Identification of Health Beneficial Chemical Constituents from the Hydrosol and Condensate of *Aquilaria Malaccensis* Prior Soaking

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Abstract— Agar wood (Aquilaria Malaccensis) is a fragrant wood containing economically important essential oil. It has been traded for many purposes including for incense, perfume and traditional medicine. However, the byproduct after hydro distillation which known as distillate (hydrosol) and condensate are, considered as waste. Therefore, this research was aimed to identify the remaining beneficial compounds that present in the hydrosol and condensate after hydro distillation rather than to be disposed. Mostly researchers are focused on analyzing the chemical constituent in essential oil only rather than the hydrosol and condensate. These agar wood bulks are much expensive in market so more study is compulsory on analyzing the content of hydrosol and condensate as well as the essential oil. Before hydro distillation process is carried out towards the agar wood, soaking process were conducted as pretreatment by varies its pH condition from acidic to base (pH 3, 4, 7, 11 and 12) at 25 °C. The soaking duration was standardized and set for one week for all samples. 100 g of grinded agar wood was put in the round bottom flask to run hydro distillation process by using 800 ml of distilled water as solvent. The hydrosol and condensate sample were taken after nine hours run through hydro distillation. Thermogravimetric analysis (TGA) was conducted to evaluate organic loss in the chips after being hydrolyzed. The condensate and distillate samples were analyzed with Gas Chromatography Mass Spectroscopy (GC-MS) to detect the compounds present. The result showed that the most suitable soaking condition for agar wood is at pH 3 with acidic condition. Analyzing by using GC-MS had shown that the composition of compound present after hydro distillation process was differed from each condition of soaking water. The major compositions of agar wood present in hydrosol and condensate are Piperidine-1-methyl, cis-a-Terpinol, Citronellol, Arginine, Rhodinol, 4-Methoxyphenol and Mequinol.

Keywords— Aquilaria Malaccesis, agarwood, hydrosol, condensate, hydro distillation, soaking

I. INTRODUCTION

Gaharu is a plant resin based which can be found in Thymelaeaceae tree family of Gongystylis, Aetoxylon, Gyrinops, and Aquilaria [4][2][16]. Aquilaria is well known by various names such as aloeswood, eaglewood, calambac, agar wood and oud [2]. These different names happened due to the language and philosophy of the country in which it is found. In Malaysia, "Karas" is the nickname of this species. These species can be found in the jungle of Kelantan, Perak, Pahang and Terengganu. There are five species of Aquilaria are recorded in Peninsular Malaysia; A. malaccensis, A. microcarpa, A. hirta, A. rostrata and A. beccariana oud [2]. The high demands for these products are reported in the region of South and East Asia and the Middle East [1]. These species enable to produce resin which use as a parameter for grading the agar wood oil. A. malaccensis contains compound of sesquiterpenes, sesquiterpene alcohols, oxygenated compounds, hydrocarbons and acids [3]. Sesquiterpene compounds that present in the agar wood make this species have its own good fragrance [11]. This species has been commercialized in perfumery products, pharmaceutical purposes, religious ritual activities and aromatic fragrances [4]. Therefore, agar wood based products are highly valuable and required in market. Currently, the issue regarding agar wood turns a new trend to be discussed in media. New technology extractions have been used such as microwave, supercritical fluid extraction, accelerated solvent extraction and ultrasonic extraction [4]. The old method which is hydro distillation is still being applied by traders. However, the efficiency of hydro distillation is lower compare to the others and chemical constituents present are very difficult to analyze [16]. To the best of our knowledge, there are few studies on analyzing the content in hydrosol and condensate. Due to its unpleasant smell, the sample of hydrosol and condensate in agar wood extraction process commonly are drown out and no further analysis. The condensate sample is brownish in colour and has a strong odor while the hydrosol just a colourless sample. The latest research shows that the condensate is acidic in properties due to the compounds that secreted via the pore [16]. These discharge samples might be re-used for the next soaking operation to recover energy, water and leachate essential compounds [4]. Therefore, the objective of this study is to identify the remaining health beneficial constituents in hydrosol and condensate of Aquilaria Malaccensis. The components exist in hydrosols may compose monoterpene, alcohols, aldehydes and ketones [16].

II. METHODOLOGY

A. Materials

The agar woods from the species of *Aquilaria Malaccensis* were obtained from a plantation in Kuala Krai, Kelantan, Malaysia.

B. Pre-treatment techniques

Agar wood barks were grinded to small pieces by Hammer Mill, CF158. One sample was set as control without any soaking process. The agar wood chips were soaked for 7 days prior extraction. The experiment was continued by soaking with different soaking medium of pH 3,4,11 and 12. 1M of Sodium Hydroxide (NaOH) and 1M of Sulphuric Acid (H₂SO₄) were used to adjust the pH of soaking water. After soaking process, agar wood samples were dried for 24 hours under room temperature.

C. Hydro distillation extraction

The distillation apparatus consisted of condenser, electronic heater and a round bottom flask with capacity of 1 litre. This experiment was run for nine hours. The condenser is continuously filled with running water. Agar wood chips and water were added into the still by the ratio of 1:10. It was left for one hour to let agar wood chips fully immersed with the water before heat was applied onto it.

D. Chemical analysis

i) Gas Chromatography Mass Spectroscopy (GC-MS)

GC-MS was used to identify the presence of chemical compounds of soaked and non-soaked agar wood. The soaking samples were mixed with hexane in a separation funnel with the ratio of 1:2. Double layer is formed whereby hexane will be floated on top will be taken for analysis with GC-MS. The GC-MS analyses were performed via Varian 450 gas chromatograph with attached Varian 240 mass spectrometer. This apparatus was equipped with DB-1 capillary column (30 m × 0.25 mm × film thickness 0.25 μ m). Injector temperature was set at 230 °C and oven temperature was programmed between 50 and 230 °C at a rate of 3 °C min-1. Injection volume was 1 μ L with split ratio 20:1. Helium was used as carrier gas and the flow rate was set at 1 mL min-1 [4].

ii) Thermogravimetry analysis (TGA)

Hemicellulose, cellulose, lignin and extractives are major compartment that presence in agar wood structure. Thermogravimetric and derivative thermogravimetric curves can interpret the temperature profile for each compartment. This practice was conducted with a Mettler Toledo analyser. The experiment was carried out with 20 mg of agar wood samples, heated from 30 to 900 °C at a rate of 10 °C min-1 under nitrogen gas flow of 60 mL min-1 [4].

III. RESULTS AND DISCUSSION

Soaking process is conducted during agar wood essential oil extraction which contributes to the opening and enlargement of pore size on agar wood structure as well as the oil yield percentage [16]. Soaking in water should then be one of the unit operations to extract essential oil from agar wood. To disturb the structure of the cell wall, the environment and surrounding condition need to be changed or altered by introducing to acidic or alkali solvent to allow the movement desired compounds from high to low concentration. By altering the external and internal factors, it can contribute the secretion of compound to the soaked water environment [16]. During soaking process, the cell has expanded and finally burst, thus releasing the content out to the soaking medium. The solvent enters cell wall by simple diffusion and increase the turgor pressure. The solvent becomes more acidic along the time due to the released compounds and corrodes the cell wall. It damages the structure of the cell wall. However, too long soaking time causes more compounds content wasted to the solvent. Most suitable soaking time for agar wood oil extraction is 7 days [15]. During the soaking process, it will change the structure or morphology of agar wood chips in order to let out the compound inside the agar wood chips. The extraction process is set for nine hours. A try and error experiment has been done for hydro distillation duration more than nine hours. However, the results are still the same so all the samples are fixed to be conducted for nine hours. The temperature of hydro distillation need to be controlled and not exceeded 120°C to avoid any rupture on the agar wood structure [3]

A. Soaked and Non-Soaked Agar Wood

In this experiment water (pH 7) is used as the soaking solvent. Water is the common and suitable solvent that is cheap, safe, environmental friendly to use and do not produce any pollution [4]. Common industry used tap water for soaking while for advance purposes, specific chemicals are used [16]. As the water used for soaking process is in large quantity, it might be recycled for other purposes. After being soaked, the sample will undergo hydro distillation. This method contributes to antimicrobial activities compare to other method [11]. The major compounds that can be detected for soaked agar wood are Piperidine,1-methyl (2.184%), Citronellyl propionate (16.423%), and alpha-Guaiene (3.056%) while for non-soaking agar wood, Piperidine,1-methyl is detected just 5.805%. These compounds mostly have abroad potential in medical purposes. Citronellyl propionate can treat nervous system problem [16]. Piperidine-1-methyl is reported can treat throat disorder [16]. Alpa-guaine is a member of sesquiterpene family; an antiasthmatics agent [16]. From Table 1, it shows that the compounds that present in both solutions are Piperidine-1methyl and Rhodinol. Rhodinol, an aromatic compound is used for perfume based products [16]. Alpha-Guaiene can be found in water soaked agar wood only. If the researchers are searching for alpha guanine compounds, soaking the agar wood with water is recommended to obtain the compounds. Total compounds that can be detected in soaking agar wood are shown in Figure 1. More compounds can be detected in soaked agar wood compare to non-soaking agar wood. The pore of the agar wood has been enlarging due to soaking process [16]. The non-soaked agar wood need more heat to be absorbed to rupture and damage the parenchyma cell on the cell wall of the agar wood [15]. Cellulose, hemicellulose, lignin and pectin are the main composition in cell wall of agar wood which is hard to break [4]. It is very strong compartment and act as a wall or barrier that need to be broken to extract the essential oil. So that is why, this non-soaked agar wood is more hard compared to soaked agar wood and less compounds can be extracted [4]. It shows that the extraction process can be boosted by soaking process. From the result of TGA from Table 2, it is shows that by soaking process as pretreatment, agar wood chips has lost their weight more compared to unsoaked agar wood which indicates that more compounds are extracted from the soaked agar wood chips but it is still not satisfied as less in amount of compounds are detected from the result of GC-MS.



Fig. 1: Relative Percentage Composition of the Essential Oil of AgarWood for Hydrosol and Condensate

Table 1: Relative Composition of the Essential Oils Based on pH

Compound	Un soaked	pH 3	pH4	pH 7	pH11	pH 12
Piperidine-1- methyl-	Present	Present	Present	Present	Present	Present
Rhodinol	Present	Present	Present	Present	Present	Present
Arginine	-	Present	Present	-	-	-
Citronellol	-	Present	Present	-	-	-
cis-á-Terpineol	-	Present	Present	-	-	-
4- Methoxyphenol	-	-	-	-	Present	Present
Aromadendrene	-	Present	-	-	-	-
Ferrocene	-	-	Present	-	-	-
alpha-Guaiene	-	-	-	Present	-	-
á-Pinene	-	-	-	-	Present	-
Camphor	-	-	-	-	-	Present
Squalene	-	-	-	-	-	Present
Mequinol	-	-	-	-	Present	Present

Table 2: Relative Weight Loss Based on Different pH

Type of agar wood	Residue before (mg)	Residue after (mg)	Weight loss (mg)	
Control	20	18.6772	1.3228	
Non soaking agar wood	20	16.6772	3.3228	
pH 3	20	8.7534	11.2466	
pH 4	20	10.6772	9.3228	
Soaked with water (pH 7)	20	13.6772	6.3228	
pH11	20	9.2587	10.7413	
pH 12	20	9.0104	10.9896	



Fig 2: GC-MS result on non-soaking agar wood



Fig 3: GC-MS result on pH 7 agar wood



Fig 4: GC-MS result on pH 3 distillate agar wood



Fig 5: GC-MS result on pH 3 condensate agar wood



Fig 6: GC-MS result on pH 4 distillate agar wood



Fig 7: GC-MS result on pH 4 condensate agar wood



Fig 8: GC-MS result on pH 11 distillate agar wood



Fig 9: GC-MS result on pH 11 condensate agar wood



Fig 10: GC-MS result on pH 12 distillate agar wood



Fig 11: GC-MS result on pH 12 condensate agar wood

A. Pretreatment with acidic soaking solution

In this experiment, acidic solution of pH 3 and 4 of 1M H₂SO₄ are used for soaking solvent. From Figure 1, it shows that more compounds can be detected in agar wood pH 3 soaking solvent compare to pH 4. Terpene-4-ol is the most abundant compounds that can be found in pH 3 at 19.843%. For medical purposes, it is an anti-asthmatic agent and it can treat wounds [16]. Referring to Table 1, the similar compounds that can be found in acidic soaking environments are Piperidine-1-methyl, Rhodinol, Arginine, Citronellol and cis-α-Terpineol. Arginine can treat heart and circulatory diseases [16]. Cintronellol and terpene-4-ol are anti-asthmatic agents and they can treat wounds [16]. Acidic environment is recommended to extract these compounds in future for further studies. Aromadendrene and Ferrocene are compounds that can be found in pH 3 and 4 only. Aromadendrene can act as antiseptic and cancer preventive while Ferrocene can trigger the absorption of iron in liver [16]. From these results, the acidic environment contributes to the enlargement of pore size and allows more compounds to be released to environment [11]. Acidic medium degrades the cell wall of the agar wood to allow more compounds to be released compare to water [10]. The use of Sulphuric acid to increase the pH better than other acid as it is an excellent dehydrating agent [4]. The acidic environment will help to break up of parenchyma cells on the cell wall as a result of soaking process, thus enhance more compounds to be released out [4]. By comparing these two results, more compounds are extracted by immersing in pH 3 condition compared to pH 4 conditions. From TGA result, the weight loss incurred in pH 3 is more compare to pH 4 which indicates that higher acidic environment can enlarge the size of pore opening. However too acidic of the environment can damage the pore structure of agar wood instead of trigger the compounds to be extracted.

B. Pretreatment with alkali solvent

In this experiment, alkali solution of pH 11 and 12 of 1M NaOH are used for soaking solvent. Based on Figure 1, Rhodinol has the largest composition in pH 11 (20.738%) while Ecgonine and Camphor dominate in pH 12 (14.502%). Ecgonine can overcome problem of diabetic [16]. The similar compounds that present in base soaking conditions are Piperidine-1-methyl, Rhodinol and Terpinen-4-ol as shown in Table 1. Base soaking condition is recommended and preferred to extract these compounds; á-Pinene, an anti-asthmatic agent [16] only present in pH 11 soaking condition while Camphor an ron ion binding agent [16], Squalene an antiseptic agent and Mequinol, an anti-asthmatic agent are the only compounds that can be found in pH 12 [16]. TGA result shows that extracted compound is less in base condition compare to acidic environment.

IV. CONCLUSION

It has been proven that soaking can trigger the opening of the pore to secrete essential oil instead of being waste product. The result shows that there are still existing remaining beneficial compounds that being released to hydrosol and condensate sample during the hydro distillation process that needed further treatment for extraction. Each soaking environment has its own abilities to extract certain compounds. Certain compounds only can be released from the pore of the agar wood at particular environment. Piperidine-1-methyl and Rhodinol will be secreted from the pore at any condition. As the conclusion, soaking process is a good pre-treatment to enlarge the pores of agar wood to extract the beneficial compounds. Acidic condition is the most suitable soaking solution in term of extracted yield. The rate of leaching of compounds from agar wood to soaked water can be affected by the condition of pH of soaking water. Therefore, the solution of hydrosol and condensate should be kept very well to prevent any loss of the beneficial compounds. From this finding, it has been proven that beneficial health compounds are present in hydrosol and condensate sample of hydro distillation. Hydrosol and condensate can be classified as a new source of valuable product that has a big potential in pharmaceutical and medical purposes [12]. For the recommendation, it is proposed to run until to 7-10 days for hydro distillation and use bulk amount of agar wood sample to obtain the essential oil instead of just hydrosol and condensate because hydro distillation method require more high energy for extraction process [11]. However, over-soaking can let the essential components in agar wood to be wasted to the soak water. Although, yield can be increase by elongation of soaking time, but over-soaking process could result to lower yield. To avoid this circumstance, it is recommended to soak the agar wood chips in a short time. It is compulsory to study the optimum time to avoid any losses of important compounds [16]. In the real industry, supercritical fluid carbon dioxide extraction method or known as SFE is more recommended rather than hydro distillation. This method has been proven as non-flammable, non-toxic, chemically stable and less energy consumption. Furthermore, it is more stable than traditional method in term of viscosity, transport properties, extraction time and purity of yield [11]. It has been commercialized to extract lot of sample in the field of pharmaceutical and medical purposes.

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