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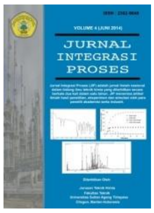

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INFLUENCE OF SOLVENT POLARITY ON ULTRASOUND-ASSISTED EXTRACTION OF RUBBER SEED OIL: YIELD, CHEMICAL COMPOSITION, AND PHYSICOCHEMICAL CHARACTERISTICS

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Abstract

Rubber seeds contain fatty acids that can be used as industrial mixtures and processed into biodiesel, soap production, and animal feed. So far, the utilization of rubber seeds has not been optimal, so rubber seeds can be processed into oil using an ultrasound-assisted extraction (UAE). The study aimed to determine the characteristics of oil from rubber seed extract, such as color, odor, and chemical compound content. In addition, the study seeks to assess the effect of solvent type (n-hexane, ethyl acetate, and ethanol), extraction time (30, 45, and 60 minutes), and ratio of material :solvent (1:5, 1:6, and 1:7 w/v) on the yield (%) of oil extract from rubber seeds. The results showed that oil from rubber seed extract obtained with n-hexane exhibited a yellow color and had a rubber seed aroma, ethyl acetate solvent produced a cloudy yellow color and had an ethyl acetate aroma, and ethanol solvent produced a brownish yellow color and had a rubber seed aroma. The most significant oil extraction result from rubber seeds is ethanol solvent with a time of 45 and 60 minutes, and a ratio of material to solvent of 1:7 w/v. The rubber seed oil content was tested using GC-MS taken from the three best samples of each type of solvent (n-hexane, ethyl acetate, and ethanol) at an extraction time of 45 minutes and a ratio of material to solvent of 1:7 w/v. The oil compound content in rubber seed extract was 90.64% using hexane, 35.95% using ethyl acetate, and 25.19% using ethanol as solvents. The oil compounds extracted using n-hexane solvent consisted of 9-octadecanoic acid, methyl ester at 63.917%, those using ethyl acetate solvent were acetic acid, butyl ester at 30.67%, and those using ethanol solvent were n-hexadecanoic acid at 9.15%.

Keywords: Extraction; Rubber seed oil; Solvent; Yield

Abstrak

Biji karet mengandung asam lemak yang dapat dimanfaatkan sebagai bahan campuran industri, pembuatan sabun, diolah menjadi biodiesel, dan pakan ternak. Selama ini pemanfaatan biji karet belum optimal sehingga biji karet dapat diolah menjadi minyak menggunakan proses ekstraksi dengan metode ultrasound-assisted extraction (UAE)*. Tujuan penelitian adalah untuk mengetahui karakteristik minyak dari ekstrak biji karet seperti warna, bau, dan kandungan senyawa kimia. Selain itu, penelitian bertujuan untuk mengetahui pengaruh jenis pelarut (n-heksana, etil asetat dan etanol), waktu ekstraksi (30, 45, dan 60 menit), rasio massa dan volume pelarut (1:5, 1:6, dan 1:7 b/v) terhadap yield (%) ekstrak minyak dari biji karet. Hasil penelitian menunjukkan bahwa minyak dari ekstrak biji karet yang menggunakan pelarut n-heksana menghasilkan warna kuning dan beraroma biji karet, pelarut etil asetat menghasilkan warna kuning keruh dan beraroma etil asetat, pelarut etanol menghasilkan warna kuning kecoklatan dan beraroma biji karet. Hasil ekstrak minyak dari biji karet terbesar adalah pelarut etanol dengan waktu 45 dan 60 menit, serta rasio massa bahan dan volume pelarut yaitu 1:7 b/v. Kandungan minyak biji karet diuji menggunakan* gas chromatography-mass spectrometry (GC-MS*) diambil dari 3 sampel terbaik dari masing-masing jenis pelarut (n-heksana, etil asetat, dan etanol) yaitu pada waktu ekstraksi 45 menit dan rasio massa bahan dengan volume pelarut 1:7 b/v. Komposisi total senyawa minyak pada ekstrak biji karet yang didapatkan menggunakan pelarut* *heksana sebesar 90,64%, etil asetat sebesar 35,95%, dan etanol sebesar 25,19%. Komponen senyawa minyak yang dihasilkan dengan pelarut n-heksana yaitu 9-octadecanoic acid, methyl ester sebanyak 63,917%, pelarut etil asetat yaitu acetic acid, butyl ester sebanyak 30,67%, dan etanol yaitu n-hexadecanoic acid sebanyak 9,15%.*

Kata Kunci: *Ekstraksi; Minyak biji karet; Pelarut; Rendemen*

1. INTRODUCTION

Rubber plantations in Indonesia have a total area of 3,338,162 ha, and about 60% of the land can be planted with rubber plants, which yield 2,035,058 ha of rubber seeds and 2,951,110 tonnes of sap. Rubber plants (*Hevea brasiliensis*) are dicotyledonous plants that can grow up to tens of meters high, depending on the surrounding environmental conditions (Hakim & Mukhtadi, 2018).

Rubber seeds contain fatty acids such as linoleic, oleic, palmitic, arachidic, and stearic fatty acids. Although rubber seeds have many benefits, such as mixed materials in industries such as paint mixtures, processed into biodiesel, factis, alkyd resins, soap making, animal feed, and processed foods such as tempeh, rubber seeds are still underutilized (Yuliantari et al., 2017). Rubber seeds can be processed into oil using an extraction process. Oil extracted from rubber seeds can be used on an industrial scale, one of which is biodiesel. Various extraction methods have been used to extract oil from rubber seeds in biodiesel production, using chemical extraction with polar, non-polar, or both solvents (Oyekunle et al., 2024). Chemical oil extraction is preferred over mechanical and enzymatic processes because the oil yield obtained is higher, and the cost is lower (Oyekunle et al., 2024). Several standard extraction methods, including maceration and soxhlet, are commonly used. However, the ultrasonic method is a safer, faster option that also increases yield (Djaeni, 2017).

The manufacture of rubber seed oil from rubber seeds using a mechanical method, screw pressing, with an extraction temperature of 70 °C and a screw speed of 200 rpm, only produces 10.11% oil (Hakim & Mukhtadi, 2018). Mangosteen peel extraction is carried out using a conventional method, namely maceration, using ethanol solvents, which also only produces a yield of 17.58% (Pratiwi et al., 2016). Rubber seed oil extraction has also been carried out using n-hexane in an ultrasonic extraction at a temperature of 40 °C for 20 minutes, which only produced an oil yield of 30.7% (Mabayo et al., 2018). Several previous studies show that oil extraction from natural materials is rarely carried out using the ultrasonic method. In this study, rubber seed oil will be extracted using ultrasonic extraction. Variables used were types of solvents (n-hexane, ethyl acetate, and ethanol), material mass to solvent volume ratio (1:5, 1:6, 1:7), temperature, and extraction time (30, 45, and 60 minutes). This study aimed to determine the effect of solvent polarity on the extraction efficiency and yield of rubber seed oil, and to identify the optimal conditions for ultrasonic extraction. The objectives of this study are to determine the effect of solvent polarity on the characteristics of rubber seed extract produced using ultrasonic method, understanding the impact of solvent type (n-hexane, ethyl acetate, and ethanol), extraction time (30, 45, and 60 minutes), and material to solvent ratio (1:5, 1:6, and 1:7 w/v) on the percentage yield of rubber seed extract by ultrasonic method, and knowing the composition of the rubber seed oil produced.

2. MATERIALS AND METHODS

The n-hexane (90%), ethyl acetate (99%), and ethanol (96%) were purchased from a local market, and the rubber seeds were obtained from Bajaratu Way Pengubuan, Lampung Tengah.

2.1 Work Procedures

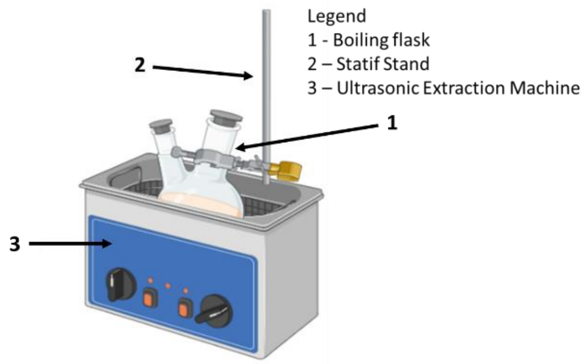
2.1.1 Preparation of rubber seeds and solvent

The collected rubber seeds were cleaned of any remaining dirt or soil with running water. The rubber seeds are separated from their shells and dried in an oven for 4 or 5 hours to obtain a constant dry mass. The solvent is prepared with 90% n-hexane, 99% ethyl acetate, and 96% ethanol.

2.1.2 Extraction process

The rubber seeds that have been oven-dried are then ground with a mortar. The ground rubber seed powder was sieved using a 50-mesh sieve. Fifteen grams of fine rubber seeds were put into a two-necked flask containing 75 mL of solvent (1:5 w/v). The procedure was repeated with a ratio of material composition and solvent of 1:6 and 1:7 w/v. The two-necked flask containing rubber seeds and solvent was put into an ultrasonic device with a temperature set at 50 °C and an extraction time of 30 minutes, then repeated at 45 and 60 minutes. The resulting rubber seed extract that contained residue and remaining solvent was filtered to separate the solids from the extract. The extract volume and mass of the residue were recorded, and the experimental procedure was repeated on ethyl acetate and ethanol solvents. The resulting rubber seed extract was then purified using a rotary vacuum evaporator.

Evaporation was carried out using a rotary evaporator at a temperature of 80 °C, a rotation speed of 70 rpm, and a vacuum strength of 250-400 mmHg. The volume of the rubber seed extract was recorded, its color characteristics were observed, and its yield was calculated. The UAE apparatus and rotary vacuum evaporator used in this study are shown in Figure 1.



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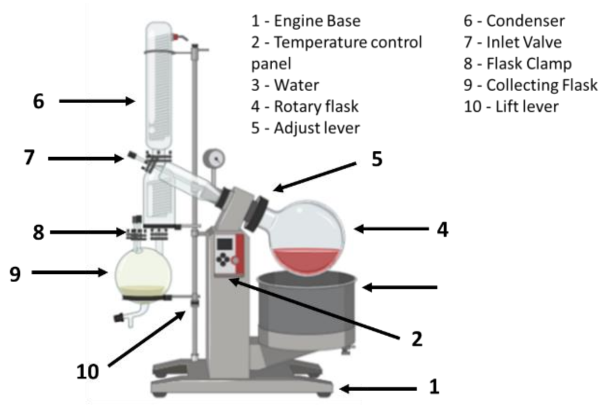


(b)

Figure 1. (a) Ultrasonic-assisted extraction, (b) Rotary vacuum evaporator

2.1.3 Extraction compound analysis

The extract was analyzed for its color, yield, and composition. The color was visually observed. GC-MS (PerkinElmer) was used to determine the composition of the extract.

3. RESULTS AND DISCUSSION

3.1 Physical Characteristics of Rubber Seed Extract

The extract color obtained from the three solvents used, n-hexane (non-polar), ethyl acetate (semi-polar), and ethanol (polar), is shown in Figure 2.

(a) (b) (c)

Figure 2. Extracted oil using (a) n-hexane, (b) ethyl acetate, (c) ethanol

N-hexane solvent produces yellow extracts, scented with rubber seeds. Ethyl acetate solvent produces cloudy yellow extracts and is ethyl acetate scented. Ethanol solvent produces brownish-yellow extracts and smells like rubber seeds. The color of these extracts is almost similar to that of previous studies, yellow and brownish yellow (Puspitasari & Proyogo, 2016). The extract odor depends on the odor of the solvent, for n-hexane has an odor like benzene, but not so pungent that the extract produces an odor that tends to smell of rubber seeds (Mabayo et al., 2018). Ethyl acetate has a smell like fruit, but is so pungent that the extract tends to smell like ethyl acetate (Ayatusa'adah et al., 2023). Ethanol has an odor like alcohol, but not so pungent that the extract produces an odor that tends to smell of rubber seeds (Riniati et al., 2019).

From the three types of solvents, it can be seen that extracts with different polarity solvents can affect the results, especially the color of the extract; the higher the polarity value of the solvent, the darker the color of the extract. The color difference occurs due to the degradation of other chemical components contained in the oil. Apart from the formation of oil extracts, different processes can occur, such as the browning process, which is the extraction of carbohydrates with amino groups from protein molecules (Nurhadi et al., 2020). It can be seen that extraction using n-hexane, a non-polar compound, produces the lightest color. Solvent polarity values will affect the extract results (Sari et al., 2021).

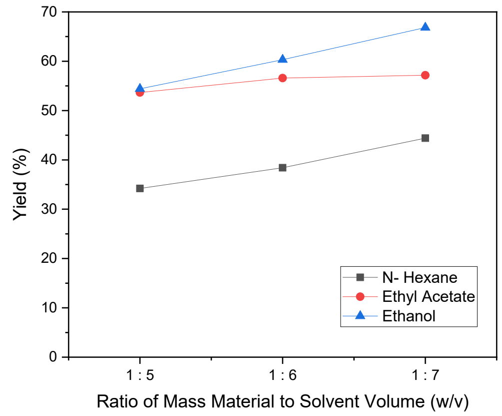
3.2 Effect of Ratio of Material Mass to Solvent Volume on the Yield (%) of Rubber Seed Extract

The effect of the material mass:solvent volume ratio on the yield (%) of rubber seed extract at extraction times of 30, 45, and 60 minutes is shown in Figure 3.

Based on Figure 3, it can be seen that each extraction process carried out at 30, 45, and 60 minutes with several solvents (n-hexane, ethyl acetate, and ethanol) has the highest extract yield at a material :solvent ratio of 1:7 w/v. The greater the material mass and solvent volume ratio, the greater the rubber seed extract produced.

This is in line with previous research on the effect of ethanol on cocoa fruit extract; the ratio of the mass of the material to the solvent affects the extract yield, which increases as the ratio increases (Witono et al., 2022). The different yield (%) changes in each type of solvent occur due to the length of the extraction time. The longer the extraction time, the greater the extract yield produced. This is due to the occurrence of equilibrium that has been achieved in the extraction process (Ridwan et al., 2017).

In this study, the highest yield of rubber seed extract was 70.97% using ethanol solvent for 60 minutes. While the smallest yield was 34% for 30 minutes using n-hexane with a 1:5 w/v mass-to-solvent ratio. This yield is higher than previous studies on the UAE extraction process, which can only produce a yield of 30.3% with n-hexane solvent of 75 mL (Mabayo et al., 2018).



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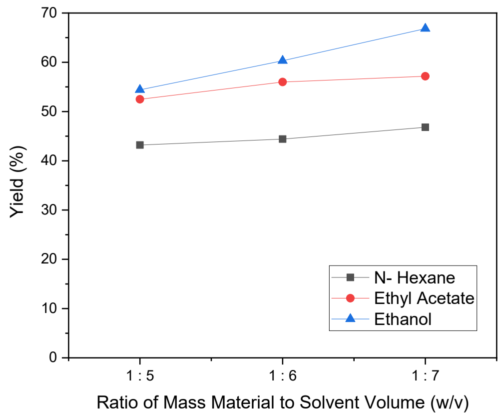

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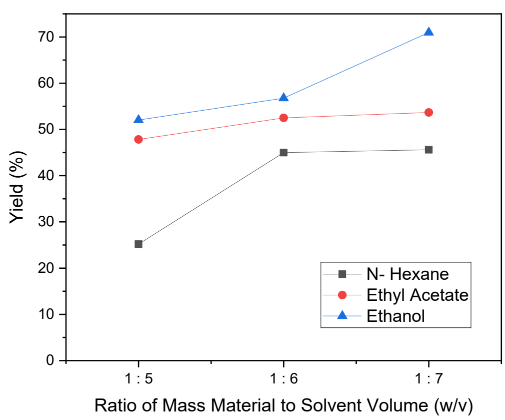
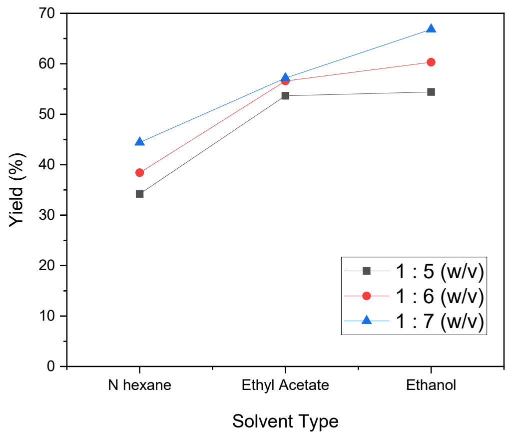


(c)

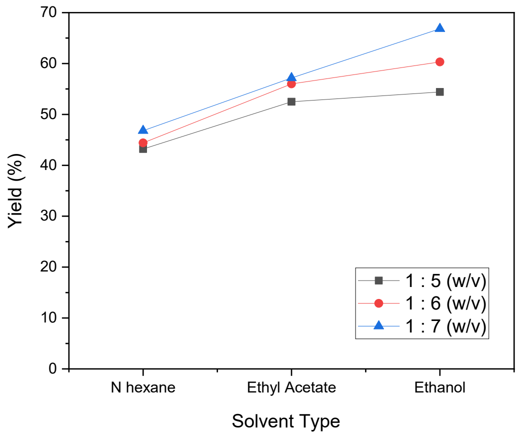
Figure 3. Effect of ratio of mass material to solvent volume against yield (%) at extraction time (a) 30, (b)45, and (c) 60 minutes

3.3 Effect of Solvent Type on the Yield (%) of Rubber Seed Extract

The effect of the solvent type on the yield (%) of rubber seed at extraction times of 30, 45, and 60 minutes is shown in Figure 4.



(a)



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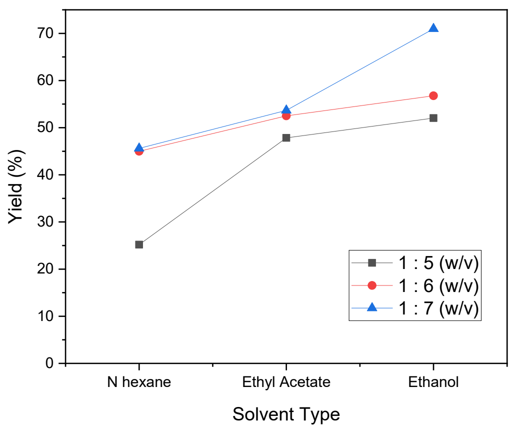


(c)

Figure 4. Effect of solvent type against yield (%) rubber seed extracts at extraction times (a) 30, (b)45, and (c) 60 minutes

Based on Figure 4, in the three extraction processes produced at times 30, 35, and 60 with three variations of mass and volume ratios used (1:5; 1:6; 1:7) w/v has the highest extract yield in ethanol solvent with a mass ratio of material and volume of 1:7 w/v for 60 minutes of extraction. In previous research by Mohd-Setapar et al. (2014), rubber seeds were extracted using the soxhlet method with ethanol and water solvents 70:30 v/v. The yield obtained was 45%, quite effective. This aligns with the extraction results obtained in this study, where the oil components may dissolve better in ethanol as the solvent reaches its boiling point..

The types of solvents used in this study have different levels of polarity, namely, n-hexane has non-polar properties, ethyl acetate has semi-polar properties, and ethanol is polar. In the study, the highest extract yield obtained in ethanol solvent was 70.97%, ethyl acetate solvent 57.17%, and n-hexane solvent 46.8%. Across all time points, ethanol consistently showed the highest average values, followed by ethyl acetate, while hexane had the lowest performance. These results indicate that ethanol is the most effective solvent under the conditions tested, likely due to its higher polarity and ability to dissolve a broader range of compounds (Mohd-Setapar et al., 2014).

This trend aligns with previous findings by Carré et al. (2018), who demonstrated that polar solvents like ethanol exhibit superior extraction efficiency compared to non-polar solvents, like hexane, due to stronger solvent-solute interactions. The maximum oil yield was obtained at a solid-to-solvent ratio of 1:7 w/v, while the minimum yield was observed at a ratio of 1:5 w/v. It can be concluded that a higher solid-to-solvent ratio leads to an increased oil yield. This occurs because a greater concentration gradient between the solid and liquid phases enhances mass transfer efficiency (Jisieike & Betiku, 2020).

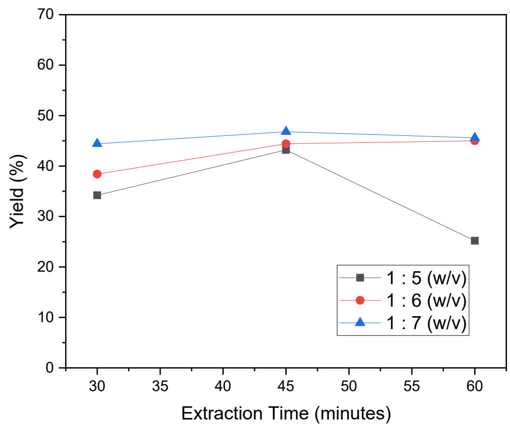
Ethanol's polarity makes it suitable for extracting polar active compounds such as flavonoids. Semi-polar solvents such as ethyl acetate can extract small amounts of semi-polar compounds in rubber seeds, namely phenols, terpenoids, alkaloids, aglycones, and glycosides. While non-polar solvents such as n-hexane can extract compounds in the form of wax, lipids, and volatile oils (Hidayah et al., 2016). The polarity properties in the three solvents, ethanol, ethyl acetate, and n-hexane, have also been tested against antioxidant activity in the extraction process of dedak hanjeli (Nurhadi et al., 2020).

3.4 Effect of Extraction Time on Yield (%) of Rubber Seed Extract

The effect of extraction time against yield (%) of rubber seed extract at each solvent type (n-hexane, ethyl acetate, ethanol) is shown in Figure 5.

Figure 5 data presents measurements recorded at three different times in 30, 45, and 60 minutes by using three solvents: hexane, ethyl acetate, and ethanol. For each solvent, three replicate values were recorded and averaged.

The optimal extraction time was 45 minutes using a ratio of material to solvent of 1:7 w/v. The longer the extraction time, the higher the yield obtained. In previous studies by Mohd-Setapar et al. (2014) rubber seed extraction has been carried out using the soxhlet method in petroleum ether solvents with extraction times of 4, 6, and 8 hours. The most significant yield was obtained at an extraction time of 6 hours, 55% compared to 4 hours. This is because the oil concentration in the extraction process increases along with the length of the extraction time.



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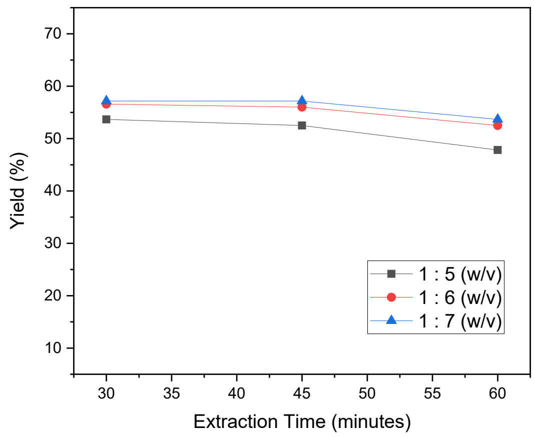

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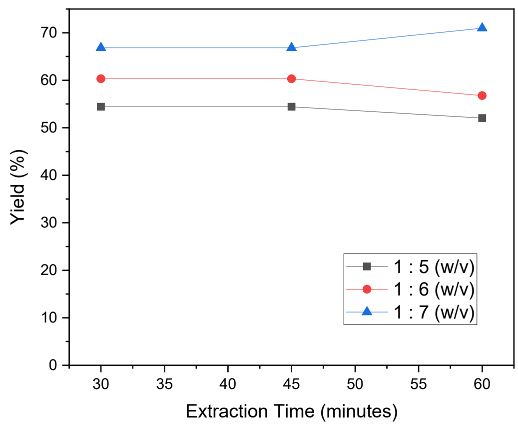

(c)

Figure 5. Effect of extraction time 30, 45, 60 (minutes) against the yield (%) of rubber seeds extract at each solvent type (a) n-hexane; (b) ethyl acetate; (c) ethanol

The increase in yield is influenced by the time the solvent needs to penetrate the rubber seed cells. Therefore, the oil can be extracted entirely over time

(Tanrisannah et al., 2023). The phenomenon has also been observed in previous studies on basil leaf oil extraction using the MAE method (Tanrisannah et al., 2023). Extraction time greatly affects the resulting extract compounds; the proper extraction time will produce optimal compounds. Longer extraction times will cause the extract to hydrolyze, while an extraction time that is too short will cause not all active compounds to be extracted from the material (Waskito et al., 2019).

3.5 Simultaneous Effect of Extraction Time, Ratio of Mass-Material to Solvent Volume, and Solvent Type on Yield (%) Extract Rubber Seeds

Based on Figure 6, the yield (%) obtained will increase if the material mass to solvent volume is greater. The yield (%) in the three types of solvents has different values according to the polarity of the solvent, with the highest yield (%) of rubber seed extract produced using ethanol solvent as much as 70.97%. Furthermore, the best ratio of material mass to solvent volume is 1:7 w/v, while the best extraction time is 45 minutes.

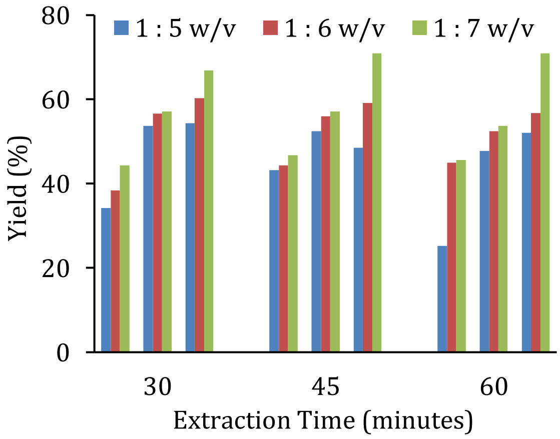

Figure 6. Rubber seed extract yield (%)

3.6 Composition of Rubber Seed Oil

The samples with the highest yield from each solvent were analyzed using GC-MS. This GC-MS test was conducted at 30 minutes of retention for each sample. The following are the results of the spectra of n-hexane, ethyl acetate, and ethanol solvents presented in Figure 7.

Based on Table 1 it can be seen in the spectrum (a) n-hexane shows a retention time of 22.51 minutes with the content of 9-Octadecanoic acid, methyl ester, (E)- as much as 63.917%; (b) ethyl acetate shows a retention time of 7.04 minutes with the content of acetic acid, butyl ester as much as 30.677%; and (c) ethanol shows a retention time of 21.46 minutes with the content of n-hexadecanoic acid as much as 9.150%. The best rubber seed extract composition was achieved using n-hexane, with a total oil composition of 90.640%. This aligns with the theory that oil is a non-polar compound, and therefore, the oil content in rubber seeds is most effectively extracted using n-hexane, which is also non-polar (Raudah et al., 2024). In contrast, ethyl acetate, a semi-polar compound, and ethanol, a polar compound, are less effective solvents. N-hexane is considered the best solvent because of its strong affinity for binding oil compounds in rubber seeds, while it does not extract other non-oil components, thereby maintaining the purity of the oil extract (Sari et al., 2021).

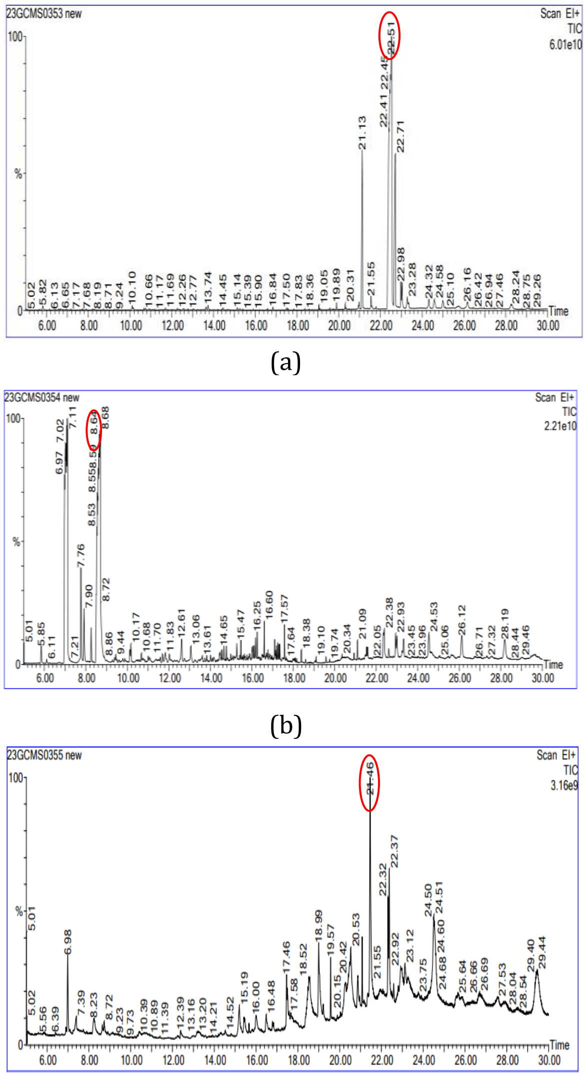

Figure 7. Spectra of (a) n-hexane, (b) ethyl acetate, and (c) ethanol

Table 1. Oil content in extracts using n-hexane, ethyl acetate, and ethanol

compound	solvent			compound	solvent		
	n-hexane	ethyl acetate	ethanol		n-hexane	ethyl acetate	ethanol
N-Acetyl-L-glutamic acid (glutarat acid)	0,118			9-Octadecanoic acid, methyl ester, (E)- (oleic acid)	63,917		
Acetic acid, 1-methylpropyl ester (ethanoate acid)		0,260		Octadecanoic acid, methyl ester (stearic acid)	11,682	0,284	
Butanoic acid			1,281	9,12-Octadecanoic acid, ethyl ester (linoleic acid)	1,304	0,809	
Methyl Tetradecanoate (myristic acid)	0,203			n-Hexadecanoic acid (palmitic acid)			9,150
Acetic acid, butyl ester (alkanoic acid)		30,677		Cis-vaccenic acid			3,495
Propanoic acid, 2-hydroxy-2-methyl-, methyl ester (propanoic acid)			0,476	(E)- 9-Octadecanoic acid, ethyl ester (oleic acid)		0,905	
9-Hexadecenoic acid, methyl ester, (Z)- (palmitic acid)	0,128			Octadecanoic acid, ethyl ester (stearic acid)	0,830	0,318	
Hexadecanoic acid, methyl ester (palmitic acid)	9,488	0,373		Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester (benzenepropanoat acid)	0,091		
Octanoic acid (asam kaprilat)			1,617	Heptadecanoic acid, methyl ester	0,119		
Nonanoic acid (pelargonic acid)			0,808	9,12,15-Octadecanoic acid, methyl ester, (Z,Z,Z)-	0,998		
Hexadecanoic acid, ethyl ester (palmitic acid)	0,667	0,350		Eicosanoic acid, methyl ester (arachidic acid)	0,847		
9-Octadecanoic acid, (Z)- methyl ester (linoleic acid)		1,972	3,199	Docosanoic acid, methyl ester (behenic acid)	0,248		
n-Decanoic acid (capric acid)			1,379				
Dodecanoic acid (lauric acid)			3,791				
				Total %weight	**90,640**	**35,948**	**25,196**

4. CONCLUSION

The characteristics of rubber seed extracts using n-hexane produce a yellow color, ethyl acetate produces a cloudy yellow color, and ethanol produces a brownish yellow color. The most significant oil extract from rubber seeds is ethanol solvent with a time of 45 minutes, and the ratio of material mass and solvent volume is 1:7 w/v. The total composition of oil compounds in rubber seed extract obtained using hexane solvent is 90.64%, ethyl acetate solvent is 35.95%, and ethanol solvent is 25.19%. While the content of oil compounds produced with n-hexane solvent is 63.917% of methyl oleate (9-octadecanoic acid, methyl ester), ethyl acetate solvent is 30.67% acetic acid, butyl ester, and ethanol is 9.15% n-hexadecanoic acid. It is recommended that rubber seeds be extracted using n-hexane to obtain extracts with high oil content. In contrast, to achieve the highest yield, it is best to use ethanol solvent with material mass to solvent volume ratio of 1:7 w/v at 45 minutes.

5. ACKNOWLEDGMENT

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