SEPARATION AND FRACTIONATION OF *AQUILARIA MALACCENSIS* OIL USING SUPERCRITICAL FLUID EXTRACTION AND THE CYTOTOXIC PROPERTIES

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ABSTRACT

Aquilaria Malaccencis is a type of agarwood. This species is commonly use to extract oil. The extract oil that produces from the plant is call essential oil. The essential oil can be used in various function. To extract oil form this plant, supercritical fluid extraction method was used. Supercritical fluid extraction is use supercritical fluid as extracting solvent. In this research CO_2 was used as extraction solvent. CO_2 was used because it much cheaper than other supercritical fluid. This research was carried by manipulate temperature and pressure. The temperature that was use is 30, 40, 50, 60 and 70℃. For pressure, 200, 250, 300, 350 and 400 bar was used. This pressure was selected based on literature review that was done. The optimum temperature and pressure that can produce high yield of oil is 50℃ and 400 bar respectively. From the GCMS analysis there is no arsenic wavelength in the extract oil. Therefore, there is no cytotoxic property in the extract oil. GCMS can detect all metal compounds that exist in the extract oil.

Keywords— *Aquilaria Malaccencis,* supercritical fluid, cytotoxic properties.

I. INTRODUCTION

In a traditional ways, commonly used are 'gaharu wood' which is can be extracted into essential oil to relieve some local pain, fever and asthma (Ibrahim, et. 2011). 'Gaharu wood ' or known as *Aquilaria Malaccensis* are mostly found in Malaysia, Indonesia, Iran, Singapore and Myanmar. Essential oil that had been produced by this plant can be used in various type perfume, cosmetic, disinfectants, candles and even body products. Its advantages in our industrial field requires this plant to undergoes some difficult phase to be fully extracted.

Essential oil can extracted via several method such as hydro distillation, solvent extraction and supercritical fluid extraction. Hydro distillation is a method of extraction, which is sometimes used instead of steam distillation. This process of extraction is one of the most used traditional methods of extraction. In this process rather than having steam pass via powdered wood in hydro distillater, the powder is soaked in to water. Then heat the water container till the steam comes out and then let it be cold and then collects the oil from the top of the hydrosol. Hydro distillation of essential oil takes several hours with low yield thus making the process low effective (Mazni, 2007).

Extraction is process of separate desire substance from it mixed. Extraction process is categorized in to two types. The first type is solvent extraction and the second type is supercritical fluid extraction. Solvent extraction was carried by using water or organic solvent as extracting solvent. Solvent extraction is one old method of separation. Supercritical fluid extraction (SFE) is a process of separating one component (the extractant) from another (the matrix) using supercritical fluid as the extracting solvent. Supercritical fluid extraction is the most effective and efficient way to extract valuable constituent botanicals (SAPKALE et al, 2010).

Supercritical fluid extraction is one of separation process. Extraction can be dividing into two types which are solvent extraction and supercritical fluid extraction. Solvent extraction is use water and organic solvent as extract solvent while supercritical fluid extraction is use supercritical fluid as extract solvent. For solvent extraction, methanol, ethanol and water are commonly use while carbon dioxide is commonly used as supercritical fluid for extract solvent. Extraction with organic solvent can hardly render an extract free of trace of organic solvent.

Using solvent extraction also will dissolve some contaminant compound. Supercritical fluid extraction can obtain high purity and non-toxic solvent leave no harmful residue. Supercritical fluid extraction using carbon dioxide is carried out to obtain high yield of extract oil. This type of extraction also depend on temperature and pressure to obtain good quality and quantity of product. To get the temperature and pressure that can obtain good quantity of product, it must be determine by experiment. Most experiment that has been done by solvent extraction process obtains less quality of oil. Therefore, supercritical fluid extraction is use to extract oil from *Aquilaria Malaccensis.*

Aquilaria Malaccensis is an aromatic plant. This plant can produce essential oil that commonly use in traditional medicine to relive pain, fever and asthma. The essential oil was extract from the bark of the plant. The leaves of this plant are commonly used to produce tea. Some experiment also was conduct to extract oil from the leaves but the oil that obtain in small quantity. Most of natural product contain cytotoxic compound but in low concentration. Cytotoxic compound can be obtained in high concentration by using fractionation process. Cytotoxic properties are the properties that the compound will be toxic toward cell. The cytotoxic property is measure using MTT assay. MTT assay is a procedure to determine the cytotoxic properties inessential oil. It needs to be determine the concentration that can be toxic to living cell. Therefore the oil that obtains from *Aquilaria Malaccensis* must undergo the cytotoxic test.

In this research, the experiment objective are include to extract the Aqualaria Malecensis oil using supercritical fluid extraction co2 as extracting solvent by using different temperature .Besides that, our main objective is also to identify the cytotoxic properties of extracted oil by using the HPLC equipment. The purpose of this research study is to determine the oil yield from the extraction process of *Aquilaria Malaccecis*

II. METHODOLOGY

Marterial: *Aquilaria malaccencis* stem bark, ethanol, liquid CO2

Apparatus: Supercritical Fluid Extraction machine, grinder,

analytical balance.

***Aquilaria Malaccencis* extraction**

Aquilaria Malaccencis stem bark were grinded into powder form to allow the extraction process occur in high rate. About 10g of powder was filled into the kitbag that was created before filled in the sample vessel. Liquid CO2 was used as the extract medium and ethanol as the co-solvent. 10% of co-solvent was use in this extraction process. The extraction process occur when the liquid CO2 is allow to flow through the sample vessel by opening MV2 valve. MV4 valve also was opened to allow the co-solvent through the sample vessel. The temperature and pressure were stated on the screen of the desktop before running the process. The temperature that was used is 30, 40, 50, 60 and 70 ℃. The pressure that was used is 200, 250, 300, 350 and 400 bars. The extraction product was collected after 1hour process running. It was collected by opening the MV3 valve of collecting vessel. The extraction product needs to go through rotary evaporator to remove the co solvent.

Figure 1: supercritical fluid extraction equipment

Co-solvent separation

After finish the extraction process, the product need go through the separation process. This process is needed to separate co-solvent that use in the extraction process. This process was carried out using rotary evaporator. This process takes time 30-45 minute to separate the ethanol. It not take time too long because the product yield from extraction is not in big quantity. Therefore this process is short. The rotary speed that was use is between 90-125 rpm. The speed cannot be too fast because if the higher speed use, the product that needed will evaporate as ethanol.

Cytotoxic properties determination

For cytotoxic properties determination, GCMS equipment was used. In the GCMS arsenic compound must be detected to detect the cytotoxic properties. CGMS will show the wavelength of compound that exists in the extracted oil. For this research arsenic wavelength must be determine. If there is arsenic compound in the extracted oil, the extracted oil have cytotoxic properties.

III. RESULTS AND DISCUSSION

In this research, the oil yield in from the extraction process need to determine. The oil yield from the extraction process will be tested to determine the cytotoxic properties in the oil. To determine the cytotoxic properties, arsenic compound must be determine.

Figure 2: the product yield from extraction process.

Temperature (℃)	Pressure (bar)	Oil yield (wt%)
30	300	1.33
40	250	1.04
40	350	1.95
50	200	0.98
50	300	1.68
50	400	2.48
60	250	0.66
60	350	1.32
70	300	1.13

Table 1: the oil yield from extraction

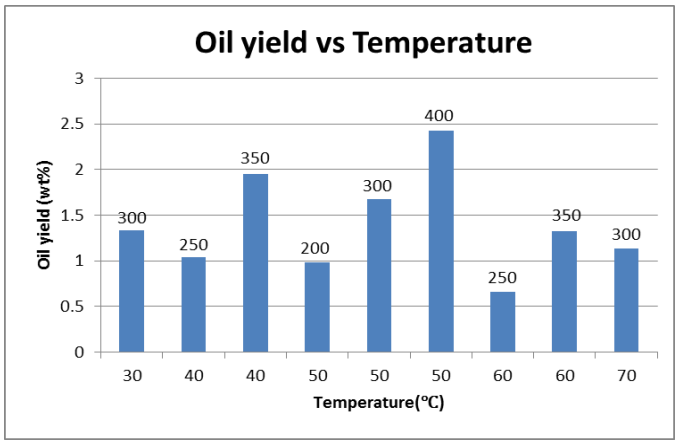

Figure 3: graph show the oil yield in the different temperature

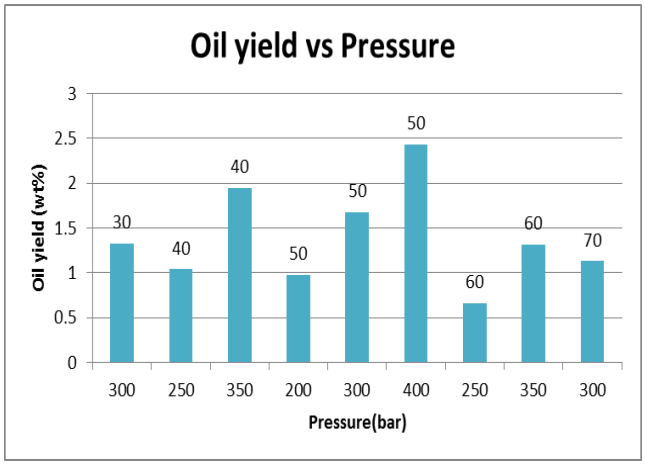

Figure 4: graph show the oil yield in the different pressure

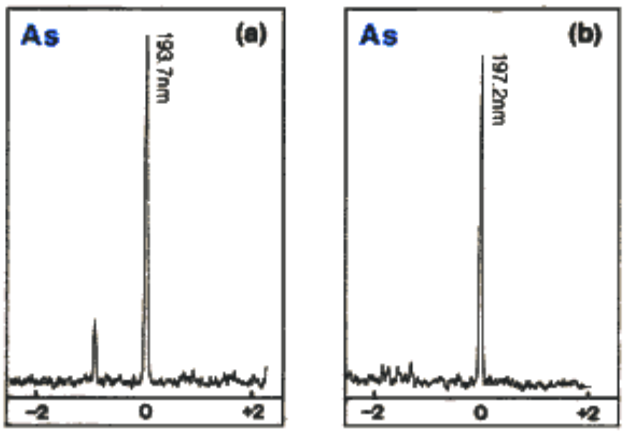

Figure 5: show the arsenic wavelength

DISCUSSION

In this experiment, temperature and pressure was manipulated. The temperature and pressure was selected by literature review of previous experiment that was done by other researcher. This research was carried using supercritical fluid extraction. The oil that yield from this experiment is in small quantity. This because some limitation of equipment. The supercritical fluid extraction equipment has maximum temperature and pressure that can be used. The maximum temperature that can be used is not more than70 degree Celsius while for the pressure that can be used is not more than 400 bar. If the temperature and pressure that was used is exceeding the maximum limit, pump of the equipment will damage. Therefore, the temperature use is not exceeding the limit.

The co-solvent was used in this experiment. The co-solvent that was used is ethanol. The co-solvent used to increase the extraction process efficiency. If there is no co- solvent used, the oil that can be produce will be much lesser. The co-solvent that was use is only 10%. This ratio is set at the screen of desktop. All the value of the experiment such as temperature, pressure, flowrate of extraction solvent and ratio of co-solvent must be filled in the screen of desktop. Before running the experiment, the equipment must be flushing first. It is necessary to avoid other contamination in the oil that will be produce.

After flushing method, the experiment can be run using *Aquilaria Malaccencis* as sample. The sample vessel only can fill with 10gram of sample. The sample also must fill into kitbag before filled in the sample vessel. Only 10 gramof sample canbe used. This is also a limitation of equipment. If the sample that can be used is in much quantity, the oil that yields also can be more. The sample that was use is in powder form. This small particle size can enhance the extraction process. The liquid CO_2 will have more contact with the sample. The small size if sample can give more surface area.

For cytotoxic properties determination, the arsenic compound must be determined. This is because, arsenic is toxic compound. If arsenic compound enter the human body, it will give some disease to the human. Therefore the cytotoxic properties must be determined. To detect the arsenic compound, GCMS equipment was used. GCMS can detect all metal compounds by giving the wavelength of compound. For arsenic compound, the wavelength is 193.7nm. But, after finish the analysis, there is no arsenic compound in the extract oil that was produced. Therefore the extract oil that produces not have cytotoxic properties.

IV. CONCLUSION

From the experiment that was carry out, the oil yield increase when the temperature and pressure increase.. At temperature 50℃ and pressure 400 bar, the oil yield is higher. This is because the pressure 400 bar is optimum for extraction using supercritical fluid. GC/MS is the most frequently used technique for analyzing essential oil composition. This method of testing requires an analytical component, a gas chromatograph, coupled with a detection component, a mass spectrometer. From the GCMS the wavelength of compound can be determined. For my experiment, wavelength of arsenic must be determine, but there is no arsenic wavelength in the extraction oil of *Aquilaria Malaccencis*

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