Effect of *Aquilaria malaccensis* Leaves After Undergoes Vacuum Far-infrared Drying

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***Abstract*— Vacuum Far-infrared Radiation (VFIR) is a technique of drying that has been used actively widely in food processing industry to replace conventional drying as it proven its effectiveness and many other benefits. The VFIR of Aquilaria Malaccensis leaves took place in order to determine the value of this agarwood parts compared to the normal studies of its bark. The purpose of this experiment is to determine the concentration of antipyretic inside the leaves. Antipyretic reagent can be found mainly in acetaminophen product such as paracetamol. Samples are dried under vacuum constant vacuum condition 200 mbar divided to three temperatures, 40 °C, 50 °C and 60 °C for 4 hours. The moisture content of each sample was taken to discover the moisture loss from the samples. Then, the leaves undergone extraction procedure using soxhlet method and analysed by High Performance-Liquid Chromatography (HPLC) under wavelength 200 nm to determine concentration of antipyretic in the leaves using mobile phase Acetonitrile-deionized water (30:70). From the findings, the concentration of antipyretic varies at different temperature of drying. The most optimum to preserve the active compound using VFIR set to be 60 °C based on findings.**

Keywords—Vacuum Far-infrared Radiation, Aquilaria Malaccensis leaves, Soxhlet Extraction, High Performance Liquid Chromatography.

I. INTRODUCTION

Aquilaria Malaccensis is one of the plants under Thymelaeaceae family that mostly found in Bangladesh, Bhutan, India and South-east Asia country and been categorized as one of the threatened plant due to its habitat loss (Barden, 2000). This plant widely known as agarwood and also been given to many names that are including gaharu, aloe(s)wood, eaglewood or kalambak is famously or well-recognized for it contribution for production of medicine, incense and perfume to whole over asia and middle east (Heuveling van Beek, 1999) Agarwood commonly used for medicinal, religious puposes and aromatic chemistry in production of fragrance.

In Malaysia, it has been known used primarily for traditional medicine, pharmaceutical, perfumery, fragrance, ceremonial and spiritual rituals (offerings burnt, idols, etc) and decorative carvings. It has been traded in overall from leaf to root. The name entitled only to refer to resin-impregnated pieces of wood from grade c and above that has been partially shaved at least from non-impregnated wood (Chua, 2008).

Drying of Aquilaria malaccensis is undertaken to preserve any active components and extent the life of the sample by removing water or moisture content and thus the active component least to degrade. Samples can also be dried under solar exposed but past experiments stated that the samples are easily contaminated from foreign particles such as dust and insects. The drying mechanism also thrust to inconvenient quality same as using conventional drying due to non-constant drying rate (Liu Yunhong, 2016).

The revolution of heating and drying process has significantly contributed by the existing vacuum far-infrared radiation (VFIR) under existing Infrared Radiation (IR). It provides momentous advantages towards for reducing heating time, uniformly heating, reducing quality losses and nonexistence of solute migration in food material, versatile, simple and compact equipment and majorly in energy saving over the using of conventional heating.

Various of processing can be utilized using the Infrared heating those are including drying, baking, blanching, pasteurization and sterilization (Kathiravan Krishnamurthy, 2008). It getting its fame due to high thermal efficiency and fast heating rate and response time compared to conventional heating as well as its characteristics of making more effective than the latter; the efficiency, wavelength and reflectivity to set it apart (Sakai N, 1994). Thus FIR drying have been experimentally and applied for the recent years of operation to many types of food and vegetable such as potatoes (Masamura A, 1988), kiwifruit (Fenton GA, 1998), onions (Mongpreneet S, 2002) and apples (Nowak D, 2004).

The application of laboratory vacuum far-infrared (VFIR) used for material and product of hot solidification as well as dry de-watering in aspects of pharmaceutical, chemical, food, farming, side product and aquatic product. The samples are including raw material medicine, crude drug, powder, dehydrated vegetable or even dried fruit piece.

It shows that the drying time could be decrease as if the pressure of the chamber decrease and the radiation heater temperature elevated. Most of medicinal materials and some type of food are really heat sensitive yet easily degradation at the presence of oxygen and thus, it is longing for it to dry at minimally oxygen content as well as low temperature in order to maintain the quality of the product (Liu Y, 2015). Generally, FIR radiation is profitable for nourishment handling on the grounds that most sustenance parts retain radiative energy in the FIR locale (Sandu, 1986). The interfaces of light and surface to the food substances are vital because reflection, light scattering are occurs.

Firstly, regular reflection occurs at the surface of material. Then, body reflection happen when light penetrates the material and diffuse caused by light scattering that leads to absorption. As the regular reflection polished and shined the surface material, the body reflection causes changes in colors as well as patterns and

structure that can virtually be seen. FIR shows only 10% from overall radiation reflected back (Skjoldebrand, 2001). In all organic type of materials, mostly only 4% reflects back from the sum of total reflection to generate shine and polish surfaces while the rest happen as the radiation enters the foods and disperse to produce many kinds of colors and patterns (Dagerskog, 1979).

The uses of FIR drying might help solving the current issues facing by industrial process due to dried vegetables has lower rehydration rate when using conventional hot-air dryer. Thus, FIR drying is projected to represent a new innovative invention or an improvement to create a high quality dried food at low cost. This is correspondingly to the creation of freeze drying that gives same quality, unfortunately, it is very expensive (Sakai N, 1994). FIR drying proves to provide less time consuming drying duration, alternate energy source, efficiently used energy saving, uniformity of temperature, high quality product, less air supply, good monitoring level and less space used with cleaning workspace and environmentally free (Sakai N, 1994); (Mongpreneet S, 2002).

By and large, solid materials can absorb infrared radiation in a thin layer surface but, moist porous and permeable material can have radiation penetrates to some profundity or depth with transmissivity relies upon its moisture content. Studies showed that on the enhancement of the FIR drying procedure for shrimp dehydration found that the impact of plate distance on the drying are insignificant while the drying rate expanded monotonically with an increase in the plate and air temperature (Fu WR, 1998).

Biological effects of FIR required an understanding with knowledge of interaction between these two towards biological structure such as cells, plasma membrane, cell fluids such as water, DNA and protein. At the cellular level, the primary biophysical mechanisms of the interaction of Far Infrared radiation to living cells can be justify in terms of altered cell membrane potentials and altered mitochondrial metabolism (Sheppard AR, 2008). FIR energy can be absorbed by vibrational level of bond molecules.

According to (Mongpreneet S, 2002) whose research on VFIR on onion welsh, radiation intensity level affected to the quality of product as well as the energy consumption. Many of mathematical model distribution on drying kinetics are based on Weibull Distribution Function since 1939 in terms of material, thermodynamics and many more. However, the Weibull Method is not proved mathematically yet ever since on VFIR and (Liu Y, 2015) experiment is objectively to relate the method to the experiment.

The objectives of this experiment are to examine the drying behavior of combination far infrared drying under vacuum condition towards Aquilaria malaccensis using Vacuum Far-Infrared device, effect of temperature and pressure towards moisture content, color and texture as well as the analysis of antipyretic activity using high performance liquid chromatography.

II. METHODOLOGY

A. *Materials*

Fresh Aquilaria malaccensis leaves were obtained from farm in Jalan Kebun, Shah Alam. The leaves chosen must be in good condition without any diseases and it desired to choose matured (dark green) leaves. The leaves are kept cool so that the texture and structure as well as moisture weren't disturbed before the experiment started. The leaves measurement in terms of sizes and thickness must averagely the same; about 3-5 cm in width and 6-10 cm in length and the thickness cannot be more 4 mm The leaves then cleaned using tap or distilled water to clean the surface so that there no other particles, suspended solid or layer of wax (some plant) on the leaves. Cleaned leaves then were dried with clean tissue or filter paper.

B. *Chemical*

Ethanol is used in this experiment mainly as a solvent for soxhlet extraction procedure. A sum of 750 ml 97% ethanol used for the soxhlet comes from Merck Ethanol Brand. Acetonitrile-deionized water (30:70) used as mobile phase for HPLC analysis and 4-Hydroxyacetanilide or acetaminophen used for standard solution after diluted with ultra pure water. The chemicals and reagents used are analytical reagent grade.

C. *VFIR dryer*

The leaves samples were dried in infrared vacuum oven under far-infrared radiation tray. The oven consists of both near-infrared radiation (upper) and far-infrared radiation (middle) thus, the middle tray way chose to dry. The oven comes with pressure regulator ranging from 0 to 1200 mbar. The stainless steel tray with dimension 45 cm x 25 cm was spread with 50.0 $\pm$ 0.05 g of leaves weighted using weighing balance and under duration time of 4 hours for 3 different temperatures for 3samples respectively. The temperatures were 40 $^{\circ}$C, 50 $^{\circ}$C and 60 $^{\circ}$C under constant pressure 200 mbar. The surface of leaves facing upward for equal spread radiated.

Figure 1 shows the Infrared Oven for VFIR drying

D. *Moisture Content*

After all the samples were dried, each of sample's moisture content were ought to find. The samples were taken the weight using weighing balance to measure the after dry weight. Using the formula;

$$Moisture\ Content, M_c = \frac{M_i - M_f}{M_i} \quad (1)$$

Where M_i is weight initial, M_f is weight after dried. The moisture content determination is to find the moisture loss from drying.

E. *Grinding*

The dried samples were then grinded using Restech Grinding Machine with 10 μm sieve grinder as a sieving plate. The samples were let to be grinded for at least an hour per sample in order to maximize size reduction to become powder so that better extraction can be collected from the sample.

Figure 2 shows Restech Grinding Machine

F. *Soxhlet Extraction*

Soxhlet method is a method to extract active compound from material by heating solvent of 250 ml 97% of ethanol in a flask and connected to a soxhlet extractor. The powdered samples were put into a thimble at weight 14.0 g each as a constant experimental procedure then placed in the soxhlet extractor. The water connected to extractor to condensate evaporated solvent and the extraction was left to be extracted for at least 2 hours for maximize extraction.

G. *High Performance Liquid Chromatography analysis*

The extracted samples were brought to HPLC to analyze the concentration of antipyretic in the leaves. A 100 μg/ml of standard solution containing 4-Hydroxyacetanilide or acetaminophen; a chemical that can be found in paracetamol was prepared prior HPLC as a baseline or benchmark to focus only to concentration of acetaminophen in leaves. The HPLC equipped with UNIPOINT software and chromatographic column of 120 x 4.6 mm with a flowrate 1 ml/min. Samples were filtered using 0.46 μm membrane to remove impurities. The extracts were injected into HPLC at 20 μL using auto sampler under Acetonitrile-deionized water (30:70) as mobile phase with condition orthophosphoric acid (pH 3.1). The samples were exposed to UV detection of wavelength 200 nm at pressure 1400 psi. The retention time of each sample set at 5 mins and the areas of peak from the samples were taken. The extracted samples then diluted at 10 times and 100 times and the standard solution was diluted to 10 μg/ml, 30 μg/ml and 50 μg/ml.

III. RESULTS AND DISCUSSION

A. *Texture and Color*

The texture of samples were observed visually as the sample from 40°C, namely A has least brownish than sample from 50°C (B) and 60°C (C) which C has greater dark brown than the other. The texture for sample C showed more wrinkle than A and B.

B. *Moisture Content*

Moisture content from the drying VFIR is calculated by comparing weight before and after drying. And the value of moisture content collected from samples.

Table 1 shows moisture content from samples

Temperature °C	Mass In (g)	Mass Out (g)	Moisture Content Loss (%)
40	50.00	16.28	0.67
50	50.00	15.47	0.69
60	50.00	14.23	0.72

Based on the table (1), it shows that the moisture loss increases as the temperature increases. This obeys the Stefan-Boltzmann Law (A.S, 1969) stating that increase in temperature will increase the energy due to radiation, heat flux that emitted to the surrounding and how the samples could absorb more heat quantities. At that point the absorption of energy offers ascend to a fast temperature increment of the samples, bringing about the ascending of water vapor pressure in the sample, and making a higher drying rate. Besides, FIR energy could penetrate to depth of 1-4 mm for samples such as leaves, thus the penetration of heating into the sample could deliver more uniform and better heating conditions contrasted to customary conventional heating (Afzal T M, 1998).

Recent experiment from (Liu Yunhong, 2016) stating that at constant temperature, the drying rate increases as the pressure decreases. Subsequently, water evaporates at low temperature causing to escape from the samples easily. This applies as same as increasing temperature at constant pressure as drying rate increases respectively as the increment of temperature.

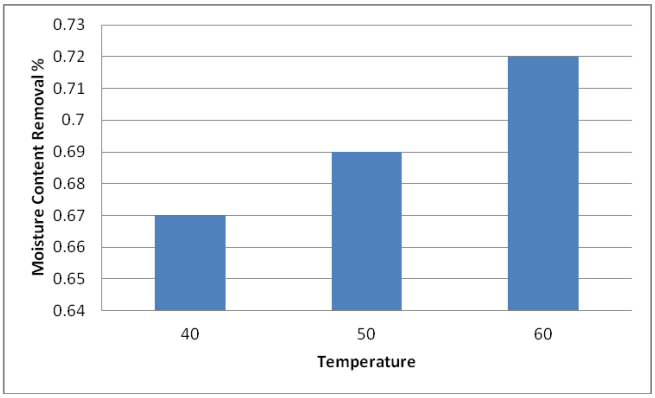

Figure 3shows Moisture Content Removal against temperature

C. *HPLC analysis*

The standard solution containing 4-Hydroxyacetanilide or acetaminophen in ultra fine water is first tested as baseline to discover concentration of acetaminophen inside leaves. Solution of 100 μg/ml was injected to HPLC through 20.0μL of and ran under machine for 5 minutes brings about such data as figure 1 and table 2. The solution then diluted to 10 μg/ml, 30 μg/ml and 50μg/ml to determine the standard curve for the standard solution. This method is used to analyze the concentration of acetaminophen in the samples as the parameters are linearity and accuracy. This method is purposely to ensure the metabolized quality of concentration taken from the extraction. Calibration curve is used to determine the exact acetaminophen concentration in the extracted leaves. The method is also known as external standard preparation. The Figure 5 shows a calibration curve after dilution the standard concentration.

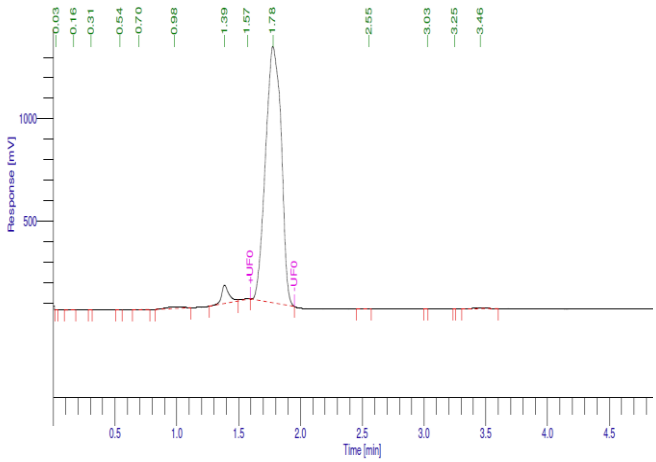

Figure 4 shows standard solution of acetaminophen in HPLC at retention time 5 minutes.

By viewing the graph, it can show that the best time where the concentration of acetaminophen at peak is at time 1.7 to 1.8 minutes. Thus the sample is best to view the peak concentration at time particular time.

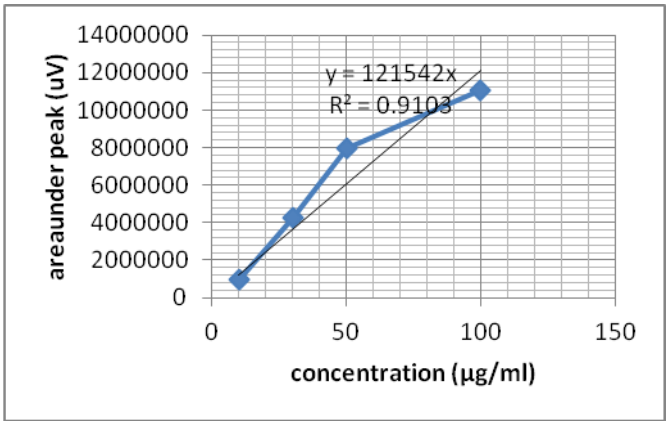

Figure 5 shows the area under graph for standard solution

Regardless the value of R^2 obtained from the experiment is not equal to 1, but the value above 0.9 shows promising and more recommendation and precaution need to be taken for the next experiment. Based on graph, the value of linear equation is;

$$y = 121542x \quad (2)$$

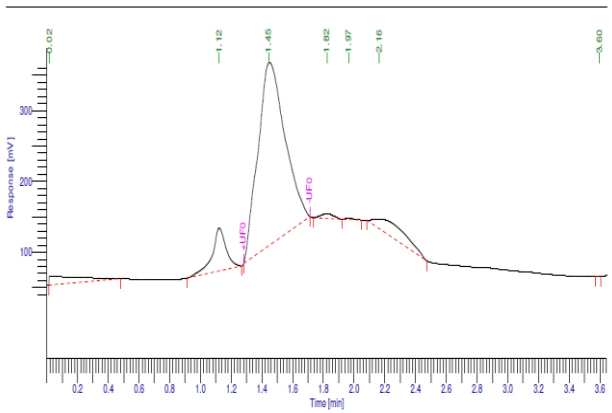

Figure 6 is the sample A at 100x dilution

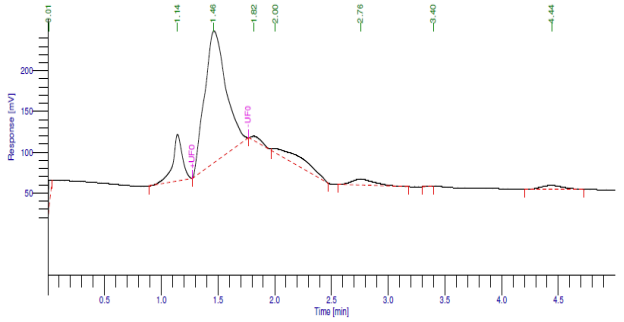

Figure 7 is sample B at 100x dilution

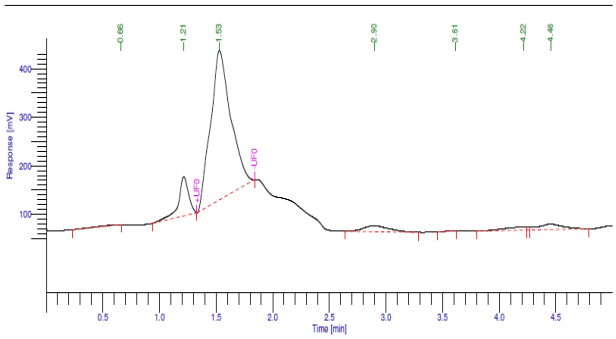

Figure 8 is sample C at 100x dilution

Table 2 is the area under a peak represents concentration content of acetaminophen in leaves after 100x dilution

Temperature (°C)	Time (min)	Area under a peak (mV)
40	1.445	3150112.85
50	1.462	2103141.50
60	1.527	3817663.20

Figures 6, 7 and 8 show the area under a peak for each of sample under 100x dilution and table 3 is the summary value of area under a peak. The real reason why the samples were diluted to 100x because the area under graph too large consequently showing no peak. For instance Figure 7 is a pure sample under HPLC showing large area under 0.6 – 3.2 minutes which cannot be concluded the area belongs entirely towards acetaminophen or any other substances.

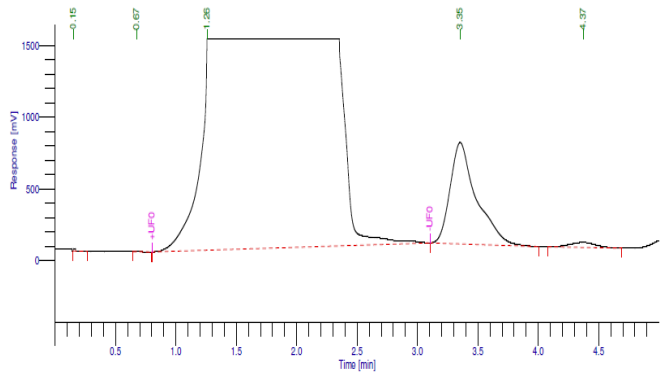

Figure 9 shows area under the peak pure sample A

The sample also cannot be verified the concentration of acetaminophen under 10x dilution because the peak area still too large causing dilution takes place to 100x dilute. The Figure 8 is an example of area under a peak of sample A that too large.

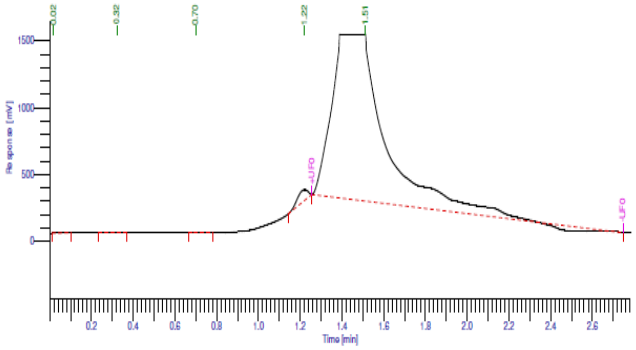

Figure 10 is area under the peak of sample A under 10x dilution

From table 3, we can determine that the concentration of acetaminophen presence utmost at sample C, which is at temperature 60°C. Sample C contains concentration of acetaminophen as its area under peak achieved 3.8 x10^7 mV which can be compared to dilution of 10 µg/ml to 30 µg/ml meaning the concentration of acetaminophen can be ranging between these two concentrations.

From the equation (2), the concentration of antipyretic is calculated as y = 121542x where Y is the area under the graph and X is the concentration.

Y = 121542x

For Sample A;

3150112.85 = 121542x
X = 3150112.85/121542
X = 25.90 µg/ml

For sample B;

2103141.50 = 121542x
X = 2103141.50/121542
X = 17.30 µg/ml

For sample C;

3817663.20 = 121542x
X = 3817663.20/121542
X = 31.41 µg/ml

From the calculation above, we can determine that the presence of antipyretic mostly found in sample C showing it is the optimum temperature to preserve active compound using Vacuum Far-infrared as the concentration of antipyretic presence is 31.41 µg/ml compared to 25.90 µg/ml in sample A and 17.30 µg/ml in sample B.

It is recommended that for upcoming experiments and researches, the concentration of antioxidants, analgesics, and anticoagulant can be a baseline to be search and experimented. The drying time, temperature and pressure can be varies alongside the different type of sample such as leaves from other species, bark from tree as well as animal. The analyzation using FTIR, SEM and GCMS can also be proposed for future determination so that nature can be optimized thoroughly.

IV. Conclusion

The objectives of study is to study the effect of Aquilaria malaccensis leaves achieved as the effect of drying towards moisture content showing as increment of temperature, the moisture content loss is also increases. The samples contain antipyretic reagent as same as paracetamol and many antipyretic other antipyretic drugs.

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