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Phytochemical Screening and Characterization of Agarwood (Aquilaria malaccensis) Chips Wood Grade as Incense Headspace Volatile Compounds by GC-MS.Ms.Q.TOF, SPME

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Abstract

Agarwood (Aquilaria malaccensis) is an Asian tree traditionally used for many medicinal purposes, its oil constituents and inhaled vapor has sedative, antidepressant and anxiolytic activities. Gas Chromatography and quadruple time of flight –mass spectrometry (GC.MS.MS/Q.TOF) in the MS-MS mode has been used in screening and identification of multiple compounds of three different grade of agarwood chipwood, using DB-1 column which led to identification of 96 compounds while the majority of the compounds about 500 compounds remain UN knowing to the difficulties of confirming or rejecting them . The generated results were so complicated and very difficult to be interpreted for the presence of isomers with similar matched spectrums; the absence of official standard references for these compounds make the judgment of confirming the identified compounds more complicated and very hard to absolute identification to be assessed. The compounds were extracted by applying a solvent less technique; trapping incense and volatile headspace. The total Ion chromatogram detected compounds are distributed among different array over the chemical families among monoterpenes, sesquiterpenes, hydrocarbons, oxygenated monoterpenes, sesquiterpenes, norterpenoids and diterpenoids ,short chain glycols, carboxylic acids and others. The flowing compounds are the major terpene, sesquiterpene and oxygenated sesquiterpenoid found in all grades of A. malaccensis chipswood under investigation, furthermore; the study represented that the characterization of compounds from agarwood chips wood is highly possible through incense volatile sample.

Keywords: agarwood, incense smoke, volatile headspace, solid phase microextraction GC-FID, GC-MS, Q.T.O.F, SPME

1. Introduction:

1.1 Aquilaria malaccensis (Lam): Is the main source of the fragrant wood which called agarwood, the Common names found are agar-wood, aloeswood, eaglewood, gaharu and karas (Indonesia and Malaysia). Twelve to fifteen other native species, belonging to the family Thymelaeaceae, are thought to produce agarwood [1,2,3,4,5].

A. malaccensis are distributed from India, Myanmar, Sumatra, Peninsular Malaysia, Singapore, Borneo and the Philippines [6,7,8]. Agarwood is used in medicinal, aromatic and religious purposes in many culture and religion as Buddhist, Jewish, Christian, Muslim and Hindu societies.[3,9,10,11]. The diverse and complex chemical components of agarwood contribute to the diversity of bioactivity and pharmacology; including neural activity, gastrointestinal regulation, antibacterial, anti-inflammation, and cytotoxicity [12].

The Asian traditional use of Agarwood as a tranquilizer and an excitement reducing agent has oriented the recent research towards its neural activity. pharmacological studies have shown that agarwood has an active effect on the nervous system.

A benzene extract of *A. malaccensis* agarwood reduced spontaneous motility, prolonged

hexobarbiturate-induced sleeping time, and decreased rectal temperature, whereas petroleum ether, chloroform, or water extracts did not have that effect.

A further bio-guided isolation of a benzene extract found that jinkoh-eremol and agarospirol were the main active constituents. Inhalation of agarwood essential oil vapor is sedative to mice, in which the main volatile constituents were benzylacetone, α-gurjunene, and (+)-calarene. As benzylacetone has a sedative effect, a number of derivatives are synthesized and assessed for a sedative effect.

The results demonstrated that benzylacetone-like compounds has sedative activities, and their intensities varied depending on the functional group in the carbon chain, the substituent in the benzene ring, and their combinations. both the ethanol extract and essential oil of agarwood,

induced by the whole-tree agarwood inducing technique in A. Sinensis trees, has a sedative-hypnotic effect, where its potential mechanism is related to regulating the gene expression of GABAA receptors and potentiating the GABAA receptor function. Agarofuran, derived from

agarwood essential oil have an anxiolytic and anti-depression activity in mice. To explore a potential drug for treating anxiety, depression, a series of

agarofuran-like derivatives are synthesized and the activity screened, among which, buagafuran

was an effective compound for anti-anxiety and anti-depression, with low toxicity and a high safety coefficient.

The potential mechanism might be through modulating central neurotransmitters, such as dopamine. Until now, phase II clinical trials are being conducted on buagafuran. Furthermore, many other activity screening results have also shown that compounds from agarwood have an effect on neural activity and neural protective activity against both glutamate-induced and corticosterone-induced neurotoxicity in PC12 pheochromocytoma and human U251 glioma cells. Agarwood compounds are a promising drug candidate in the future [12].

The chemical constituents of agarwood originated from the genus Aqularia include sesquiterpenes, chromone, aromatic compounds, terpenes, sesquiterpenes in the form of the flowing chemical families ,Agarofurans, Agarospiranes, Guaianes, Eudesmanes Eremophilanes; and Prezizananes [3,6].

1.2.1 GCMSMS.Q.TOF: Recently GC.MS.Q.TOF has been widely used to identify chemical constituents' in plant secondary metabolism, usually scientists used gas chromatography systems based on quadrupole mass spectrometer analyzers, in both GC.Q.MS or

GC.MS/MSMS.Q.TOF Four poles are connected into the electrical circuit that generates

radiofrequency field. The change in the electrical field enables to generate scanning in time. Which lead to generate fragments in the ion source through ion optics enters the analyzer. The ions separation is accomplished by applying alternating RF frequency and DC voltage to diagonally opposite ends of the quadrupole, which in turn allows a specific mass fragment to pass through the quadrupole filter [1].

1.2.2 Basic principle of GCMS. Q.TOF: Gas chromatography mass spectrometer with time of flight detector separate ions in different principle when compared with normal gas chromatography quadrupole mass spectrometer [1] where in TOF system it measures the times taken by ions to pass along an evacuated tube as a means of measuring m/z values and therefore of obtaining a mass spectrum so the main features of this technique are: All ions starting entering the quadrupole tube at the same time. The ions separation process depended on ions velocity which is proportional to

its mass. The ions arrive at the detector in procession and strictly in the order of increasing m/z values. Ions of the smallest m/z values arrive first, followed successively by others of increasing m/z value Both mass spectrometers GC.Q.MS and GC.MS.Q.TOF are operated on the same gas chromatographic system by splitting the column effluent to both detectors.

The Q. MS has to be operated in optimized single ion monitoring (SIM) mode to achieve high

sensitivity which can be a compete with the TOF MS. The TOF MS has provided full mass range information in any acquired mass spectrum

without losing sensitivity. Whilst the Q MS has showed the performance already achieved in earlier tests, the sensitivity of the TOF MS is on average higher than that of the Q.MS in the "operational" SIM mode by a factor of up to 3, reaching detection limits of less than 0.2 pg. Measurement precision determined for the whole analytical system is up to 0.2 % depending on substance and sampled volume. The main advantage of coupling a TOF MS to agas chromatograph (GC) over using the QP MS is the intrinsic full mass range acquisition and the better mass resolution and accuracy. The identification of unknown peaks is significantly facilitated by these advantages and the use of more narrow mass intervals is expected to reduce interferences and background noise. In addition, much higher data acquisition rates are possible using TOF MS, which is an advantage for fast chromatography. A TOF MS instrument can measure more than 10000 mass spectra per second.

They are added up and averaged over a certain time period to yield the desired time resolution. The possibility of operating the TOF MS at high

data rates is also of high interest for fast chromatography and narrow peaks, for which the operating frequency of quadrupole instruments

(especially when measuring several ions) can be a limiting factor.

The maximum time resolution for the TOF MS used in this study is 50 Hz. An increase in the data frequency will lead to decreased signal-to-noise levels. The data frequency must therefore be optimised to provide a sufficient number of data points per chromatographic peak while keeping the signal-to-noise level as high as possible. In contrast, a QP MS is a mass filter and will only measure one mass at a time. It needs to scan many individual masses sequentially to register a full mass spectrum.

To achieve high sensitivity, Q. MS are therefore often operated in single ion monitoring (SIM) mode in which the instrument is tuned to only one or a few selected ion masses and all the other ions

do not pass the quadrupole mass filter. Regardless of these limitations of the QP MS, it is widely used in analytical chemistry due to its stability, ease of operation, high degree of linearity, good reproducibility as well as sensitivity.

Especially for Volatile compounds monitoring the advantage of obtaining the full mass information from the TOF instrument might allow retrospective quantifications of species which were not target at the time of the measurement. For this purpose, the TOF MS must be well characterised (in particular with respect to linearity) and the calibration gas used during the

measurements must contain measurable amounts of the retrospective sub- stances and be traceable to an absolute scale.

2. Material and Methods:

2.1 *A. malaccensis* (agarwood) sample: Three different grade of agarwood chips (high grade, medium and low of (*A. malaccensis*) were collected from Kedaik Agarwood Sdn. Bhd., a well-known Malaysian agarwood supplier. Those chipwood were obtained from Endau-Rompin Forest Reserve, Pahang. C7-C20 n-alkanes were supplied by Tokyo Chemical Industry Co., Ltd. (Toshima, Kita-ku, Tokyo).

2.2 Instrumentation:

2.2.1 Gas Chromatography-Flame Ionization Detector (GC-FID): Chemical analyses were performed by gas chromatography-flame ionization detector (GC-FID) Agilent 7890B (Agilent technology. USA), equipped with DB-1 (100% dimethylpolysiloxane) capillary column, 30 m × 0.25 mm ID × 0.25 µm film thickness. Spilitless mode was used with narrow SPME inlet liner at 220 ºC injector temperature, carrier gas Helium at 1.2 mL/min and 250 ºC for both detectors' temperature.

2.2.2 GC.MS.MS.Q.TOF: Sample separation were conducted by Agilent 7890A system in coupling with 7200 series quadrupole time of flight mass spectrometer (Agilent technology

.USA), equipped with DB-1 (100% dimethylpolysiloxane) capillary column, 30 m × 0.25 mm ID × 0.25 µm film thickness.

The Ms transfer line temperature was 250˚C ,carrier gas was Helium (purity 99.999%) at flow rat 1.4ml.min-1 ,oven temperature was pr ogrammed ,60 C initial (hold for 0.5 min) at 3C/min to 100C (hold for 3min) then to 200C at5C/min (hold for 3min) and finally to 250 at 5C/min , injection temperature ,200C, injection volume 1µl and in spilitless mode. The Q.TOF system was used under Total Ion Chromatogram (TIC) mode; temperature of ion source was 250C, ionization voltage was fixed at constant ionization energy of 70eV. the mass range was 40-500 Dalton. Data acquisition and processing was done by Agilent Mass Hunter.

2.2.3 The Solid Phase Microextraction (SPME): sampling apparatus was equipped with a 50/30 µm divinylbenzene-carboxen-polydivinylmethylsiloxane (DVB-CAR-PDMS) purchased from Supelco Inc., Bellefonte, PA and USA. This fiber was used to extract volatile compounds emitted from incense smoke and headspace volatile of agarwood respectively. That SPME fibre was pre-conditioned at gas chromatography injection port using SPME narrow inlet for 30 minutes at temperature 200 Co before collecting the volatile compounds.

2.2.4 Data handling: Data acquisition and processing was performed by Agilent MSD ChemStation version 2.0 and Mass Hunter version B.06.00 Agilent technology, spectrum searching was performed with National Institute of Standard and Technology (NIST) 11 MS spectrum library.

3. Results and Discussion:

The analysis of the oil or wood composition in agarwood usually achieved by GC.MS with GC-FID [13] and that clearly can be observed through the published worked handling the investigation of chemical constituents of agarwood, since GC–MS is regarded as a well-established and reliable method for profiling of plant constituents. However, agarwood samples can be too complex

for the normal GC-FID and GC-MS separation, which can lead to in incomplete resolution or absolute identification of all chemical constituents.

3.1 Agarwood high grade results:

A total of 96 ions were identified in the three samples of agarwood chipwood through the GC.MS.Q.TOF. 51 compounds have been identified in the first sample of high grade, detailed data are mentioned in table [1}, the dominate compounds among these sample are Longifolene aldehyde 26 %, 25 Epiglobulol, Eremophila-1(10),11-diene 7%, Guaia-1(10),11-

diene 6%, α-Gurjunene 6%, β-Eudesmol 5 %, and beta Humulene 5%

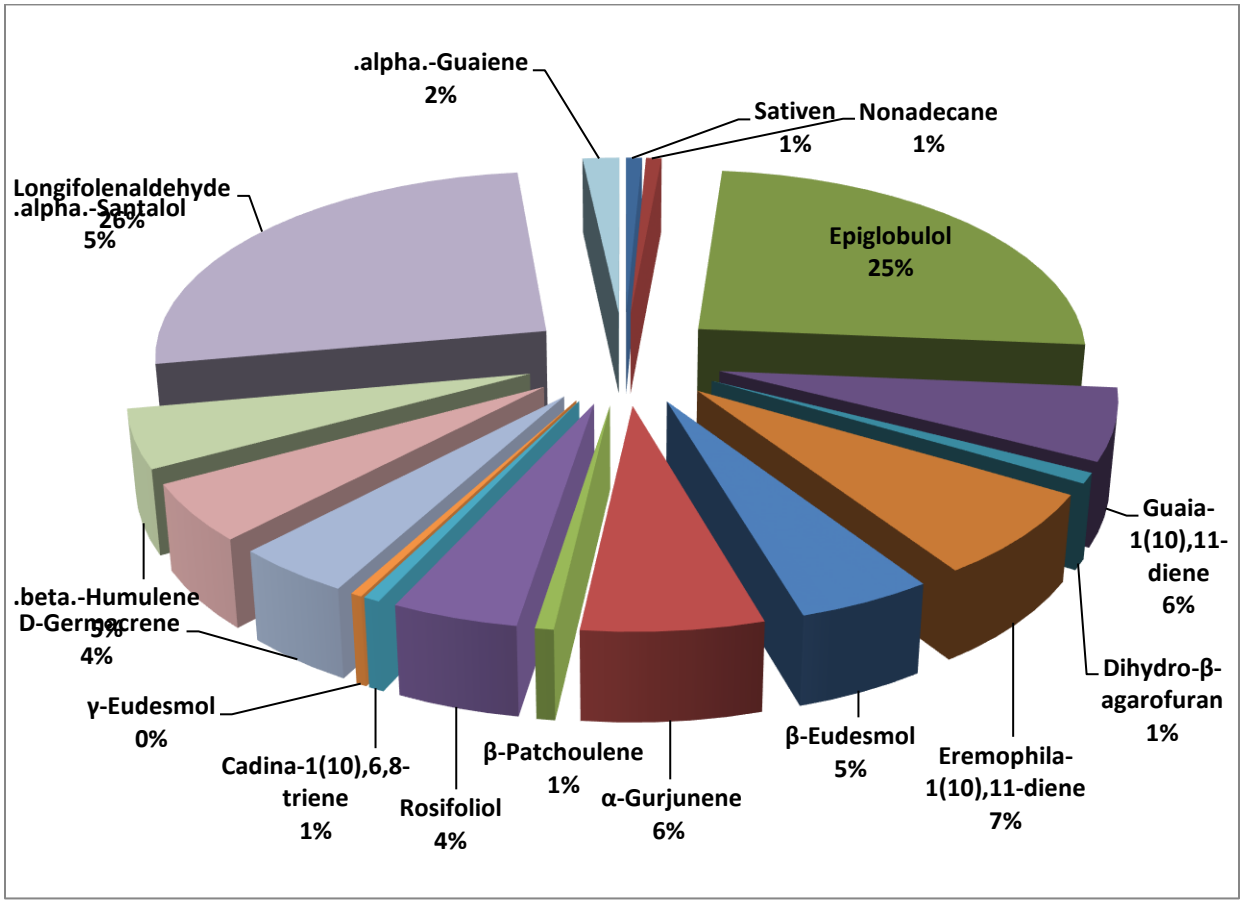


Figure 1: Agarwood chipwood high grade chemical compounds sample 1

3.1 Agarwood medium grade results:

The second sample of agarwood chipwood from *A. malaccensis* reveals 76 compounds as in table [2] dominated by beta-Guaiene 32%. Flowed isoledene 24%, (-)- aristolene 14%, Rotundone 7 %, Valencene 4%, Gamma-Muurolen 3%, Caryophyllene and alpha Guaiene 2% respectively.

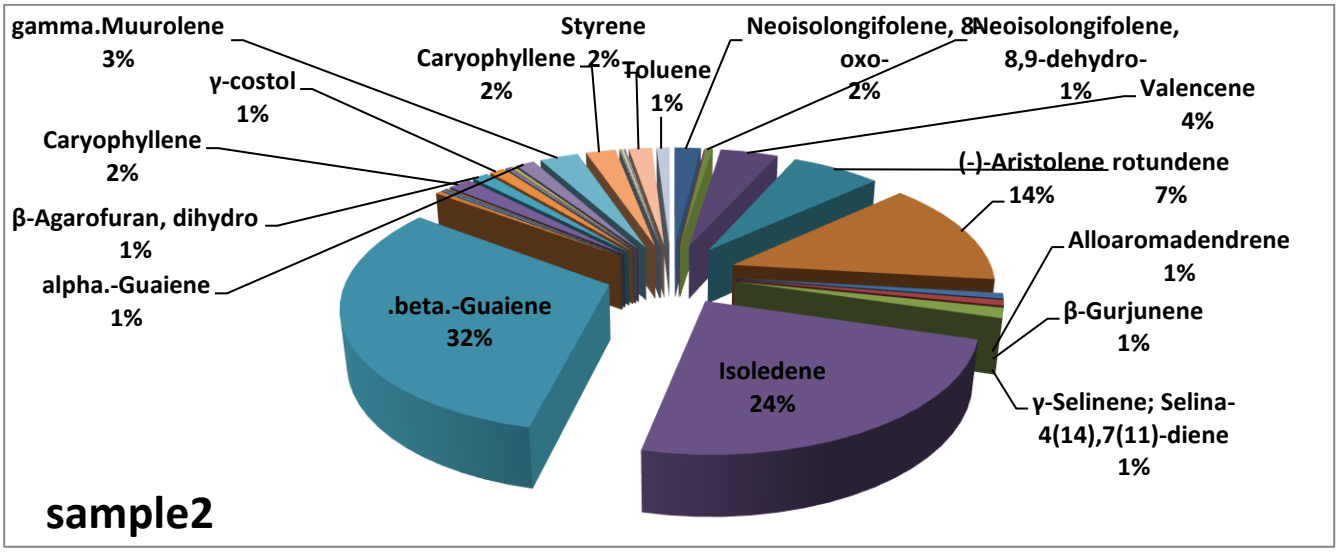


Figure 2: Agarwood chipwood medium grade chemical compounds sample 2

3.2 Agarwood low grade results:

The third sample of agarwood chipwood from *A. malaccensis* show the present of 52 compounds, its remarkable to be mentioned that the peak abundance in the third sample is less than the all previous samples, but there are similarly in the compounds identified, another different is the dominate compounds, Aristolene 20%, is the dominate compounds among the low grade compounds while in first sample it was dominated by Longifolene aldehyde and beta-Guaienein the second sample. The third sample constituents are dominated respectively by Aristolene 20% , Epiglobulol 17%, γ-Selinene(Selina-4(14),7(11)-diene) 16% , Spatulenol 14% , λ costol 11% and Guaia-1(10),11-diene(α-Bulnesene) 5%

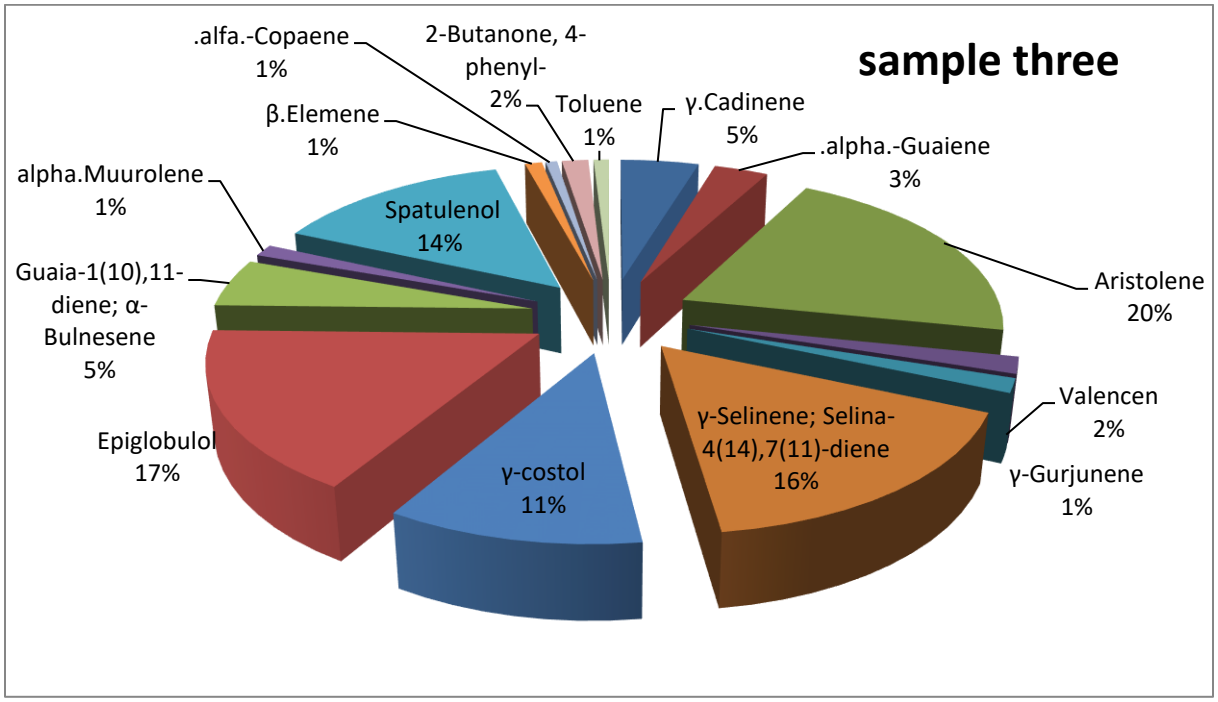


Figure 3 : Agarwood chipwood low grade chemical compounds sample 3

3.1 Discussion:

Many of compounds detected in the main chromatograms of these samples are pyrolyzed products, these products as Toluene o-Xylem, Benzaldehyde, Phenol, Acetophenone and Naphthalene; some of the sesquiterpenes are also paralyzed products from the resin. These findings also confirmed by Ishihara [14], and many of these pyrolyzed form are also reported by Pripdeevech [15]**Error! Reference source not found.** .The main different from the three samples results are the abundance; in the first sample is greater than the other two samples; while the second sample compounds are moderate in their value of abundance; at last the third sample get the lowest abundance value through all the compounds. Second observation can be added; It's the present of certain compounds seem to be exclusive to the category, since Sativen, Nonadecane, Eremophila-1(10), 11-diene, β-Eudesmol, Rosifoliol γ-Eudesmol , D-Germacrene, Clovene , .alpha.-Santalol and beta.-Humulene are detected only in the first sample agarwood chipwood from *A. malaccensis*;

Table 1 **: sample 1** Agarwood chipwood high grade Q.TOF compounds

No	RT	Compound Name	Formula	Area%
1	1.293	Propanal	C3H6O	0.04
2	1.309	Propane, 2-methoxy-	C4H10O	0.02
3	1.676	2-Propenal	C3H4O	0.02
4	2.368	Toluene	C7H8	0.02
5	4.051	Styrene	C8H8	0.02
6	6.123	Phenol	C6H6O	0.04
7	13.24	Naphthalene	C10H8	0.02
8	13.66	.alpha.Terpineol	C10H18O	0.01
9	16.88	4-Phenyl-2 butanol	C10H14O	0.03
10	21.76	n-Decanoic acid	C10H20O2	0.03
11	23.77	β-Elemene	C15H24	0.03
12	24.64	γ-Cadinene;	C15H24	0.01
13	24.93	beta cubebene	C15H24	0.01
14	25.41	α-Muurolene	C15H24	0.03
15	25.41	Alloaromadendrene	C15H24	0.36
16	26.08	Elemol	C15H26O	0.03
17	26.10	β-Patchoulene	C15H24	0.11
18	26.33	Sativen	C15H24	0.71
19	26.50	.alpha.-Cubebene	C15H24	0.01
20	26.66	Selina-3,7(11)-diene	C15H24	0.03
21	27.77	.alfa.-Copaene	C15H24	0.11
22	28.10	4-epi-cis-Dihydro-β-agarofuran	C15H26O	0.23
23	28.82	Nonadecane	C19H40	0.70
24	28.87	Guaia-1(10),11-diene	C15H24	5.86
25	28.90	Cycloisolongifolene, 8,9 dehydro-	C15H22	0.01
26	29.30	Butylated Hydroxytoluene	C15H24O	0.01
27	29.52	Spathulenol	C15H24O	0.42
28	29.73	Epiglobulol	C15H26O	23.7

29	30.42	.alpha.-Calacorene	C15H20	0.02
31	30.91	Dihydro-β-agarofuran	C15H26O	0.86
32	32.46	Eremophila-1(10),11-diene	C15H24	6.87
33	32.69	Isoledene	C15H24	0.45
34	32.81	β-Eudesmol	C15H26O	4.56
35	33.27	α-Gurjunene	C15H24	6.11
36	33.55	Di-epi-.alpha.-cedrene	C15H24	0.41
37	33.97	β-Patchoulene	C15H24	0.63
38	34.08	Rosifoliol	C15H26O	4.25
39	34.25	Selina-3,7(11)-diene	C15H24	0.10
40	34.25	γ-Gurjunene	C15H24	0.28
41	34.57	Ledene alcohol	C15H24O	0.00
42	34.66	Cadina-1(10),6,8-triene	C15H22	0.56
43	34.65	γ-Eudesmol	C15H26O	0.39
44	35.36	D-Germacrene	C15H24	3.99
45	35.62	Cadalene	C15H18	0.09
46	35.72	Clovene	C15H24	1.29
47	35.73	.alpha.-Santalol	C15H24O	4.70
48	35.97	.beta.-Humulene	C15H24	4.77
49	37.92	Isolongifolen-5-one	C15H22O	0.00
50	38.28	Longifolenaldehyde	C15H24O	24.91
51	40.17	.alpha.-Guaiene	C15H24	1.64
52	40.44	Cadin-1,3,5-trien-5-ol	C15H22O	0.01
53	41.23	Selina-4(14),7(11)-diene	C15H24	0.25
54	42.38	n-Decanoic acid	C10H20O2	0.02
55	42.65	γ-costol	C15H24O	0.08
56	45.67	n-Hexadecanoicacid	57-10-3	0.13

Table 2 Agarwood chipwood medium grade Q.TOF compounds sample 2

No	RT	Compound label	Formula	Area
1	2.361	Toluene	C7H8	0.876
2	4.043	Styrene	C8H8	0.360
3	6.147	Phenol	C6H6O	0.239
4	8.580	p-Cymene	C10H14	0.048
5	10.28	m-Xylene	C10H14	0.005
6	13.23	Naphthalene	C10H8	0.374
7	13.66	.alpha.-Terpineol	C10H18O	0.049
8	16.08	2-Butanone, 4-phenyl-	C10H12O	1.500
9	22.15	Isolongipholene	C15H24	0.047
10	22.71	.alfa.-Copaene	C15H24	0.663
11	22.93	cis-muurola-3,5-diene	C15H24	0.104
12	23.76	beta.-Neoclovene	C15H24	0.095
13	23.77	β.Elemene	C15H24	0.971
14	23.99	Cyperene	C15H24	0.044
15	24.51	α-Gurgujene	C15H24	0.029
16	25.87	Longifolene-(V4)	C15H24	0.007
17	25.91	.alpha.-Guaiene	C15H24	0.106
18	26.12	β-Patchoulene	C15H24	0.129
19	26.29	Longifolenaldehyde	C15H24O	0.001
20	26.83	Alloaromadendrene	C15H24	0.039
21	27.33	Spatulenol	C15H24O	13.477
22	27.77	alpha.Muurolene	C15H24	1.220
23	27.97	α.Curcumene	C15H22	0.292
24	28.63	beta.Vatirenene	C15H22	0.031
25	28.88	Guaia-1(10),11-diene	C15H24	4.912
26	29.24	4-epi-cis-Dihydro-β-agarofuran	C15H26O	0.366

27	29.72	Epiglobulol	C15H26O	16.127
28	30.43	γ-costol	C15H24O	10.190
29	30.71	Elemol	C15H26O	0.338
30	31.19	Dihydro-β-agarofuran	C15H26O	0.183
31	32.46	Rotundene	C15H24	0.158
32	32.48	Guaia-3,9-diene	C15H24	0.021
33	33.04	Humulene	C15H24	0.349
34	33.46	γ-Selinene; Selina-4(14),7(11)-diene	C15H24	15.752
35	33.66	Guaia.9.11.diene	C15H24	0.004
36	33.68	beta.Guaiene	C15H24	0.008
37	33.91	Agarospirol	C15H26O	0.005
38	33.96	γ-Gurjunene	C15H24	1.430
39	33.96	α.Elemene	C15H24	0.293
40	34.12	Valencen	C15H24	1.678
41	34.24	β.Gurjurene	C15H24	0.261
42	34.44	Aristolene	C15H24	19.564
43	34.65	.alpha.-Guaiene	C15H24	3.074
44	34.77	γ.Cadinene	C15H24	4.442
45	39.24	Cadalene	C15H18	0.020
46	39.90	10.epi.gama.eudesmol	C15H26O	0.009
47	40.92	Neoisolongifolene, 8-oxo-	C15H22O	0.078
48	42.95	gamma.HIMACHALENE	C15H24	0.028

Table 3 : Agarwood chipwood low grade Q.TOF compounds sample 3

No	RT	Compound Name	Formula	Area%
1	1.696	Benzene	C6H6	0.049
2	2.361	Toluene	C7H8	0.907
3	4.044	Styrene	C8H8	1.617
4	5.522	1.Hexen.3.ol	C6H12O	0.01
5	10.27	3-Nonen-1-ol, (Z)-	C9H18O	0.082
6	11.8	Camphor	C10H16O	0.004
7	13.25	Naphthalene	C10H8	0.077
8	13.67	.alpha.-Terpineol	C10H18O	0.006
9	16.14	2-Butanone, 4-phenyl-	C10H12O	0.141
10	20.54	α.Ionene	C13H18	0.011
11	21.68	cis-muurola-3,5-diene	C15H24	0.081
12	22.71	Ylangene	C15H24	0.214
13	22.92	.alpha.-Cubebene	C15H24	0.016
14	23.66	cis-muurola-3,5-diene	C15H24	0.02
16	23.77	β-Elemene	C15H24	0.049
17	24.95	Caryophyllene	C15H24	2.085
18	25.91	Aciphyllene	C15H24	0.125
19	26.1	β-Patchoulene	C15H24	0.104
21	26.26	β.Ionone	C14H22O	0.02
22	26.45	.gamma.-HIMACHALENE	C15H24	0.008
24	26.51	gamma.Muurolene	C15H24	2.7
25	26.59	Isolongifolene-5-ol	C15H24O	0.005
26	27.56	beta.Panasinsene	C15H24	0.306
27	27.74	.gamma.-HIMACHALENE	C15H24	0.007
28	27.77	.alpha.-Guaiene	C15H24	1.317
29	28.26	delta.-Selinene	C15H24	0.075
30	28.39	(+)-epi-Bicyclosesquiphellandrene	C15H24	0.607

31	28.89	Guaia-1(10),11-diene; α-Bulnesene	C15H24	0.279
32	28.89	Cadina-1(10),4-diene; δ-Cadinene	C15H24	0.239
33	29.2	4-epi-cis-Dihydro-β-agarofuran	C15H26O	0.084
34	29.26	Longifolenaldehyde	C15H24O	0.051
35	29.27	Cadina-1(2),4-diene	C15H24	0.137
36	29.61	Cadina-1,3,5-triene	C15H22	0.153
37	30.44	γ-costol	C15H24O	1.06
39	30.91	β-Agarofuran, dihydro	C15H26O	1.133
40	31.99	Caryophyllene	C15H24	1.622
41	32.22	Di-epi-.alpha.-cedrene	C15H24	0.417
42	32.22	10s,11s-Himachala-3(12),4-diene	C15H24	0.104
43	32.44	Selina-3,7(11)-diene	C15H24	0.092
44	32.45	delta.Neoclovene	C15H24	0.436
45	32.68	(-)-Aristolene	C15H24	0.075
46	32.77	Cedrol	C15H26O	0.009
47	32.79	Caryophyllenyl alcohol	C15H26O	0.074
48	32.8	Caryophyllene-(I1)	C15H24	0.283
49	32.84	Nootkaton-11,12-epoxide	C15H22O2	0.005
51	32.95	(+)-epi-Bicyclosesquiphellandrene	C15H24	0.671
53	32.96	Cyperene	C15H24	0.409
54	33.36	α-Curcumene	C15H24	0.001
55	33.46	.beta.-Guaiene	C15H24	30.112
56	33.74	Isoledene	C15H24	22.826
57	33.97	γ-Selinene; Selina-4(14),7(11)-diene	C15H24	1.246
58	34.11	β-Gurjunene	C15H24	0.679
59	34.25	Alloaromadendrene	C15H24	0.665
60	34.44	(-)-Aristolene	C15H24	13.034
62	34.65	Rotundone	C15H24	6.231
63	34.76	Valencene	C15H24	4.04

64	35.22	9-Cedranone	C15H24O	0.183
65	35.29	Neoisolongifolene, 8,9-dehydro-	C15H22	0.499
66	35.5	Humulene	C15H24	0.38
67	35.53	Isolongifolene, 7,8-dehydro-8a-hydroxy-	C15H24O	0.123
68	36.2	Cedranoxide8.14	C15H24O	0.012
69	37.07	3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide	C15H18O4	0.005
70	38	Murolan-3,9(11)-diene-10-peroxy	C15H24O2	0.024
71	38.88	Cadina-1(10),6,8-triene	C15H22	0.026
72	38.93	Cyclolongifolene oxide, dehydro-	C15H22O	0.071
73	39.24	Cadalene	C15H18	0.036
74	40.93	Neoisolongifolene, 8-oxo-	C15H22O	1.833

From the table 1 above The present of 2-Butanone -4-phenyl, Dihydro β-agarofuran are significant at all the samples of the three grades table 4 contain the compounds which can be found in all grades of *A. malaccensis* chips wood under investigation with their relative area in each grade. We can see that the area of these flowed compounds are garater in high grade agarwood chipwood compounds than in low or mid-grade; alpha.-Guaiene, alpha.-Terpineol, Alloaromadendrene, Cadalene, Dihydro-β-agarofuran A, Dihydro-β-agarofuran A, Guaia-1(10),11-diene, Longifolenaldehyde , β-Patchoulene while Styrene and Toluene are dominate only in mid-grade, in the same time 2-Butanone, 4-phenyl and β-Element and Naphthalene are dominate in the low grade.

Table 4: common terpine, sesquiterpene and oxygenated sesquterpinoied found in all grades of *A. malaccensis* chips wood under investigation

Compound	**Class**	**S1**	**S2**	**S3**
.alpha.-Guaiene	C15H24	1.64	0.106	1.317
alpha.-Terpineol	C10H18O	0.01	0.049	0.006
2-Butanone, 4-phenyl-	C10H12O	0.03	1.500	0.141
Alloaromadendrene	C15H24	0.36	0.039	0.665
Cadalene	C15H18	0.09	0.020	0.036
4-epi-cis-Dihydro-β-agarofuran A	C15H26O	0.23	0.366	0.084
Dihydro-β-agarofuran	C15H26O	0.86	0.183	1.133

Guaia-1(10),11-diene	C15H24	5.86	4.912	0.279
Longifolenaldehyde	C15H24O	24.91	0.001	0.051
β-Elemene	C15H24	0.03	0.971	0.049
β-Patchoulene	C15H24	0.11	0.129	0.104

Figure 4: Area % of common compounds found in all sample of *A. malaccensis* chips wood under investigation

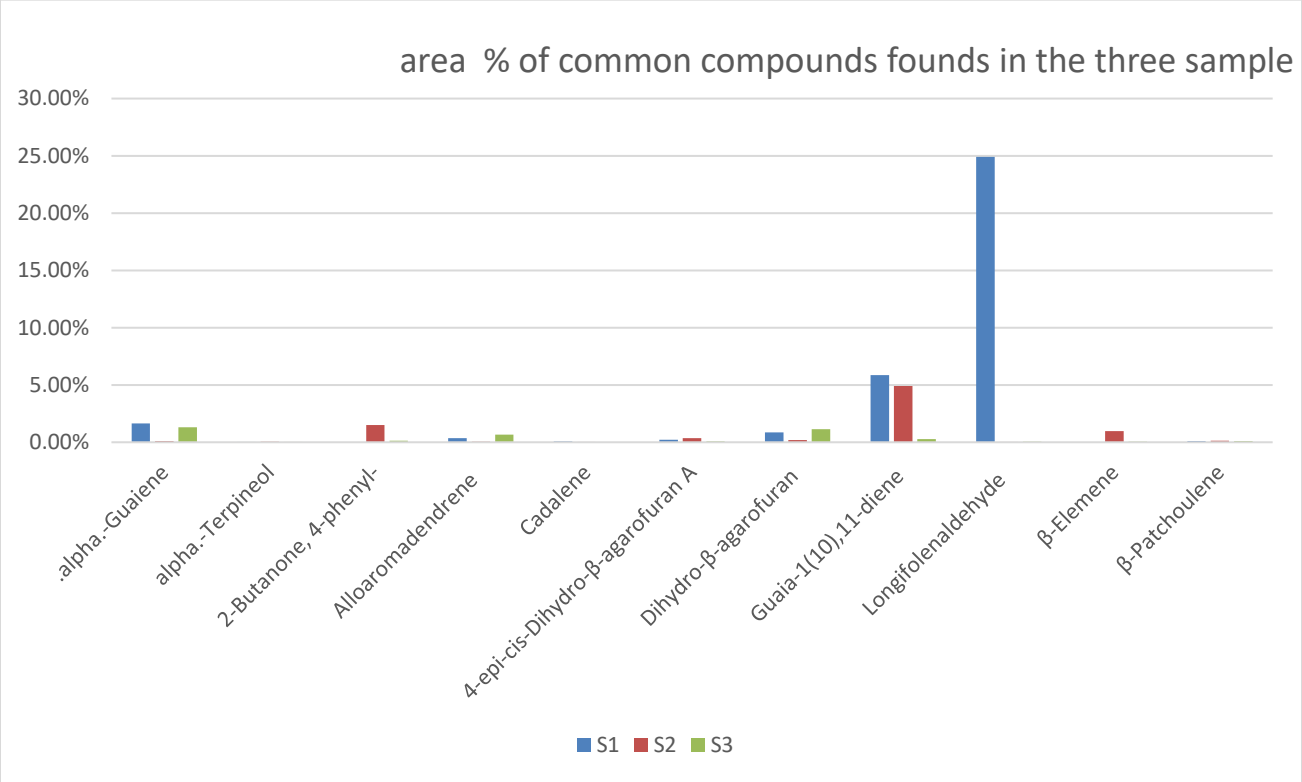
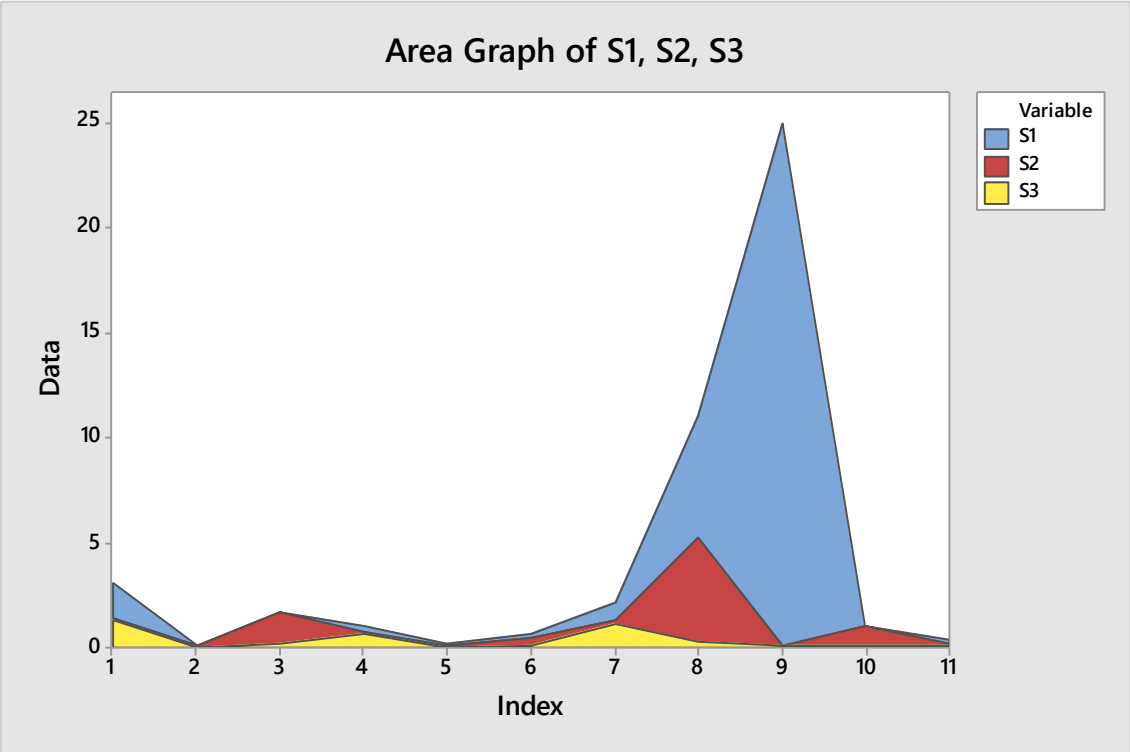


Figure 5: area plot of three different grade



4. **Conclusions**:

A reliable result obtained from the agarwood chipwood analysis by using GC.MS.Q.TOF in the MSMS mode due to the high resolution and sensitivity of the Q.TOF. The work can be more enhanced in the future in case of availability of official reference standards beside Q.TOF MS library for quantitative and qualitative analysis. The results reveal more than 590 peaks are observed. The generated results are so complicated and very difficult to be interpreted for the present of isomers with similar matched spectrums; the absence of official standard references for these compounds make the judgment of confirming the identified compounds more complicated and very hard to absolute identification to be assessed. A total of 96 ions are identified in the three-sample low, high and middle grade of agarwood chipwood of *A.malaccensis* from Malaysia through the GC.MS.Q.TOF. The total Ion chromatogram detected compounds are distributed among wide range over the chemical families including monoterpenes, sesquiterpenes, hydrocarbons, oxygenated monoterpenes, sesquiterpenes, norterpenoids, diterpenoids, short chain glycols, carboxylic acids and others. To the best of our knowledge, this is most complete scan of agarwood chipwood of Aquilaria malaccensis chemical profile of three different samples. One of the very few works that provide complete information about agarwood chipwood of *A.*

malaccensis, which represents a valuable data. Furthermore; this study revealed that the characterization of compounds from agarwood chips wood are highly possible through incense volatile samples; since 1993 up to date there are no scientific systematic investigation has addressed this issue.

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