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SHORT COMMUNICATION

Production of Agarwood Essential Oil: Study on Effectiveness Pre-Treatment Technique of Hydrodistillation Extraction

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ABSTRACT

There are many uses of agarwood trees in a number of countries around the world. However, lack in extraction process efficiency was affected to the lower product quality and oil yield. Therefore, this research was aimed to investigate the effect of pre-treatment techniques of soaking with water and soaking with three fungi on agarwood oil yield. The oils produced were compared with industrial samples. Analysis of chemical compounds is done by using Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS). As a result, the sample treated with fungi *Phanerochaete chrysosporium* achieved the highest average oil yields of 0.58% with content of 80.13% of total chemical compounds compared to sample treated with water only consist of 0.27% oil yield and 72.58% of total chemical compounds was detected. The sample soak with fungi is more advantageous than soak with water in terms of oil productivity, energy saving and efficiency process. The main component present in all parameter studied were epoxybulnesene (1.64%-7.54%), tetradecanal (1.02%-6.10%), agarospirol (0.29%-1.34%), bulnesol (0.59%-8.07%), selina-3,11-dien-9-ol (1.10%-4.02%), oxo-agarospirol (1.12%-10.80%) and eudesmol (0.19%-9.17%).

Keywords: Agarwood, analysis, essential oil, hydrodistillation method, pre-treatment technique

In Malaysia, the famous name agarwood tree is called as "karas" and its fragrant wood is known as "gaharu". Agarwood is known by different names around the world such as aloeswood, eaglewood, calambac, garoo wood and oud (Blanchette, 2006). These various names occur according to the language and philosophy of the country in which it is located. Besides that, agarwood are many different used in a number of countries around the world such as in pharmaceutical purpose, aromatic fragrance, religious ritual activities and perfumery product. Therefore, products derived from agarwood have more growth potential in market demand. Thus makes agarwood is one of the highly valuable products in the world.

Hydrodistillation is a popular extraction technique used for obtaining essential oils from plants. However, now the traditional agarwood oil distillations are reported low in process efficiency and data of chemical analysis of agarwood oil distillation are very rare (Jutarut *et al.*, 2011). Additionally, the extraction process is not always complete and the heat is not stable to control. According to Liu *et al.* (2008) stated that hydrodistillation extraction is safe to operate and environmentally friendly but its disadvantage is a time consuming process and needs a large amounts of plant material. For this purpose need more extensively studied to solved quality of agarwood oil. It's because the trading of agarwood oil is according to its quality (Nurlaila *et al.,* 2014) and the low quality oil is cheap and high quality oil is expensive.

Pre-treatment techniques are important prior extraction process to improve agarwood oil. Several studies to investigate the effect of cell

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wall breakage during pre-treatment technique have been carried out. Most of previous studied have been developed in examines of cell wall expansion by using water, ethanol, acid alkaline, ultrasound, oxidation, ionizing radiation and steam explosion in soaking process. Each technique has advantages and disadvantages. But now there are a new ways to improve production essential oil by applying microbial in soaking process known as enzymatic hydrolysis.

Enzymatic hydrolysis can be defined as a process of breakdown the cell wall in molecule with the presence of water. This process involved of enzymes as biological catalyst that are used to break down cellulose in lignicelluloses (Biology Online, 2007) and was first introduced by Hitze *et al.* (1972) mentioned that use of enzymes as a support in the oil extraction process.

Most of the microorganisms such as fungi and bacteria have been found are able to degrade cellulose and other plant cell wall fibres (Dashtban *et al.,* 2010). The entire fungal differs depending on which fungal species the cell comes from, cell type, and developmental stage. Fungi also believed to help increase digestibility of agarwood. In order to facilitate its extraction from plant cells, it is necessary to degrade the agarwood cell walls to increase the permeability for oil. A previous studied from Arsat (2008) indicates that oil production of agarwood using fungi as technical enzyme gave the highest yield compare to extraction without enzyme pre-treatment. The cell wall degradation by enzymes treatment can be enhanced oil extractability of agarwood chips.

Therefore, this research focus on enzymatic treatment to increase oil production and the oil obtained was compared by treatment with solvent (water) to better understand of the effect of wood cell expansion. Without soaking agarwood acted as control in this research. The average of yield were calculated to evaluate the significance of oil quality and compared with industrial oil.

Plant materials – The chip woods of *Aquilaria malacenssis* used in this study were obtained from Rompin, Pahang. The sample was verified from the herbarium of the Institute of Bioscience, UPM and voucher specimen No SK 2422/14. Two agarwood oils from industrial extraction also were procured from Rompin, Pahang and Linggi Melaka, Malaysia. The fungi were obtained from Choice Care Sdn. Bhd and were stored at Microbiology Faculty of Industrial Sciences and Technology, UMP. In this research, there are three fungi used namely, *Phanorocaete crysosporium* (ATCC 24725), *Trichoderma reesei* (ATCC 56763) and *Fusarium solani* (ATCC 36031).

Pre-treatment techniques – Sample of agarwood were ground to 0.5 cm and submerged with water, three various fungi and without soaking parameter. The agarwood chips were initiated soaked with water in a round bottom flask for 7 days prior extraction (Bhuiyan *et al.,* 2009; Liu *et al.,* 2008) and the oil produced were compared to the oil extracted from without soaking. The experiment was continued soaked with three various fungi and shaken by using double stack shaker at 150 rpm (Arsat, 2008).

Hydrodistillation extraction – Approximately 300 g agarwood chip was performed with clevenger type apparatus for 9 hours in a round-bottom flask 5 L at a ratio of 1:10 (Tajuddin & Yusoff, 2010; Fazila & Halim, 2012). The extractions were conducted in triplicates and the average oils ware calculated. The oils were then stored in sealed containers under refrigeration prior to analyse by chromatography techniques.

Chemical analysis – The chemical constituents of agarwood oil obtained by two chromatography technique of Gas Chromatography-Flame Ionization Detector (GC-FID) and Gas Chromatography-Mass Spectrometry (GC-MS).

Both GC-FID and GC-MS was performed using an Agilent 7890A network system gas chromatography. GC-MS were attached to a mass spectrometer (Agilent 5975C) using a DB-1MS capillary column (30 m length x 0.25 mm diameter; 0.25 μm film thickness). The GC-FID instrument was equipped with a flame ionization detector (FID) and detector in full scan mode under electron impact

ionization (EI, 70eV) was used in GC-MS. However, both chromatography techniques are same in operating condition. Temperatures of the injector and detector were set at 250°C. The oven temperature was programmed at 60°C for 3 min, ramped at 3°C/min to 240°C and then held for 10 min. The helium as a carrier gas was set at a flow rate of 1.2 ml/min. The volume of the sample injected was 1.0 µL. In GC-MS, the components were identified based on the mass spectra with published data (Joulian & Konig, 1998) and also based on 90% correlation of the mass spectrum with the pattern of the NIST library (Adams, 1995). Whereas GC-FID, the components were identified based on basis of comparison of their retention indices. Retention indices were calculated using a homologous series of *n*-alkanes (C7-C24).

Statistical analysis – The variation in yield content was analysed using general linear model procedure by one-way analysis variance followed by post Hoc analysis (IBM SPSS statistics 20). All data were presents in means ± standard error and was considered to be significantly different at $p<0.05$.

The effects of various pre-treatment techniques are important to obtain the effective ways in soaking technique. Two oils extracted by industrial scale were used for comparing with pre-treatment hydrodistillation extraction in order to determine the effect of oil yield and chemical compounds. From the results, there are several similar and varying of components and oil yield can be observed. The lists of components are shown in Table 1 and 51 compounds were identified in this analysis. Based on the analysis by GC, oxygenated sesquiterpenes showed the highest constituents than other terpenes compounds. The comparisons of oil yield between all parameters studied are shown in Figure 1 and it can be seen that the data were presents in means ± standard error.

In terms of chemical analysis, the total of constituents for sample soaked with water and without soaking is lower than sample soaked with fungi at 72.58% and 66.24% respectively. Although the sample soaked with water is less in total of hydrocarbon but highest in the number of constituents identified which consists of 36 compounds compared to others parameter. This statement was proved by GC chromatogram at Figure 2. In industrials sample, the oils showed the difference in the amount of hydrocarbon from Kedaik (industrial oil I) and Linggi (industrial oil II) consists of 75.85% and 68.78% respectively. Both of the oils are lower than the number of hydrocarbon in all pre-treatment technique

In terms of oil yield extraction with sample soak produced more average oil yield of 0.27%±0.10 as compared to without soaking with only 0.19%±0.18. But both of the oil produced is lower than sample treated with fungi. Comparisons of these oil yields are shown in Figure 1. A possible explanation for these results is the sample which immersed in water for 7 days can help in removing a lot of wax associated with the agarwood and assist in extracting agarwood oil (Azah *et al.,* 2008). Therefore, the soaked sample subjected to hydrodistillation showed a higher result compared without soaking technique but lower than oil yield from sample treated with fungi.

Conversely, the oils extracted from sample mixed with fungi *P. crysosporium* shows the greatest amount of hydrocarbon consists of 80.13% compared to the oil extracted by *T. reesei* (72.13%) and *F. solani* (76.38%). This result obtained was also higher than the oil treated with water and two industrial oils. The number of identifying compounds showed not much difference between samples soaked with *P. crysosporium* and *T. reesei* consists of 32 and 31 compounds respectively. Apparently, in terms of oil yield showed pre-treatment with fungi *P. crysosporium* (0.58%± 0.03) also has a maximum amount of oil produced followed by *T. reesei* (0.49%± 0.67) and *F. solani* (0.37%± 0.15).

In this study, the compounds oxo-agarospirol showed percentage area high detected in *T. reesei* (10.79%) and *F. solani* (10.80%) compared to *P. crysosporium* is low only 1.12%. Whereas, the compounds 4-phenyl 2-butanone (26.87%) and hexadecanol (11.17%) high detected presents in fungi *F. solani* might be due to the oil soaked with this

Table 1. Chemical compositions of agarwood obtained by industrial and pre-treatment oils.

Compounds	Pre-treatment technique						Industrial oils		Identification
				Fungi					
	DB-1	Soak	Unsoak	Ph	Tr	Fs	Ind I	Ind II	
Monoterpenes hydrocarbons									
4-phenyl 2-butanone	1210	5.03	7.03	2.66	0.35	26.87	6.2	3.51	RI, MS
Sesquiterpene hydrocarbons									
β-Maaliene	1414				2.00			0.21	RI, MS
α-guaiene	1440	2.09	0.98	0.08					RI, MS
Aromadendrene	1443			1.99	1.08	1.20			RI, MS
ϒ-gurjunene	1472	1.07		2.34		1.66		2.99	RI
β-agarofuran	1474	1.56	0.15						RI
α-selinene	1486	1.35	0.19	1.88	1.12	0.23	3.10		RI, MS
α-muurolene	1496	0.34	0.43				0.40	1.24	RI, MS
α-bulnesene	1503				1.31	1.89	1.02		RI, MS
Oxygenated sesquiterpenes									
α-elemol	1530	1.00	0.60	0.54		1.01	0.90	0.39	RI, MS
α-agarofuran	1553			0.6					RI
Norketoagarofuran	1557				4.24	0.83	8.38		RI
Epoxybulnesene	1572	1.64	5.01	7.54	2.74		2.77	2.41	RI
Tetradecanal	1593	2.95	3.67	1.02	4.86		6.10	1.17	RI, MS
Caryophellene oxide	1600		4.79		0.63			3.49	RI
Guaiol	1603	3.02	1.82	1.76	1.20			1.58	RI, MS
ϒ-eudesmol	1606		1.19			1.72	3.19	0.36	RI, MS
1,5-epoxy-nor-ketoguaiene	1614	3.22	3.19		1.22		1.86	4.91	RI
10-epi-ϒ-eudesmol	1619	1.55	2.62	4.98			0.94	1.22	RI
Agarospirol	1631	1.13	0.29	1.34	0.64	0.49	1.16	0.8	RI, MS
Epi-α-cadinol	1640	0.67	1.22	1.20					RI
Jinkoh-eremol	1643	3.35			1.07		2.42	1.23	RI
Kusunol	1650	1.93	3.07	2.67	1.26	0.27	2.59		RI
α-eudesmol	1652	2.16	1.83					1.52	RI
Bulnesol	1664	4.03	0.59		3.03	1.37	3.65	8.07	RI, MS
Dehydrojinkoh-eremol	1673	0.42	4.02	4.76	0.78		1.11	3.53	RI
epi-α-bisabolol	1678			1.07					RI
α-bisabolol	1683	0.69	0.45		1.33	2.35		0.39	RI
selina-3,11-dien-9-one	1689		0.78	2.07	3.44	6.19	1.46	0.81	RI

**Continued*

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Compounds	Pre-treatment technique						Industrial oils		Identification
				Fungi					
	DB-1	Soak	Unsoak	Ph	Tr	Fs	Ind I	Ind II	
selina-4,11-dien-14-oic acid	1728	2.61		2.39	1.29		1.77	2.04	RI
selina-3,11-dien-14-al	1735	1.03			4.93		1.36	0.17	RI
9,11-eremophiladien-8-one	1740	2.24	1.90	1.96	3.92	1.16			RI
selina-3,11-dien-14-ol	1750	1.14	1.23	1.89	0.71			2.81	RI
guaia-1(10),11-dien-9-one	1752			1.95			0.98	0.53	RI
selina-4,11-dien-14-al	1758	1.15	0.85	0.94	0.51	0.59			RI
guaia-1(10),11-dien-15-ol	1770	1.23	0.14	0.98	2.20	0.30		0.87	RI
selina-3,11-dien-14-oic acid	1775		0.59						RI
2-hexadecanone	1782	2.38	2.14	1.83	1.04				RI
dihydrokaranone	1799	0.70	0.27				2.20	0.11	RI
guaia-1(10),11-dien-15-al	1806			3.55			1.27	0.25	RI
guaia-1(10),11-dien-15-oic acid	1811			1.65					RI
Karanone	1812		0.78		3.18				RI
Oxo-agarospirol	1822	2.28		1.12	10.79	10.8		3.73	RI
Pentadecanoic acid	1842	1.29	0.22	0.79					RI, MS
Hexadecanol	1853				0.17	11.17			RI
Eudesmol	1880	5.20	0.19	9.17	2.33	3.06	5.50	0.90	RI
2-hydroxyguaia-1(10),11-dien-15-oic acid	1930	6.12	5.68	10.38				14.60	RI
n-hexadecanoic acid	1950	1.09			6.50		11.36		RI, MS
Monoterpenes hydrocarbons		5.03	7.03	2.66	0.35	26.87	6.20	3.51	
Sesquiterpene hydrocarbons		6.41	1.75	6.29	5.51	4.98	4.52	4.44	
Oxygenated sesquiterpenes		61.14	57.46	71.18	66.27	44.53	65.13	60.83	
Total		72.58	66.24	80.13	72.13	76.38	75.85	68.78	
% yield		0.27	0.19	0.59	0.49	0.37	0.20	0.14	
Number of compounds		36	35	32	31	21	26	30	

Ph = *P. crysosporium*, Tr = *T. reesei*, Fs = F. solani, Ind = Industrial oils, RI = Retention Index, MS = GC-MS library.

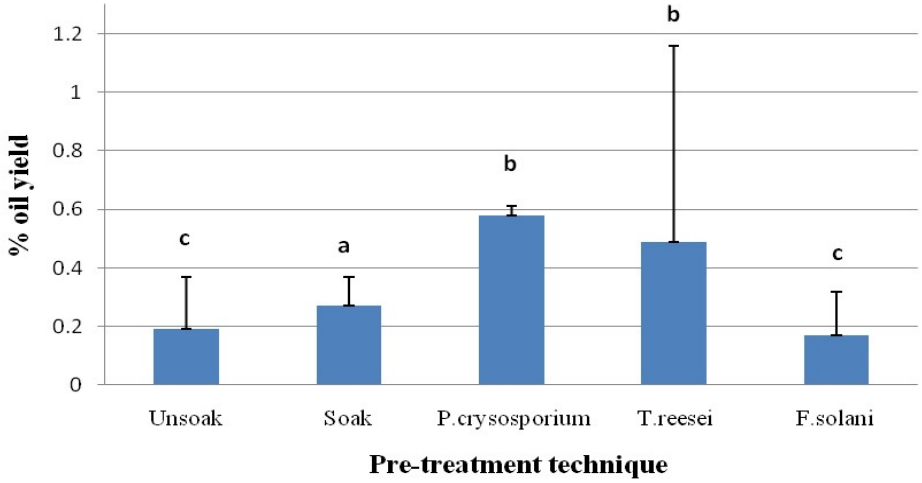

Figure 1. Graph of percentage of oil yield with standard error.

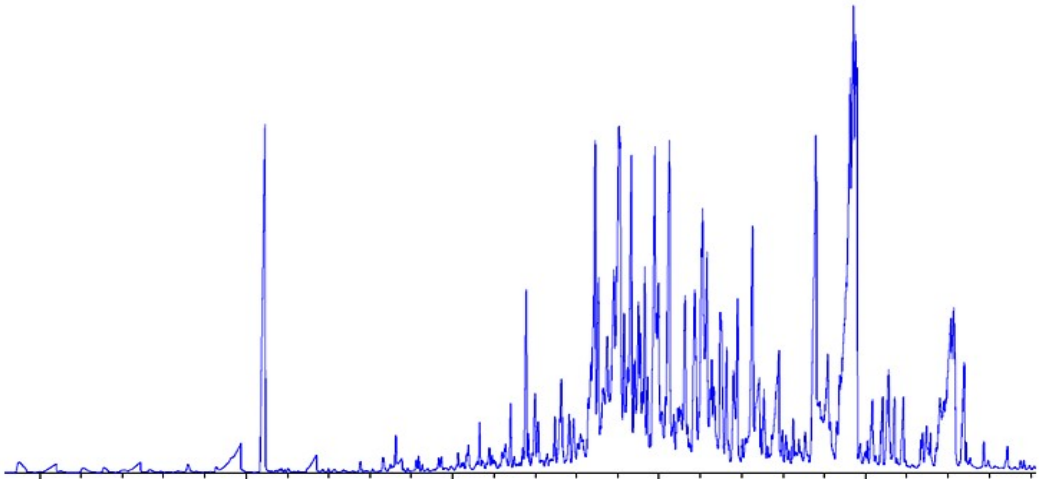

Figure 2. GC chromatogram of agarwood soaked with water.

fungi have a high volatile compounds. The reason for this statement is 4-phenyl 2-butanone is a derivative from carboxylic acid and hexadecanol is one of the alcohol group and ease to evaporate. From this research, a famous compound in agarwood namely agarospirol is high detected in oil extracted by fungi *P. crysosporium* (1.34%) compared to others parameter studied.

It is clear that *P. crysosporium* is the suitable fungus that can be applied in soaking technique and is one of a white rot fungus. This finding supported by Belkacemi *et al.* (1998) and Orth *et al.* (1991) stated that the use of white and soft rot fungi for degradation of lignocelluloses due to its specialized ability to degrade the abundant aromatic polymer lignin in wood cell. Interestingly, it does not affect the odor of oil after hydrodistillation. A possible explanation for the oil yield extracted from sample soaked with *T. reseei* decreased caused by the lower cellulose activity during the soaking process. This process affected by decreasing permeability oil of the cell membrane that caused by the lower of enzyme synthesis (Reese *et al.,* 1969) thus the lower oil yield was obtained.

Nevertheless, the sample soaked with fungi *F. solani* gave the lowest oil yield and can be classified unsuitable has been used in the pre-

treatment technique. This fungus is able to degrade lignin but producing of laccase and lignin peroxidase (Dashtban *et al.,* 2010). Additionally, the oil produced from this fungus gave uncomfortable odor. Furthermore, our results indicate for the first time that *F. solani* was used in soaking technique and more knowledge should be studied to improve oil yield.

Overall, the same compounds are identified in all parameters studied were 4-phenyl 2-butanone, agarospirol and eudesmol. Whereas epoxybulnesene (1.64%-7.54%), tetradecanal (1.02%-6.01%) agarospirol (0.29%-1.34%), bulnesol (0.59%-8.07%), selina-3,11-dien-9-ol (1.1%-4.02%), oxo-agarospirol (1.12%-10.80%) and eudesmol (0.19%-9.17%) can be classified as a major compound detected. Although 4-phenyl 2-butanone (Azah *et al.,* 2008; Tajuddin & Yusoff, 2010) is a large in percentage area detected but it's not suaitable as a main compound in agarwood oil because it's coming from a derivative carboxylic acid. Most of the present studied reported that agarospirol one of the marker compounds founds in agarwood oil extracted (Azah *et al.,* 2008; Bhuiyan *et al*., 2009; Tajuddin & Yusoff, 2010; Jutarut *et al.,* 2011).

Clearly, from this study indicates that the enzymatic hydrolysis pre-treatment will enhance yield of extracting oil (Osburn, 1944) and the largest amount of chemical compounds can be identified by using the suitable fungi compared untreated sample and treated with water. The same results are reported by Sengupta and Bhattacharyya (1996) who examined on mustard seed and canola samples and the oil yield increased with enzymatic hydrolysis pre-treatment were identified.

In conclusion, pre-treatment with fungi *P. crysosporium* gave the maximum oil yield (0.58%± 0.03) with low standard error compared sample treated with water only 0.27%± 0.10 and without soaking consist of 0.19%± 0.18. If compared with the oil produced by industry Linggi and Kedaik is low in oil yield which is only about 0.14% to 0.20% respectively. In chemical analysis, the oil pre-treated with fungi *P. crysosporium* indicates the large amount of total hydrocarbon content of 80.13% compared to without treated and two industrial oils However, seven major chemical compounds found in all extracted oils were epoxybulnesene (1.64%-7.54%), tetradecanal (1.02%-6.10%), agarospirol (0.29%-1.34%), bulnesol (0.59%-8.07%), selina-3,11-dien-9-ol (1.10%-4.02%), oxo-agarospirol (1.12%-10.80%) and eudesmol (0.19%-9.17%). Oxygenated sesquiterpenes were the major portion of all extracted essential oils. In this study, analysis of GC-FID was observed similar to the GC-MS data analysis. The sample treated with fungi *P. crysosporium* is more efficiency in extraction process, high product quality and the yield maximization produced.

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REFERENCES

Adams, R.P. (1995). Identification of essential oil components by gas chromatography-mass spectrometry. Carol Stream, IL, USA: Allured.

Arsat, S. (2008). Enzymatic-enhanced production of gaharu oil: Effect of shaking speed and water/gaharu ratio. Thesis. Unpublished Bachelor Thesis. University of Malaysia Pahang, Malaysia.

Azah, N.M.A., Chang, Y.S., Mailina, J., Said, A.A., Majid, A.J., Husni, S.S., Hasnida S.H., & Yasmin, N.Y. (2008). Comparison of chemical profiles of selected gaharu oils from peninsular Malaysia. *The Malaysian Journal of Analytical Sciences*, 12(2): 338-340.

Bhuiyan, M.N.I., Begum, J., & Bhuiyan, M.N.H. (2009). Analysis of essential oil of eaglewood tree (*Aqulilaria agallocha* Roxb) by gas chromatography mass spectrometry. *Bangladesh Journal Pharmacology*, 4: 24-28.

Belkacemi, K., Turtotte, G., de Halleux D., & Savoie P. (1998). Ethanol production from AFEX-treated forages and agricultural residues. *Applied Biochemistry and Biotechnology*, 70(72): 441-462.

Biology Online Editable Wiki Dictionary. 2007. http://www.biology-online. /dictionary/Enzymatic_hydrolysis (14 February 2014).

Blanchette, R.A. (2006). Sustainable agarwood production in trees. http://forestpathology.cfans.umn.edu/agarwood.htm. Downloaded on 23.9.2015.

Dashtban, M., Schraft, H., Syed, T.A., & Qin, W. (2010). Fungal biodegradation and enzymatic modification of lignin. *International Journal of Biochemistry and Molecular Biology*, 1(1): 36-50.

Fazila, N. & Halim, K.H. (2012). Effect of soaking on yield and quality of agarwood oil. *Journal of Tropical Forest Science*, 24 (4): 557-564.

Hitze, W., Stute, R., Woelk, H.U., Gillaue, R., & Walson, P. (1972). Enzyme aided extraction of corn germ oil. *British Patent*, 1: 402-769.

Joulain, D. & Konig, W.A. (1998). *The Atlas of Spectral Data of Sesquiterpene Hydrocarbons*. Hamburg: E. B.–Verlag.

Jutarut, P., Pakamas, C., & Chakrit, T. (2011). Mathematical modeling for extraction of essential oil from *Aquilaria crassna* by hydrodistillation and quality of agarwood oil. *Bangladesh Journal Pharmacol*, 6: 18-24.

Liu, J., Wu, J., Zhao, Y.X., Deng, Y.Y., Mei, W.L., & Dai, H.F. (2008). A new cytotoxic 2-(2 phenylethyl) chromone from Chinese eaglewood. *Chinese Chemical Letter*, 19: 934-36.

Nurlaila, I., Nor Azah, M.A., Mailina, J., Mohd Hezri, F.R., Saiful Nizam, T., & Mohd Nasir, T. (2014). A review study of agarwood oil and its quality analysis. *Journal Teknologi* (*Science and Engineering*) 68(1): 37-42.

Orth, A.A., Deny, M., & Tien, M. (1991). Overproduction of lignin-degrading enzymes by an isolate of Phanerochaete chrysosporium. *Application of Environmental Microbiology*, 57(9): 2591-2596.

Osburn, J.O. & Katz, D.L. (1944). Structure as a variable in the application of diffusion theory to extraction. *Transactions of the American Institute of Chemical Engineers*, 40: 511-531.

Reese, E.T. & Maguire, A. (1969). Surfactants as stimulants of enzyme production by microorganisms. *Application of Microbiology*, 17: 242-245.

Sengupta, S. & Bhattacharyya, N.P. (1996). Oxidative stress induced resistance in Chinese hamster V79 cells. *Biochemical and Biophysical Research Communications*, 228(2): 267-271.

Tajuddin, S.N. & Yusoff, M.M. (2010). Chemical composition of volatile oils of *Aquilaria malaccensis* (Thymelaeaceae) from Malaysia. *Natural Product Communications*, 5(12): 1965-1968.