulteration. However, *A. sinensis* (UODOI14) is contaminated. Therefore, we need to be careful when handling agarwood oils to avoid this problem. Based on total phenolic content, FRAP, β-carotene/linoleic acid bleaching assays, the essential oil of *A. malaccensis* is a better antioxidant than *A. sinensis*. Results from this study could be useful for selecting agarwood oil as an active ingredient in various ranges of cosmetic and personal care products.

Table 5 β-Carotene/linoleic acid bleaching assay of selected *Aquilaria* spp. essential oils

Essential oil	Time (min)		% Inhibition	IC_{50} (mg/mL) (1 mg/mL)
	0	60		
A. malaccensis	0.243 ± 0.002	0.078 ± 0.002	32.00	3.66
A. sinensis	0.183 ± 0.001	0.056 ± 0.002	30.84	6.25
Ascorbic acid	-	-	-	1.11

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