EFEECT OF VFIR TOWARDS *AQUILARIA SUBINTEGRA* LEAVES

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***Abstract*— The application of far infrared radiation (FIR) to vacuum drying is interesting since FIR leads to higher drying rates and yields the dried product of better quality. In this work, the effect of far infrared radiation drying towards *Aquilaria Subintegra* leaves was investigated. *Aquilaria Subintegra* leaves with an initial weight of 50g were dried at various temperatures (40, 50 and 60°C) for 4 hours of drying time and the VFIR were set to -200 Mbar to apply vacuum condition. The results revealed that temperature had significant effects on the drying kinetics and various qualities of the *Subintegra* leaves in terms of moisture content analysis. The dried sample were grinded and were extracted with Ethanol solution to investigated existence of active ingredients in sample by using Soxhlet Extraction method. From the HPLC chromatogram result there were no detection of any antipyretic compound or active ingredients in the leaves.**

Keywords*— *Aquilaria Subintegra* , *Vacuum Drying* , *Moisture Content* , *Extraction* , *HPLC

I. INTRODUCTION

Aquilaria is a species that belongs to the Thymelyaceae family. It is claimed originating to South and Southeast Asian countries which includes Bangladesh, Philippines, Singapore, Thailand, India, Indonesia, Myanmar and Malaysia. Common names are agarwood, eaglewood (England), bois d'eaigle, bois daloes (France), tram huong, Do bau (Vietnam), kritsanaa (Thailand and Laos). Subintegra is one popular species of agarwood beside of malaccencis and crassna. Agarwood usually a big evergreen tree with 15-40 m tall and 0.6-2.5 m in diameter [1]

This species is highly demand in the world because of all the parts of tree have their own advantages. This species has been commercially uses for perfumery and religious purposes, especially in the Middle East and Asian countries [2]. According to Anand Jigisha (2012), the leaves of aquilaria species can be used to make a green tea which is goof to relieve constipation and detoxifying agent. Besides that ,the Aquilaria species also nowadays have received an extensive amount of research in regards to their chemical constituents and biological activities such as antimicrobial, antibacterial, antidiabetic, anti-inflammatory, antioxidant, central nervous system activity, laxative, cytotoxicity, anti-allergic, antifungal and antitumor. Recently, many people believe herbal plants, fruits and vegetables are very usable and can be approaches for medicine and health purposes due to the active ingredients contained. Besides, in other research by Jintana Sattayasai [3], the herbal plant of species from Aquilaria genus or agarwood leaves such as Aquilaria sinensis, Aquilaria agallocha Roxb.and Aquilaria crassna, have potential in the treatment includes of various kinds of pain, cough and anaphylaxis for many of years in Asia region . Furthermore, the studies in terms of pharmaceutical revealed that the leaves of A. sinensis were contained eight α-glucosidase, which might be used as alternative medicine for the diabetes diseases.

In other hand, because of drug in modern medicine may resulting side effects to our health, it has attracted more researcher in terms of replacing the modern medicine with herbal medicine. For example , by the study by Chung-Hung Chan (2011)[10], it revealed that compound of thiazolidinedione (TZD), which is commonly to treat diabetes patient , was potentially to cause edema, abnormal water retention inside the body, and increased risk of coronary heart disease and heart attacks . Thus, the other study on active ingredients extracted from plants such as quercetin and kaempherol have reported and it is proved that the extracted ingredients can minimize the side effects of the TZD.

Far Infrared radiation appear to be an excellent drying technology because of several compromising advantages. For example is FIR drying proved to be higher drying rate, energy saving, and uniform temperature distribution which is giving a better quality product [4,6,11]. Basically, the drying rates by this radiation is increased because the electromagnetic wave produced is absorbed directly by the sample without any loss to surrounding. Hence, energy consumption is relatively lower compared to other drying process [7].

To improve the advantages of the far infared mentioned above, the combination of far-infrared radiation and vacuum condition is proposed as a drying technology for *Aquilaria Subintegra* leaves in this study. It is because several researchers stated that vacuum is a pre-treatment technique to increase the mass transfer rate between the sample and its surroundings [5]. Furthermore ,vacuum-drying also a suitable technique for materials that are sensitive to high temperatures and also offers a great potential for preserving bioactive compounds during moisture removal process [6].

II. METHODOLOGY

A. Sample

The sample, *Aquilaria Subintegra* leaves, were collected from a plantations located in Jalan Kebun, Shah Alam. For the selection of the leaves, there were several significant aspects that need to be concerned before collecting it for experiments uses which is fresh leaves and matured leaves. It is because to minimize the error during the experiments. Typically, the *Aquilaria Subintegra* leaves with around 3-5 cm in width and 6-10 cm were chosen to be used in the experiment. There were several batches for leaves collecting prior to experiment and to prevent the tree of this species losing nutrients and damaged .After collecting procedure, all the leaves were cleaned with tap water and wiped with clean tissues to remove dirt and contaminant on the leaves, Lastly, the leaves were stored in the refrigerator for overnight period before used in the drying process.

B. Chemicals

In this study, acetaminophen which obtained from Merck was set as a stock solutions for the antipyretic analysis using HPLC instrument. All the stock solution and standard solutions were prepared by diluted with the pure distilled water. Acetonitrile solution was used as a mobile phase together with water for a ratio of 30:70. The other solution were ethanol which act as solvent for the extraction of the dried leaf sample.

C. Drying Procedure

Drying was performed in a Vacuum Far infrared dryer, described in detail by Šumic´ et al. (2013) [6].The pressure was set to -200 MBar of pressure in order to kept the dryer in vacuum condition. Samples were uniformly arranged on the tray as a thin layer. Sample weight was kept constant which is 50g for each experiment. Drying of the samples were performed at different Temperatures which is 40, 50 and 60 ºC .

D. Moisture Analysis

For the effects of VFIR drying towards the moisture content in the leaves sample, the data were collected for three different set of VFIR temperature. The weight of leaves are taken before drying start and after 4 hours of drying process. The resulting difference in weight of leaves after dried in for each temperature will be the indicator for the moisture content analysis which fitted into:

(Initial weight-Dry weight)/(Initial weight)x 100 % [8] (1.1)

E. Extraction Procedure

Dried leaves samples were first grind before proceed to the the extraction process. In this experiment solvent extraction or soxhlet extraction technique was used for extract the crude/oil from the leaves by soxhlet extractor instrument where ethanol act as solvent. For this experiment, firstly, the paper thimble was weighed using weigher balance. Then, the 40ºC of leaves sample was filled into the thimble and weighed to get 10g of weight. 250ml of ethanol solvent was measured by measuring cylinder and inserted into the boiling flask. The pipe is set to be connected to the condenser to allow the movement of water which is act as cooling agent for this extraction. The solvent was heated and the vapor of the heating process was travelled up a distillation arm and floods into the thimble housing chamber. After a few minutes, the warm solvent slowly transport into the chamber. At the time chamber is almost fill with the solvent, the chamber was cleared by siphon side arm automatically and then the ethanol solvent was travelled downwards back to the boiling flask [2]. All the extraction process step mentioned above was repeated for 50ºC and 60ºC leaves sample by continuous extraction process for about 2 hours .The obtained extract was filtered under vacuum. The extraction solution of sample were prepared and transferred into a glass bottles and stored to prevent any damages to solution.

F. Active Ingredients Analysis

The content of active ingredients or antipyretic compounds in ethanolic extracts was determined by using High Performance Liquid Chromatography (HPLC) using acetaminophen as a stock solution diluted with ultra-pure distilled water made up to 1000 ppm of concentration. The stock solution were prepared by diluting the acetaminophen with ultra-pure water make up to 1000 ppm of concentration. From stock solution, the standard solution were prepared and diluted to 100, 300 and 500 ppm of concentration. Before injected the prepared standard solution and ethanolic extracts solution to the HPLC, both solution needs to filtered through a membrane filter. This is important to prevent any impurities that may interrupt the result of chromatography.

III. RESULTS AND DISCUSSION

A. The effects of VFIR to the moisture content in sample

Table 1 shows the collection of the result of leaves sample weight before and after the drying process varying with three sets of VFIR temperature which is 40, 50 and 60 T ºC. The resulting difference in weight of leaves after dried in for each temperature was used for calculate the moisture content in the leaves.

Table 1: Weight of Leaves Sample

Temperature ºC	Weight of Leaves (g)		Moisture content removal (%)
	Initial weight	Dry weight	
40	50	27.03	45.94
50	50	24.76	50.48
60	50	23.94	52.12

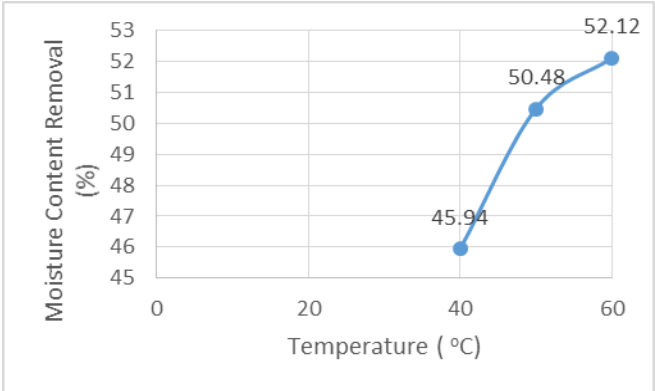

Figure 1 : Moisture content removal against temperature of VFIR

The trend of percentage of removal moisture in the subintegra leaves for a 40, 50, and 60 ºC VFIR temperature were illustrated in the figure above. Based on the graph, its show that the percentages of reduced moisture contents of the Subintegra leaves from initial weight of 50 g to final moisture were 45.94, 50.48, and 52.12 percent respectively and it was increasing as the drying temperature increased. Hence, from the range analysis of the experiments, it is clearly shown that the drying VFIR temperature had very significant effect on the removal moisture in the leaves.

B. High Performance Liquid Chromatography analysis

The procedure to analyse the acetaminophen concentration related to chromatography result is by a few parameters. The parameters called linearity and accuracy. This is important parameter in this study to ensure the better for qualitive measurement on the active component that obtained from the ethanolic extracts. These linearity parameter were generated by plotting a calibration curves of peak area against the standard acetaminophen concentration as shows in Figure 4.2 where the equation is:

$$y = 10934x + 867173 \quad (1.2)$$

Where,

y = Peak Area

x = Concentration of standard solution

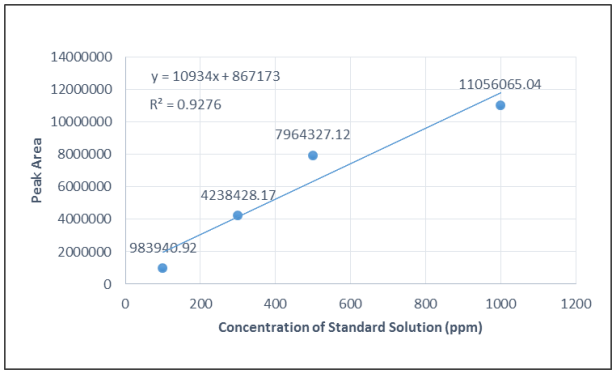

Figure 2 : Linear response of peak area against standard solution concentration

After achieved the standard calibration curve, three set extracted ethanolic sample of 40, 50 and 60ºC were then injected to HPLC for the determination of active compound in the sample. From the result show in Figure 6, for the sample of 40ºC, there were a detection of compound properties present at the retention time of 1.46 minutes. This was determine based on the highest peak of response that appear on the resulted HPLC chromatogram. Meanwhile, the retention time for 50 ºC as shown in Figure 7was 1.48 min and for 60ºC sample in Figure 8 was 1.53 min. According to the HPLC chromatogram for each result, it is show that the retention time were slightly increases as the temperature of sample were increase. Hence, the 60ºC, resulting a higher retention time than the other two samples.

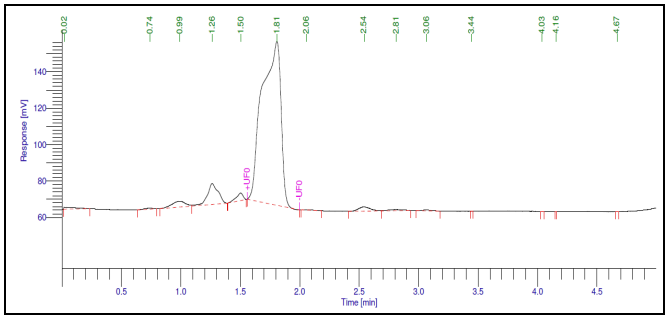

Figure 3 : HPLC chromatogram for 100ppm standard solution

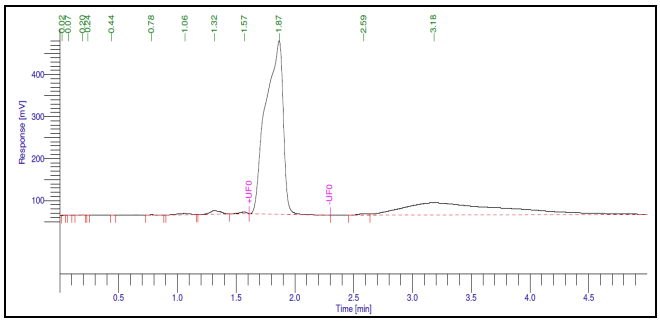

Figure 4 : HPLC chromatogram for 300ppm standard solution

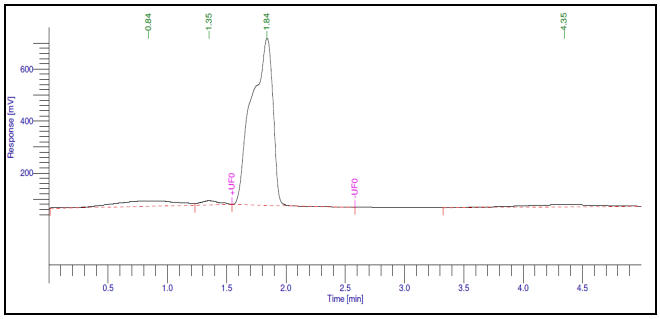

Figure 5 : HPLC chromatogram for 500ppm standard solution

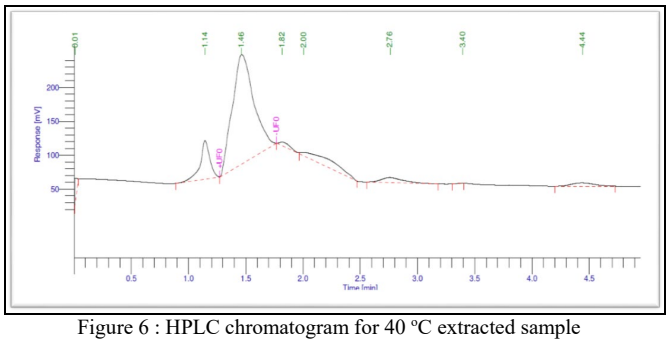

Figure 6 : HPLC chromatogram for 40 ºC extracted sample

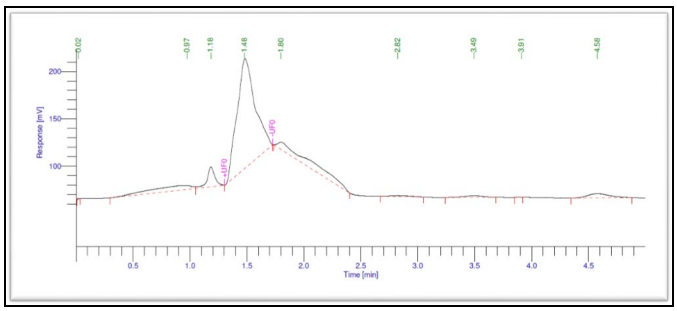

Figure 7: HPLC chromatogram for 50 ºC extracted sample

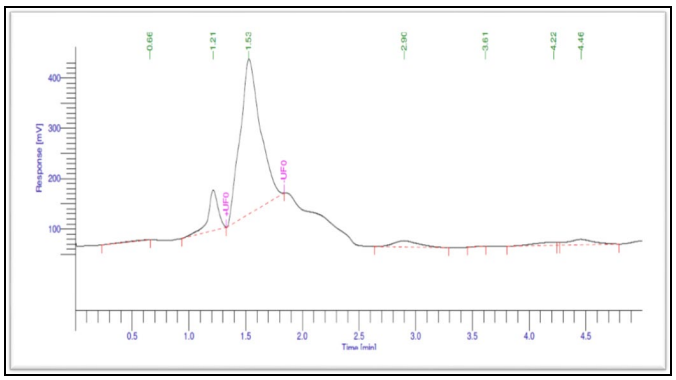

Figure 8 : HPLC chromatogram for 60 ºC extracted sample

According to the three retention time resulted on each sample, the value were at a range between 1.46 to 1.53. However, the reading of retention time for standard solution were at 1.81 to 1.87 in range as shown at the Figure 3 to 5. Hence, there are a far difference value of retention time between the three sample solution and the standard solution. Hence , it can be concluded that the active compound or antipyretic properties were not contain in the *Subintegra* samples at 40 , 50 and 60 ºC temperature conditions .However , there are possibly some factors that might had affected the three sample which resulting a less accurate result. The first factor is, before run the sample to the HPLC analysis, the extracted sample were needed to be diluted again by ethanol solution. It is due to the volatility of ethanol solution were high and might effected the result of HPLC analysis. Besides, the used of ethanol solution for extraction process in this study may not suitable as

many researcher were use methanol to extracts a leaf sample. [16,17]

IV. Conclusion

As a conclusion , the main aim for this study which to study the effect of the Vacuum Far Infrared dryer towards the Aquilaria Subintegra leaves in terms of moisture content removal was successfully achieved . Besides, for the determination of antipyretic compounds in the leaves there were no active component detected in leaves sample by the HPLC analysis.

Acknowledgment

I would like to thank to my supervisor, Miss Habsah Alwi from Universiti Teknologi Mara who provided support and expertise that greatly assisted the research in terms of particular technique and methodology and for the comments that greatly improved the manuscript.

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