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EXTRACTION OF SQUALENE FROM *Aquilaria malaccensis* LEAVES USING DIFFERENT EXTRACTION METHODS

(Pengekstrakan Skualena daripada Daun *Aquilaria malaccensis* Menggunakan Kaedah Pengekstrakan yang Berlainan)

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Abstract

Nowadays deep sea sharks species have been hunted for its liver oil as the major source of squalene because of its importance in medical field especially as an antioxidant and anticancer compound. Studies have proved the existence of squalene compound in the *Aquilaria malaccensis* leaves. Supercritical carbon dioxide (SC-CO_2) extraction was done to study the effect of SC-CO_2 operating parameters (pressure and temperature) on the oil yield as well as percentage yield of squalene compound in the oil extracted from the leaves. Both the oil yield and the squalene percentage obtained by using SC-CO_2 extraction method were compared with conventional Soxhlet extraction technique. For SC-CO_2 extraction, the temperature used were 45 °C, 60 °C and 75 °C while the operating pressures were 100 bar, 200 bar and 300 bar. Each extraction process was carried out for 60 minutes with carbon dioxide flow rate of 8 g/min. Meanwhile for Soxhlet extraction, six types of solvents were used (methanol, ethanol, ethyl acetate, n-hexane, acetone and water). Soxhlet extraction gave higher oil yield (45.66%) by using ethanol as solvent compared to SC-CO_2 (13.22%) at 300 bar and 60 °C. However, Soxhlet gave lower squalene percentage in the oil extract (0.78%) by using n-hexane as solvent when compare with SC-CO_2 (3.97%) at 200 bar and 60 °C. These findings reveal that SC-CO_2 extraction is a preferable technique used to extract oil with high percentage of squalene compared to conventional Soxhlet extraction technique with shorter extraction time and higher selectivity on squalene.

Keywords: *Aquilaria malaccensis*, supercritical carbon dioxide, squalene, gas chromatography analysis

Abstrak

Pada masa kini spesies jerung laut dalam diburu kerana minyak hatinya sebagai sumber utama skualena disebabkan kepentingannya dalam bidang perubatan terutama sekali sebagai sebatian antioksida dan antikanser. Kajian telah membuktikan kewujudan sebatian skualena di dalam daun *Aquilaria malaccensis.* Pengekstrakan karbon dioksida lampau genting (SC-CO_2) telah dijalankan untuk mengkaji kesan parameter operasi SC-CO_2 (tekanan dan suhu) terhadap hasil minyak bersama peratusan hasil sebatian skualena di dalam minyak yang diekstrak daripada daun tersebut. Kedua-dua hasil minyak dan peratusan skualena yang didapati menggunakan kaedah pengekstrakan SC-CO_2 telah dibandingkan dengan kaedah pengekstrakan Soxhlet konvensional. Untuk pengekstrakan SC-CO_2, suhu yang digunakan adalah 45 °C, 60 °C dan 75 °C manakala tekanan operasi pula adalah 100 bar, 200 bar dan 300 bar. Setiap proses pengekstrakan telah dijalankan selama 60 minit dengan kadar aliran karbon dioksida sebanyak 8 g/min. Manakala bagi pengekstrakan Soxhlet, enam jenis pelarut telah digunakan (metanol, etanol, etil asetat, n-heksana, aseton dan air suling). Pengekstrakan Soxhlet memberikan hasil minyak yang lebih tinggi (45.66%) dengan menggunakan etanol sebagai pelarut berbanding pengekstrakan SC-CO_2 (13.22%) pada 300 bar dan 60 °C. Walau bagaimanapun, Soxhlet dengan menggunakan n-heksana sebagai pelarut memberikan peratusan skualena yang lebih rendah di dalam ekstrak minyak (0.78%) apabila dibandingkan dengan SC-CO_2 (3.97%) pada 200 bar dan 60 °C. Hasil penemuan ini mendedahkan bahawa pengekstrakan SC-CO_2 adalah kaedah yang lebih baik untuk mengekstrak minyak dengan peratusan skualena yang tinggi dengan masa pengekstrakan yang lebih singkat dan pemilihan yang lebih tinggi terhadap skualena berbanding kaedah pengekstrakan Soxhlet konvensional.

Kata kunci: *Aquilaria malaccensis,* karbon dioksida lampau genting, skualena, analisis kromatografi gas

Introduction

Squalene production or manufacture is typically from shark liver oil. In the case of deep-sea sharks, the liver is the main organ for lipids storage, being in the same time an energy source and means for adjusting the buoyancy. In their case, the unsaponifiable matter represents 50–80% of the liver, the great majority there of being squalene. *Centrophorus artomarginatus* deep-sea sharks which live in waters at 600 to 1000 m depth, without sunlight, manage to survive where pressure is consistently high and the oxygen supply is very poor, due to this compound from their liver, which accounts for 25% to 30% of their total body weight. The liver of the shark *Centrophorus squamosus* represents 18.1% from the body mass; 77.2% by weight of the liver composition is oil and the squalene concentration in this oil is 79.6%. Similar values have been obtained for *Centroscymnus crepidater* [3].

Aquilaria are genera of tropical trees that produces a valuable resinous wood called agarwood. The aromatic properties of agarwood when burned or distilled are extraordinary and there is high demand for the resinous wood to make incense, perfume and as traditional medicine [1]. History of trading agarwood internationally was started as early as the thirteenth century, with India as one of the first origin of agarwood for overseas markets. Ordinarily, agarwood is exported in bulky quantities. In 1997 report, over 700 tons of agarwood from *Aquilaria malaccensis* species were involved in international trade. From these data almost among 20 reported countries, export or re-export were from Indonesia and Malaysia [2]. The leaves of *Aquilaria malaccensis* tree is being discarded as waste due to large consumption of the barks and woods. As the leaves are good sources of natural antioxidants and bioactive compounds, it can be utilized and used as ingredients of healthcare products.

As the world becomes more advanced, many researchers have explored the novel usage of these nature. Plants and plant extracts have been used as medicine, culinary spice, dye and general cosmetic since ancient times. Plant extracts are seen as a way of meeting the demanding requirements of the modern industry. In the past two decades, much attention has been directed particularly in the food pharmaceutical and perfume industries [4]. Chemical compounds originated from plants assist their effect on the human body over manners similar to the chemical compounds in man-made drugs. Therefore, herbal medicines do not have much difference from conventional drugs in terms of mechanism. This feature not only qualifies herbal medicines to have valuable pharmacology, but also gives them the same ability as conventional medicinal drugs that have dangerous side effects [5].

Rapid development of technology and knowledge has revealed another source of squalene compound in natural plant which is Agarwood plant. Based on previous research [6] done on the leaves of *Aquilaria crassna Pierre* (Gaharu), it is found that the sample contained 5-hydroxy-7-methoxy-2-(4-methoxyPhenyl)-4H-I-benzopyran-4-on, squalene and epifriedelanol. Another research done [7] also found 12 compounds that appeared in the characterization of methanolic extracts of Agarwood leaves which one of them is squalene (Table 1).

Table 1. Chemical components of agarwood leaves

Component	Molecular formula
n-hexadecanoic acid	$C_{16}H_{32}O_2$
Glycerine	$C_3H_8O_3$
2-propanone,1,3 dihydroxy	$C_3H_6O_3$
3,7,11,15-tetramethyl-2-hexadecen-1-ol (Phytol)	$C_{20}H_{40}O$
Dodecyl acrylate	$C_{15}H_{28}O_2$
2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$

Table 1 (cont'd). Chemical components of agarwood leaves

Component	Molecular formula
6-ethyl-5-hydroxy-2,3n,7-trimethoxynaphthoquinone	$C_{15}H_{16}O_6$
9,12,15-octadecatrienoic acid, (z,z,z)-	$C_{18}H_{30}O_2$
Squalene	$C_{30}H_{50}$
Octaethylene glycol monodecyl ether	$C_{28}H_{58}O_9$
1-tetradecanol	$C_{14}H_{30}O$

Currently, in order to improve product yields and quality of certain material used, many innovative technologies have been developed such as microwave assisted extraction (MAE), accelerated solvent extraction (ASE), subcritical water extraction (SWE), ultrasound extraction (Sonication), supercritical fluid extraction (SFE) and phytonics process. For this study, it is focused on the supercritical fluid extraction technology using carbon dioxide as solvent. For comparison purpose, Soxhlet extraction method was carried out too besides of SFE method.

Supercritical fluid extraction (SFE) is a substitute method in sample preparation to lessen the use of organic solvents and escalate production. The factors to be considered are pressure temperature, modifier addition, sample volume, analyte collection, flow rate and pressure control and restrictors. There are many benefits in using carbon dioxide as the extracting fluid not only to its encouraging physical properties, carbon dioxide is also economical, safe and copious. Other important properties of this solvent is that it has low surface tension, low viscosities and moderately high diffusion coefficients [8]. Besides, the extraction of product at low temperature which can avoid damages from heat and for some organic solvents, carbon dioxide does not have solvent remains and also practices natural friendly extraction procedure.

This research was done in order to investigate the effects of the operating condition of SC-CO_2 extraction such as temperature and pressure on the oil yield and percentage of squalene extracted from the *Aquilaria malaccensis* leaves as the leaves is claimed to be contained high quantity of squalene. It was also done by comparing the oil yield and the percentage of squalene obtained from *Aquilaria malaccensis* leaves by using SC-CO_2 and Soxhlet extraction.

Materials and Methods

Sample preparation

Fresh, green, healthy and matured *Aquilaria Malaccensis* leaves were selected and obtained from Gaharu Plantation in Segamat in a large quantity to ensure its same properties. The leaves were washed first using tap water to remove dirt's and unwanted substance from the surface of the leaves. The leaves were dried under sun continuously every day until the percentage humidity loss achieved 65% weight in dry basis. The dried leaves were then dried powdered using grinder (Waring, U.S) into small pieces. In order to get a particle size of 355µm, the powdered leaves were then sieved by using sieve tray (Endecotts Octagon 2000 Digital Sieve Shaker). For preserving and storing purposes, the sealed plastic bag was kept in freezer at -20 °C.

Extraction using supercritical carbon dioxide extraction

15g of sample was placed into the vessel, with glass beads and methanol as modifier. 30 glass beads (4.76g) were used to prevent clogging during the run with mixture configuration. 10% of methanol used as the modifier (10 mL) was calculated using volume basis of the vessel (1L). The experiment was conducted with pressure range (100-300 bar) and temperature range of (45-75 °C) and each set of extraction processes were run for 1 hours. Next, 99.9% pure CO_2 gas (Mega Mount Industrial Gases Sdn Bhd, Malaysia) were supplied into the system (SEPAREX Equipment, France, Unit 4397- XUO32) continuously with pump at flow rate of 8 g/min so that it is fed through high pressure. Back pressure regulator (Tescom Corp., USA) with temperature of 65 °C was adjusted to apply the pressure according to the desired value while the temperatures were adjusted by setting the oven temperature. After the pressure and temperature inside the vessel have achieved the desired value, the start time was recorded. The extracted oil in the vessel was then collected for every 15 minutes by opening the collection valve at each interval and the weights of oil extracted were recorded. The extracted oil collected was automatically separated from the CO_2 as the solvent is in gas state at back pressure regulator temperature of 65 °C and some were purged out from the system. Each run was replicated 3 times to ensure reliability of the data gained.

Extraction using Soxhlet extraction

50g of the dried powder was placed in the Soxhlet tea bag and was loaded into Soxhlet extractor chamber. The Soxhlet extractor was placed onto the round bottomed flask which contains 500 mL of analytical graded solvent. Sample to solvent ratio of 1:10 was constant for each type of solvent used. The Soxhlet extractors were set in series for simultaneous extraction by using six different types of solvents used (methanol, ethanol, ethyl acetate, n-hexane, acetone and water). The main chamber of the Soxhlet extractor was equipped with a condenser. The flow of water in the condenser was set co-currently with the flow of vapor. The water flows in from the bottom inlet and going out the top outlet of the condenser. The solvent is then heated up to its boiling point until reflux. Next, rotary evaporator (Heidolph, German) was used to remove the solvent from the mixture of oil and solvent by evaporation. After most of the solvent was removed from the mixture and only the oil left which is 5% from the initial weight, the oil was put into sample beaker and then was dried in oven for 6 consecutive days at 40 °C for further evaporation of solvent. Due to squalene high boiling point (429 °C) which is the target compound, the length of oven drying does not destroy the compound targeted. The weight of dried extract obtained was weigh and recorded.

Analysis of oil yield percentage

The comparison of oil yield percentage of *Aquilaria malaccensis* leaves extract was made in terms of mass percentage of the samples. The extracted oil obtained was placed in the collection vessel, sealed and stored in chiller at 2.7 °C after each extract to prevent any possible degradation of the product for further analysis using gas chromatography.

$$\text{Extract yield (\%)} = \frac{mass\ of\ oil\ extracted\ (g)}{mass\ of\ sample\ (g)} \times 100\% \quad (1)$$

GC-analysis of squalene

For the condition of gas chromatography, separations were performed on a 5% diphenyl/95% dimethyl polysiloxane fused silica column (30m x 0.250 mm internal diameter, 0.25-ím film thickness, Sugelabor, Madrid, Spain) with helium as the carrier gas. The GC temperature was programmed at a rate of 20°C/min from 130 to 230 °C (2 minutes) and then to 290°C (30 minutes) at 3°C /min. In all analyses, the detector temperature was set at 320 °C and the 2600 Chromatography software (Perkin-Elmer Nelson Systems) was used for data acquisition [9].

Results and Discussion

Oil yield percentage

Each set of temperature and pressure was conducted for one hour of four fractions. Each fraction is equivalent to 15 minutes. The percentage of oil yield was calculated using equation 1 and the results obtained were tabulated in Table 2.

Based on the result tabulated in Table 2, the average oil yield obtained is 3.161%. It can be seen clearly that the highest percentage of oil yield obtained is at 300 bar and 60 °C which is 13.222%. The lowest yield percentage obtained is at 100 bar and 75 °C which is 0.102% *Aquilaria malaccensis* oil.

Table 2. Oil yield obtained by using SC-CO_2 extraction

Pressure (bar)	**Temperature (°C)**	**Extraction Time (minutes)**			
		15	**30**	**45**	**60**
		Oil Yield (%)			
100	45	0.0367	0.1693	0.2013	0.2360
	60	0.2240	0.2500	0.2793	0.3113
	75	0.0200	0.0687	0.0860	0.1020
200	45	0.1667	0.1820	0.2007	0.3433
	60	3.1853	3.2227	3.2640	3.2907
	75	3.5707	3.7093	3.7240	3.7513
300	45	0.4167	0.6153	0.7227	0.8513
	60	13.0880	13.1753	13.1960	13.2227
	75	6.0520	6.2893	6.3093	6.3407

Effect of temperature at constant pressure

It can be observed that increases in temperature does not affect the oil yield obtained at 100 bar. The highest oil yield was obtained at moderate temperature of 60 °C. Meanwhile, lowest oil yield was obtained at highest temperature which is 75 °C. It can be seen that the oil yield increases as the temperature increases. From Figure 1(b), the highest oil yield was obtained at an operating temperature of 75 °C followed by temperature at 60 °C and 45 °C. From Figure 1(b) and (c), it was shown that the lowest oil yields were obtained at 45 °C, the lowest operating temperature. It can be observed that the oil yield was low at low temperature, and increases as the temperature increases. This is due to the decrease in the CO_2 density when the temperature increases at constant pressure, hence reduced the extractability of the oil into the $SCCO_2$.

On the other hand, it is anticipated that increase in solute vapor of oil occurred as the temperature increase that contributed to the high oil extraction yield. This phenomenon is similar with other studies reported [10]. The solvent approached the gas-like properties as temperature increases and this facilitate the extraction rate and produced asymptotic yield [11]. However, in Figure 1(a) the higher temperature resulted in lower oil yield percentage. This phenomenon happened due to the decrease in fluid density at increasing temperature, leading to the reduction in the fluid solvent power, eventually reducing the extraction efficiency [12]. Because of this reason, the oil yield decreases as the temperature increases from range 60 °C and above as can be seen in Figure 1 (a) and Figure 1 (c). The reduction of oil yield happened when the temperature increased from 60 °C to 75 °C, showed in Figure 1(a) and (c), while in Figure 1(b), the yield was increased as temperature increased from 40 °C to 75 °C. Although the oil yield obtained was the highest at highest temperature of 75 °C as shown in Figure 1(b), the difference was not significantly large when compared to the oil yield obtained at 60 °C.

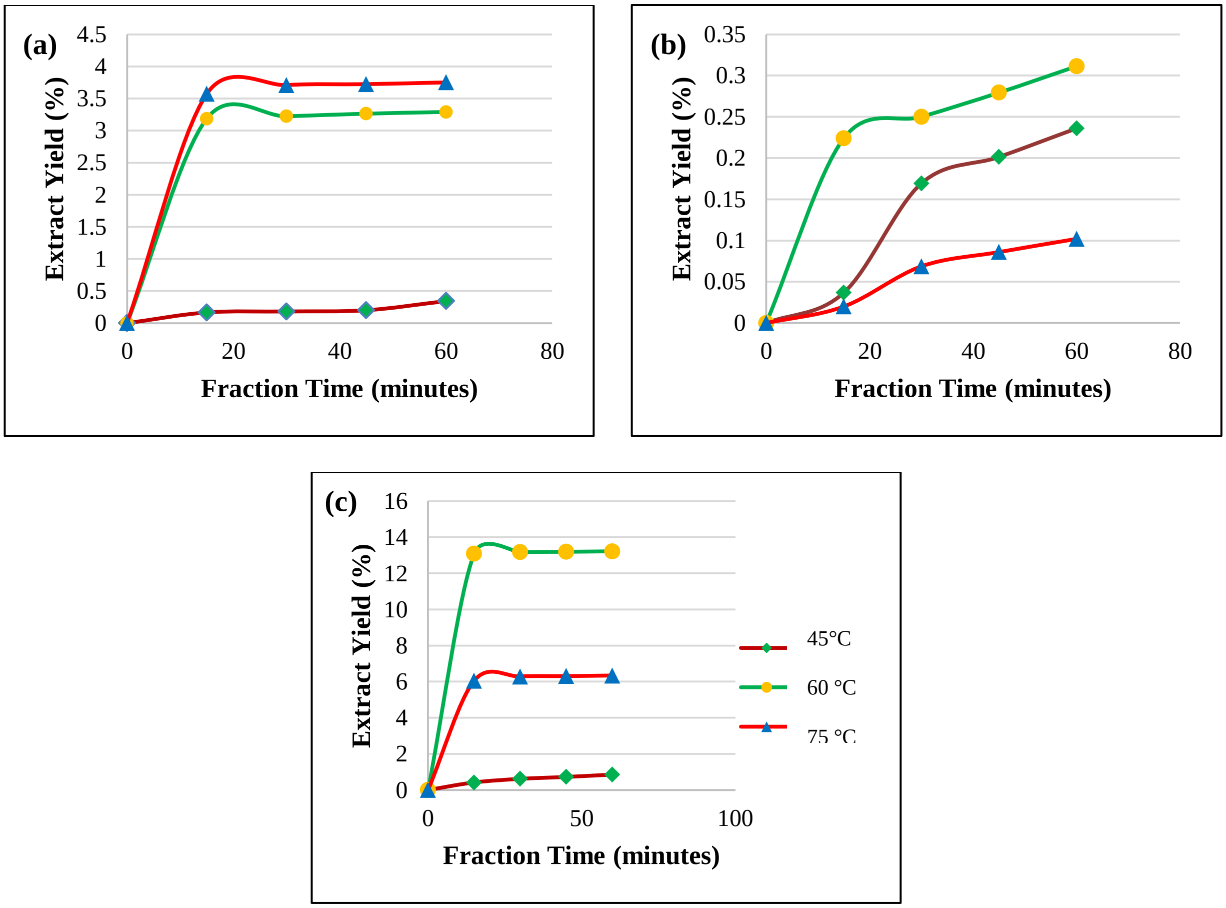

Figure 1. Percentage of oil yield at (a) 100 bar, (b) 200 bar, and (c) 300 bar

Effect of pressure at constant temperature

The highest percentage of oil yield obtained was collected at pressure 300 bar as shown in Figure 2. Meanwhile, the lowest percentage of oil yield is at pressure of 100 bar. The results represented have shown that the effect of pressure at constant temperature is noticeable. As the pressure increases, the oil yield obtained increases as well. This can be explained because some compounds appeared at certain pressure and disappeared at other pressure. The phenomenon existed because there are competition between solvent density and solute pressure. The density of solvent used increases with an increase in pressure at constant temperature, while the vapor pressure of the solute decreases as pressure decreases [13]. The increase in pressure at constant temperature will increase the solvent density and the solubility of oil components and enhance the extraction yield [14]. In addition, this was also due to the effect of diffusion rates of the extracted compounds from the matrix to the supercritical fluid medium [15]. Thus, it can be concluded that the pressure resulted in the highest oil yield of 13.222% was obtained at the highest pressure of 300 bar.

Increasing temperature also can improve the mass transfer coefficient, which is quite significant for the extracted experiments. According to the phenomenon, it shows that the oil yield increases with increasing pressures, however this characteristic has a little similarity with the effect of extraction temperature which reveals that the oil yield increases with increasing temperature [16]. Thus, it can be concluded that the increase in temperature increases the oil yield obtained to a certain level, but might decreases despite of the temperature increase. In conclusion, the best operating temperature for the oil yield is 60 °C.

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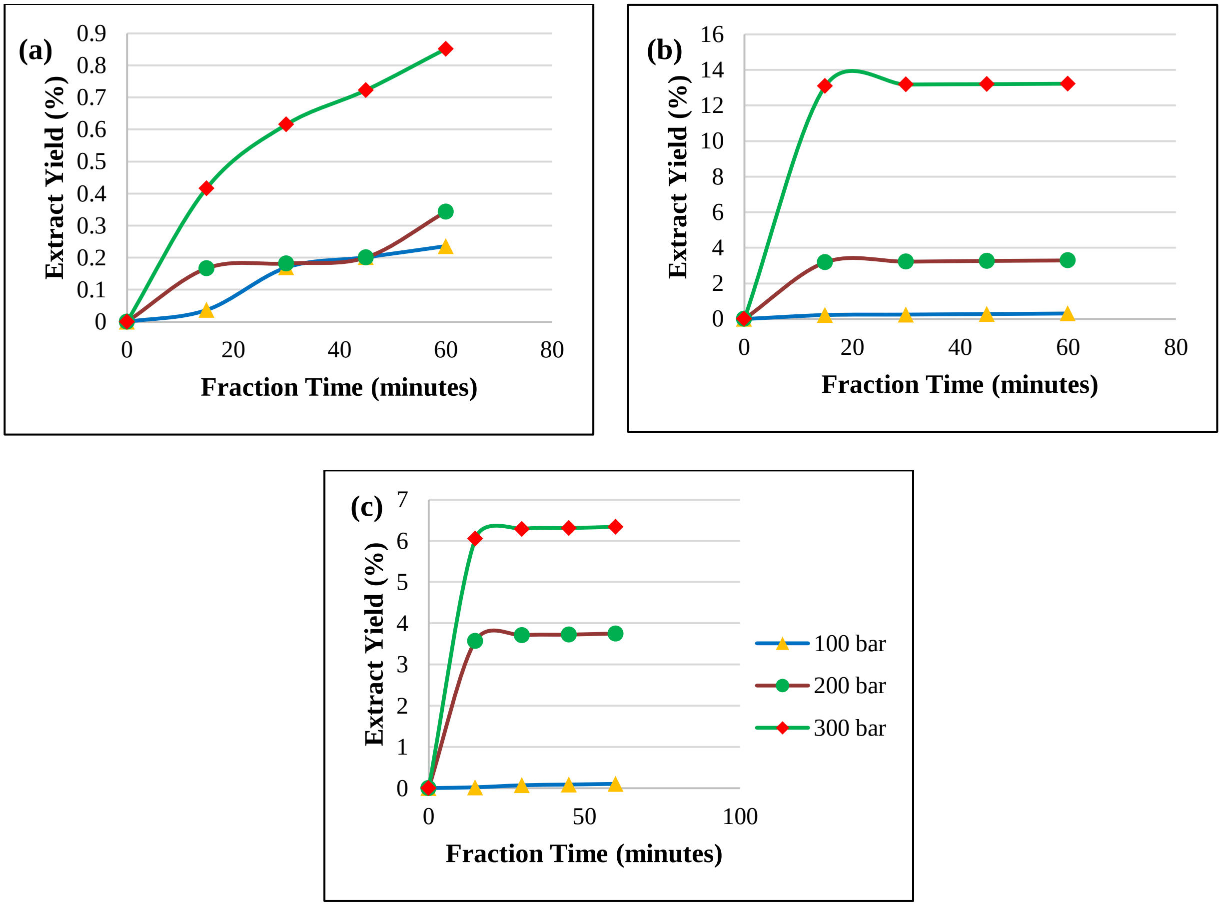


Figure 2. Percentage of oil yield at (a) 45 ^oC, (b) 60 ^oC, and (c) 75 ^oC

Soxhlet extraction

For Soxhlet extraction method, the polarity of the solvent is the most significant factor because it will influence the extraction result overwhelmingly [16]. Thus, different types of solvent were used to investigate which solvent could obtain the highest yield of oil as well as squalene from *Aquilaria malaccensis* leaves.

From the results obtained, we can see that the highest oil yield obtained by using this method is by using solvent ethanol which gave 45.66% oil yield. In contrast, the lowest oil yield was obtained by using n-hexane as solvent which gave 14.06% oil yield. Generally speaking, polar molecules are soluble in water while non-polar molecules are soluble in fat or organic substances. From Figure 3 above, methanol gives the second highest oil yield percentage which is 38.16%. Both methanol and ethanol are bipolar because they own hydroxyl group. Bipolar solvents can dissolve in a large number of oils because of the hydrogen bonds, and can dissolve in water because they possess an ionic end on the molecule [17].

Since ethanol and methanol is considerably highly in polarity and have bipolar properties, thus higher oil yield percentages were obtained. Then, water gives moderate percentage of oil yield which is 25.12%. Water is polar while the oil is non-polar. Oil is not soluble in water thus give lower oil yield when water is compared to bipolar solvents which are ethanol and methanol. The structure of oil is made up of long hydrocarbon chain and has lack of charge to attract the molecules of water [18]. Moreover, oil is non-polar while water is a polar compound. This makes oil immiscible with water and insoluble in water. This phenomenon follows the principle ‘like dissolves like’ which means that polar substances will only be dissolved in polar substance while non-polar substances only dissolves in non-polar substances.

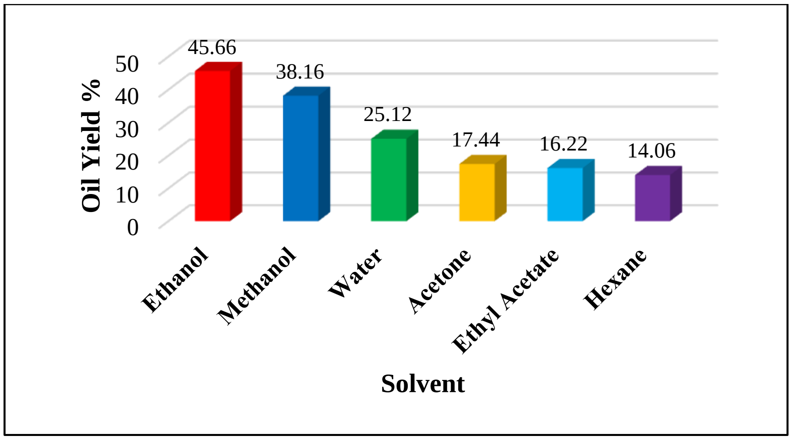
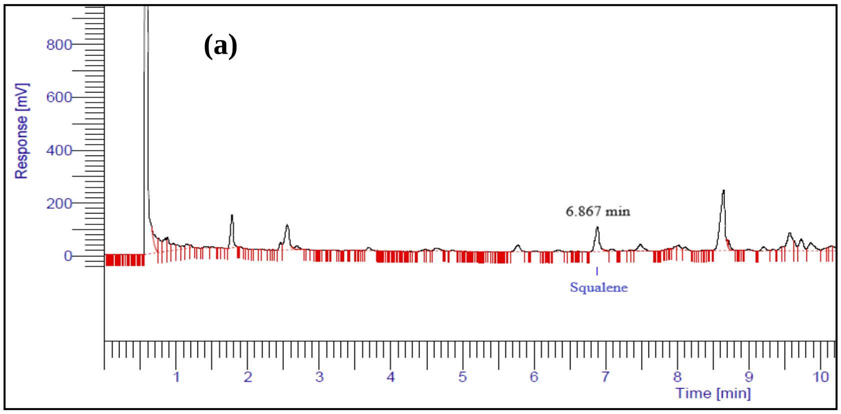


Figure 3. Percentage of oil yield obtained for different types of solvents used

The other three solvents which are acetone, ethyl acetate and n-hexane extracted relatively lower percentage of oil yield which are 17.44%, 16.22% and 14.06%, respectively. The oil yield obtained is lower compared to other solvents mentioned before because it is non-polar thus only extract non-polar components. Although oil is non-polar, the low oil yield shows that there are low numbers of nonpolar components per weight of sample during the extraction process. In conclusion, the percentage of oil yield obtained is based on the polarity of the solvent used. The polarity is the main factor contributing to the difference in the oil yield according to the principle 'like dissolves like'.

Gas chromatography analysis

During the extraction process, the vapor pressure of other compounds in the sample increases with increasing temperature. In addition, since the polarity of solvent CO_2 changes with the change in temperature and pressure, this might be the reason to the decrease in percentage of squalene yield even though both the temperature and pressure increases. The polarity of solvent supercritical CO_2 varies with variation of temperature and pressure [19]. Moreover, since the extraction process is dependent on the conditions of solubility and diffusely, competitive extraction of substances will occur [17]. Thus, it can be concluded that the highest percentage of squalene yield is 3.97% which was obtained at moderate temperature and pressure at 60 °C and 200 bar. Figure 4 shows the chromatogram of both extraction where squalene appears at retention time of 6.867 minutes.



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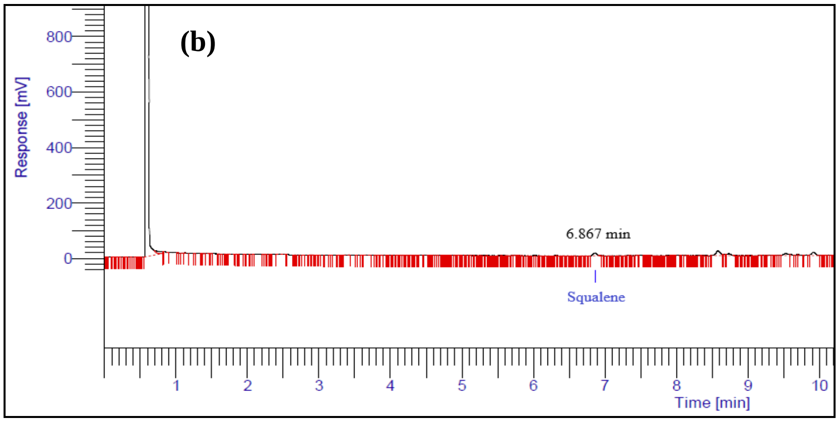

Figure 4. GC chromatogram of (a) SC-CO_2 extract at 200 bar, 60 °C and (b) Soxhlet extract using solvent n-hexane

The highest percentage of squalene obtained from Soxhlet extraction was by using n-hexane as extracting solvent with percentage of 0.78% of the total components in the extracted oil. This is reasonably true because n-hexane is the most non-polar compound thus it favors to extract squalene and most of the non-polar component from the sample. The polarity of squalene is reasonably low which almost zero and it is categorized as non-polar compound [20]. This phenomenon obeys the principle of 'like dissolves like'. The percentage of squalene yield decreases as the polarity of the solvent increases from ethyl acetate to acetone with percentage of 0.5% and 0.33%respectively. As the polarity increases, the solvent becomes least favor to extract the squalene which is a non-polar compound.

Result shows moderately high percentage of squalene in the extract by using solvent ethanol and methanol which are 0.52% and 0.26% respectively even though both compounds are high in polarity. This can be explained because of the bipolar properties of the solvents makes it possible to dissolve non-polar as well as polar compound. Squalene is slightly soluble in ethanol and methanol and thus was able to be extracted. However, the percentage is not as high as non-polar solvents such as n-hexane. In conclusion, the result shown that non-polar solvent which is n-hexane was able to easily extract highest percentage of squalene by Soxhlet extraction from Aquilaria Malaccensis leaves.

Comparison of extraction

It was observed that the highest reading of 13.22% oil yield extracted by SC-CO_2 extraction was lower than the highest oil yield of 45.66% extracted by using Soxhlet extraction by using solvent ethanol. In general, when we compare the oil yield obtained by both methods, Soxhlet extraction will give higher percentage of oil yield compared to that by using SC-CO_2 extraction. The same result was observed in the previous researcher that the extracted oil yield obtained by SC-CO_2 extraction was lower than Soxhlet extraction. The properties of solvents such as the solubility ability used in Soxhlet extraction affect the oil yield percentage. The phenomenon where the oil yield obtained is higher is most probably due to the high oil solubilization capability of solvent used as well as to the extraction of waxes and resin-like materials [21]. However, this phenomenon cannot illustrate that Soxhlet extraction is better than SC-CO_2 extraction in terms of selectivity. Since CO_2 is non-polar solvent, supercritical extraction will extract only non-polar components from *Aquilaria malaccensis* leaves, and moreover squalene is a non-polar compound. Thus, this makes SC-CO_2 extraction is better to be used as the purification and separation of squalene from other components when comparing with Soxhlet extraction [17].

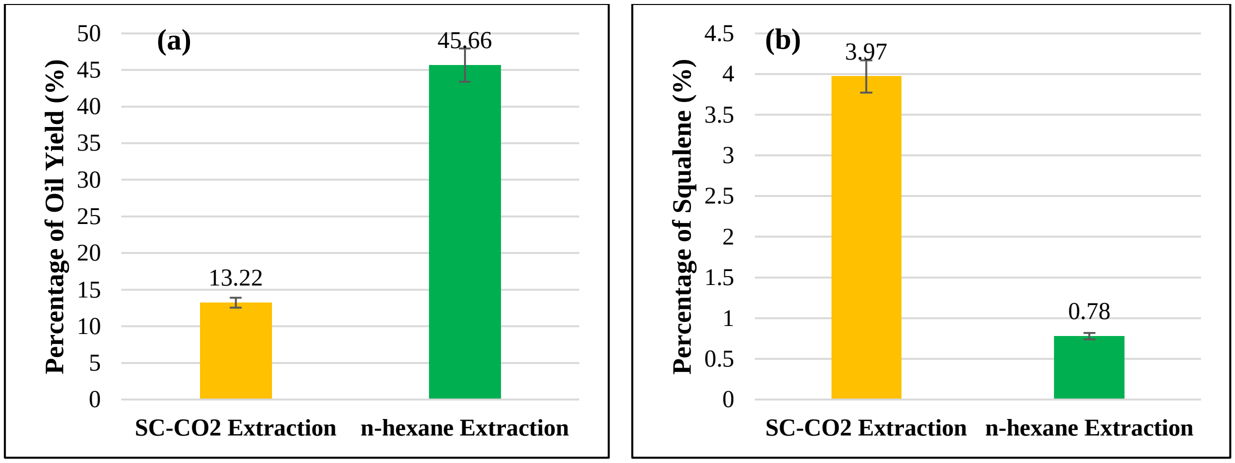

Figure 5. (a) Percentage of oil yield and (b) percentage of squalene obtained by both methods of extraction

From Figure 5, it can be seen that the SC-CO_2 extraction method successfully extract a significantly high percentage of squalene per extract compared to that of by using Soxhlet extraction method. It shows a huge difference where the highest percentage of squalene in the oil yield for SC-CO_2 is 3.97% which was obtained at 200 bar and 60 °C while Soxhlet extraction gave highest squalene percentage of 0.78% by using n-hexane as solvent. The lowest percentages of squalene in the extract yield were same for both methods which is 0.01% at condition of 100 bar and 45 °C for SC-CO_2 and solvent water for Soxhlet. The same result was proven in a previous research where the extraction of vitamin E where the percentage yield extracted by SC-CO_2 is higher compared to Soxhlet extraction method [16]. This is because CO_2 is non-polar solvent and thus it targets the compound squalene which is also a non-polar component, based on the principle 'like dissolves like', thus SC-CO_2 extraction extract more non-polar substance compared to Soxhlet extraction method [22]. Moreover, the variation of polarity of CO_2 based on the variation of temperature and pressure makes it possible for the SC-CO_2 solvent to have a high selectivity on squalene compound at the correct temperature and pressure. In conclusion, SC-CO_2 extraction method extract higher percentage of squalene compared to Soxhlet extraction method.

Conclusion

The highest oil yield obtained from Soxhlet extraction is 45.66% by using ethanol as a solvent. However, n-hexane solvent shows the highest squalene percentage of 0.78% in Soxhlet extraction. For SC-CO_2 extraction the highest oil yield obtained was 13.222% at operating condition of 300 bar and 60 °C but the highest squalene percentage was obtained at 200 bar and 60 °C (3.97%). SC-CO_2 extraction was more selective to the squalene compound compared to Soxhlet extraction as higher percentage of squalene was extracted by using SC-CO_2 (3.97%) compared to Soxhlet extraction (0.78%). Hence, it can be concluded that extraction using SC-CO_2 is more preferred and better compared to extraction using Soxhlet in getting higher percentage of target compound squalene.

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