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A binary solvent for the simultaneous *Calophyllum* oil-resin extraction and purification

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Abstract: Sustainable biodiesel production can be realised by the use of a low-cost feedstock, efficient energy and renewable raw materials. The simultaneous *Calophyllum* oil-resin extraction and its purification using a binary solvent (*n*-hexane mixed with alcohol) were examined to meet those aspects. The extraction conditions effect was investigated to determine the optimal oil yield and quality. *n*-Hexane mixed with alcohol was extracted and purified effectively the oil from *Calophyllum* seeds. The oil yield and its quality were mainly affected by the *n*-hexane-to-alcohol ratio. The oil yield enhanced as the *n*-hexane-to-alcohol ratio enlarged from 1:1 to 2.5:1. The acid value and density of the oil improved as the *n*-hexane-to-alcohol ratio declined from 2.5:1 to 1:1. The *n*-Hexane-to-alcohol ratio of 2.5:1 provided the best yield (59%) of the oil extracted at 40°C for 5 hours. The oil presented its best quality at 0.893 g·cm⁻³ of density, 41.0 mPa·s of viscosity, 8.8 mg KOH·g⁻¹ of the acid value, 88.3 g per 100 g of the iodine value, < 1% of moisture content and < 0.04% of ash content. The oil also had an inhibitory activity against *Staphylococcus aureus*.

Keywords: sustainable; biodiesel; nyamplung; *n*-hexane; alcohol

The multipurpose plant of *Calophyllum inophyllum* has been widely utilized for shipbuilding, the fabrication of furniture, the protection of abrasion and coastal demarcation, as a windbreaker, and mainly as a source of oil and a second-generation feedstock of biodiesel (CHAVAN et al. 2013; ONG et al. 2014; SILITONGA et al. 2014; AZAD et al. 2016; JAIN et al. 2018). In Indonesia, the land area for cultivation of *Calophyllum inophyllum* is growing since it may offer a novel form for development of

agriculture with the absence of competition with lands used for food production.

The fruit part of the *Calophyllum* plant has a great prospective for biodiesel production because it contains seeds with a high oil content (CRANE et al. 2005; ATABANI, DA SILVA CÉSAR 2014). The seeds contain also a toxicant resin which is comparable to myrrh (DWECK, MEADOWS 2002). Moreover, the seeds are identified to contain phytochemical constituents such as alkaloid, coumarin, xanthone, steroid, poly-

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phenol, triterpenoid, flavonoid, etc. (YIMDJO et al. 2004; DAL, MUMPER 2010; KOLB et al. 2011).

Extraction of the *Calophyllum* oil is commonly conducted with a screw press (JAHIRUL et al. 2013; FADHLULLAH et al. 2015). The amount of oil extracted is low (< 35% based on the dried seed mass), and it is tinted green, very viscous and has a high acidity because the resin is also extracted with the oil. Use of *n*-hexane to extract the oil can increase its yield (> 50%) and quality (BHUIYA et al. 2015; JAHIRUL et al. 2015).

Before using *Calophyllum* oil for biodiesel production, degumming and neutralization of the oil should be carried out to eliminate the resin and free fatty acids from it (KARTIKA et al. 2010). These processes unfortunately consume a lot of water and chemicals, and the loss of neutral oil is very high (> 30%). On the other hand, refining the oil using *n*-hexane mixed with alcohol (3:1 v/v) has been successful to get rid of large amounts of the resin from the oil, and the loss of the neutral oil is then very low (< 2%) (ANGGRAINI et al. 2014).

Based on the above facts, a comprehensive investigation of the simultaneous oil-resin extraction and its purification from *Calophyllum* seeds with *n*-hexane mixed in alcohol to obtain an oil with a high yield and quality was carried out in this study. The alcohol type (i.e., methanol and ethanol) and the *n*-hexane-to-alcohol ratio effects were examined to recognise the optimal extraction conditions leading to the best yield and quality of oil.

MATERIAL AND METHODS

Material. The Forest Research and Development Centre (KHDTK Carita, Indonesia) provided the *Calophyllum* seeds for this study while Brataco, Merck and Sigma-Aldrich (Indonesia) supplied the *n*-hexane, methanol, ethanol and other chemicals.

Oil-resin extraction and purification. The average moisture content of the seeds used in this study was $2.23 \pm 0.00\%$ (NF V 03-903). 100 g of the seeds and 100 ml of the alcohol were mixed, and, subsequently, milled for 5 min with a blender. Corresponding to the *n*-hexane-to-alcohol ratio (v/v) of 1:1–2.5:1, 350–500 ml of *n*-hexane and 100–250 ml of excess alcohol were then poured into the mixture of the seeds and alcohol. For every experiment, the ratio of the total solvent to the seeds (v/w, expressed

in ml·g⁻¹) was hence 7:1. Extraction was undertaken in a three-necked flask outfitted with a reflux system and a hot plate magnetic stirrer. The operating condition was fixed at 40°C and 800 rpm for 5 hours.

After the complete extraction was achieved, the solid was filtrated with a vacuum filter, and the filtrate was then allowed to stay overnight to separate it into two layers. The top layer was comprised of *n*-hexane and the oil had a yellow colour while the bottom layer constituted the alcohol and impurities (mainly resin) which had a dark brown colour. Recovery of the *n*-hexane and alcohol was undertaken with a rotary evaporator. The oil and resin were dried at 105°C for 1 h, and they were then weighed. The oil-resin yield, the oil yield and the separated oil percentage (% dry matter basis) were calculated from Equations 1–3:

$$\text{Oil-resin yield} = \frac{\text{Oil and resin mass after drying (g)}}{\text{Seeds mass (g)}} \times 100 \quad (1)$$

$$\text{Oil yield} = \frac{\text{Oil mass after drying (g)}}{\text{Seeds mass (g)}} \times 100 \quad (2)$$

$$\text{Separated oil percentage} = \frac{\text{Oil mass after drying (g)}}{\text{Oil and resin mass after drying (g)}} \times 100 \quad (3)$$

All the experiments were performed twice in repetition, resulting in a mean and standard deviation for all of the data. The influence of the alcohol type and *n*-hexane-to-alcohol ratio on the oil yield and quality was investigated by an experimental design of a randomised factorial and an analysis of the variance (*F*-test at *P* = 0.05).

Analytical methods. Determination of the moisture, oil, protein, ash and crude fibre contents was, respectively, conducted by NF V 03-903, NF V 03-908, NF V 18-100, NF V 03-322 and SNI 01-2891-1992. The quality of the extracted oil was investigated by analysis of the moisture and ash contents (SNI 01-2891-1992), the density (AOAC 920.212), the viscosity using an Oswald viscometer (Sigma-Aldrich, Indonesia) at the ambient temperature, the acid value (NF T 60-204) and the iodine value (AOCS-Cd 1d-92). Moreover, the quality of the resin was investigated through the analysis of the acid value only (NF T 60-204). The content of the oil and resin's phytochemical compounds using Harborne method was also analysed qualitatively (HARBORNE 1987), and the performance of the oil as antibacterial against *Escherichia coli*, *Salmonella* and *Staphylococcus aureus* was examined using the method as described by ADEWUYI et al. (2014).

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Table 1. The properties of the used *Calophyllum* seeds

Parameter	wt%
Moisture content	2.2 ± 0.0
Oil content (db)	64.5 ± 1.3
Protein content (db)	4.5 ± 0.0
Ash content (db)	1.7 ± 0.0
Fibre content (db)	25.6 ± 1.7

db – dry basis

RESULTS AND DISCUSSION

From Table 1, the oil contained in the *Calophyllum* seeds represented 64.5 ± 1.3% (dry matter basis). Based on previous study (KARTIKA et al. 2018), it composed of saturated (31.4%) and unsaturated (68.6%) fatty acids. For biodiesel production, an oil with high unsaturated fatty acids content was favourable when associated with the flow properties of a biodiesel (JAIN et al. 2018). The *Calophyllum* seeds were also quite rich in protein and fibre (Table 1).

Based on the microscopic image of the *Calophyllum* seed (Fig. 1), the oil was on the inside of the cell. An extraction method using a solvent will increase the fluidity of the oil, hence it will more easily release throughout fibred matrix toward the material's surface. A solvent system can also break the membrane of the cell and then dissolves the substances contained therein (DAI, MUMPER 2010).

The essential purpose of the oil extraction from the oilseeds is to recover triglycerides, and the use of a single non polar solvent, generally *n*-hexane, is the best method for triglyceride recovery from various oilseeds (JAIN et al. 2018). Resin recovery is also desired in the *Calophyllum* seeds extraction. The



Fig. 1. An image of the oil within the *Calophyllum* seeds (2× magnification)

latter is alcohol-soluble (DWECK, MEADOWS 2002), thus being a polar component. Because of this contrary property of solubility between the triglycerides and the resin, it is possible to extract both with a binary solvent consisting of non-polar and polar solvents. Use of *n*-hexane mixed with alcohol (i.e., methanol and ethanol) in this study was effective to simultaneously recover both from the *Calophyllum* seeds. In addition, the triglycerides could be directly purified because the resin could be separated from it only with an extra step of decantation.

The concurrent oil-resin extraction from the *Calophyllum* seeds and its purification with *n*-hexane mixed in alcohol affected the yield and quality of the oil. Table 2 shows the extraction conditions, i.e., the alcohol type and the *n*-hexane-to-alcohol ratio, that generally affected yield of the oil-resin and the oil. However, the analysis of the variance (*F*-test at *P* = 0.05) presented the *n*-hexane-to-alcohol ratio had a much higher influence on the oil yield than the alcohol type (Table 3), and both did not affect the oil-resin yield. The oil yield enhanced as the *n*-hexane-to-alcohol ratio was augmented from 1:1 to 2.5:1. This trend was also observed in the increase in the separated oil percentage as the *n*-hexane-to-alcohol ratio was augmented from 1:1 to 2.5:1 (Table 3). The alcohol type did not affect the oil yield and the separated oil percentage, meaning that methanol and ethanol had similar performance when separating the resin from the oil-resin mixture. The enhancement in percentage in the separated resin was observed for each alcohol type tested as the *n*-hexane-to-alcohol ratio reduced from 2.5:1 to 1:1. Because of the high polarity of the resin, it is readily dissolved in a polar solvent, such as methanol and ethanol.

The best oil yield of 59.5 and 58.9% was, respectively, obtained with ethanol and methanol at the *n*-hexane-to-alcohol ratio of 2.5:1. The separated oil percentage obtained from this condition was 88–90%, and the oil had a clear yellow colour. The oil extraction from the *Calophyllum* seeds using 100% *n*-hexane, i.e., the *n*-hexane-to-alcohol ratio of 7:0, could extract more oil (64.0%) but it had a greenish yellow colour due to its resin content. The highest oil yield reported in the preceding study (JAHIRUL et al. 2013; BHUIYA et al. 2015) was lower (51–54%), and it was obtained at the *n*-hexane-to-alcohol ratio of 3:0 for 16–48 hours. Here, it was attained for only 5 h but at a higher *n*-hexane-to-alcohol ratio (5:2 versus 3:0), meaning that such an optimal extrac-

Table 2. The effect of the operating conditions on the extraction performance and quality of the *Calophyllum* oil and resin

Alcohol type	<i>n</i> -Hexane to alcohol ratio (v/v)	Yield (wt%)		Separated oil percentage (wt%)	Oil density (g·cm ⁻³)	Oil viscosity (mPa·s)	Iodine value of oil (g iodine per 100 g)	Acid value (mg KOH·g ⁻¹)	
		oil-resin	oil					oil	resin
Ethanol	1.0:1	64.5 ± 3.0	51.9 ± 0.6	80.5 ± 2.7	0.894 ± 0.001	47.8 ± 5.5	85.0 ± 0.4	13.3 ± 0.2	109.1 ± 3.3
	1.3:1	69.0 ± 0.1	57.0 ± 0.3	82.5 ± 0.5	0.895 ± 0.001	48.5 ± 1.0	87.2 ± 2.7	14.8 ± 2.2	133.3 ± 4.8
	1.8:1	65.0 ± 1.0	55.4 ± 0.6	85.2 ± 0.4	0.898 ± 0.001	48.7 ± 2.4	87.7 ± 3.1	17.9 ± 0.6	132.5 ± 6.0
	2.5:1	66.7 ± 1.0	59.5 ± 0.8	89.3 ± 0.1	0.900 ± 0.000	52.1 ± 6.8	88.3 ± 0.0	19.7 ± 2.3	109.2 ± 0.5
Methanol	1.0:1	66.0 ± 0.2	51.8 ± 0.1	78.5 ± 0.4	0.893 ± 0.000	43.0 ± 0.6	86.7 ± 1.3	8.8 ± 0.7	104.0 ± 7.7
	1.3:1	64.7 ± 0.9	53.2 ± 1.3	82.3 ± 0.8	0.895 ± 0.000	46.9 ± 2.8	85.3 ± 0.0	11.4 ± 1.9	119.9 ± 6.5
	1.8:1	67.6 ± 1.4	57.1 ± 0.2	84.4 ± 1.5	0.896 ± 0.001	41.0 ± 2.2	89.0 ± 0.5	16.1 ± 2.6	119.4 ± 9.2
	2.5:1	66.8 ± 0.1	58.9 ± 0.2	88.1 ± 0.3	0.898 ± 0.001	48.1 ± 5.4	86.5 ± 2.2	16.5 ± 1.8	123.5 ± 5.2

tion condition resulted in use of 2.3 times as much solvent. But, recovery of this surplus solvent could be easily conducted for its reuse. On the other hand, compared with the oil extraction using a screw press (FADHLULLAH et al. 2015), the yield of the oil extracted in this study was much larger (59 versus 33%). This showed that the extraction of the *Calophyllum* oil using a solvent was more effective than the mechanical press, as noted in an earlier study (JAHIRUL et al. 2013; BHUIYA et al. 2015).

A high extraction efficiency was also observed on the lipid extraction from *Chlorella vulgaris* using hexane mixed in methanol (MALEKZADEH et al. 2016). The crude lipid recovery improved as the hexane-to-methanol ratio increased, and the use of this solvent mixture was more effective than the other solvent mixtures. In this study, the highest lipid recovery was obtained with the lower hexane-to-methanol ratio (1:1 versus 2.5:1) but at a higher temperature (65 versus 40°C) and a longer extraction time (24 versus 5 h). The application of the lower temperature and shorter time in the oil extraction from the *Calophyllum* seeds using this solvent mixture should reduce the cost production of this process, and it should support the aspects of the sustainable biodiesel production, i.e., a low-cost feedstock, an efficient energy and renewable raw material. In addition, this extraction technology was much simpler and more compact, plus it could produce resin with a high polyphenol content (4.3–5.5%) as a by-product (KARTIKA et al. 2018).

The *Calophyllum* oil extracted in this study presented good qualities for each extraction condition tested. It had a density of ≤ 0.900 g·cm⁻³, a viscosity of ≤ 52.1 mPa·s, an acid value of ≤ 20 mg KOH·g⁻¹ and an iodine value of 85–89 g iodine per 100 g (Table 2). Moreover, it had very poor moisture ($< 1\%$) and ash ($< 0.04\%$) content. Compared with the previous result (JAHIRUL et al. 2015), it had a lower density (≤ 0.90 vs 0.94 g·cm⁻³), a lower viscosity (≤ 52 vs ≥ 56 mPa·s) and a lower acid value (≤ 20 vs 24 mg KOH·g⁻¹). It was much better compared with the mechanically extracted oil (BOUCHER 2000; ATABANI et al. 2013), i.e., a density of ≤ 0.90 vs 0.93 – 0.95 g·cm⁻³, a viscosity of ≤ 52 vs 98 mPa·s, an acid value of ≤ 20 vs 35 – 54 mg KOH·g⁻¹ and an ash content of ≤ 0.04 vs 0.26 – 0.31% . Furthermore, its quality was relatively identical to the degummed and neutralised oil (KARTIKA et al. 2010), mainly with its density and ash content, and its viscosity

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Table 3. The *F*-value obtained from the analysis of the variance for the extraction yield and quality of the *Calophyllum* oil and resin

Source of the variation	<i>F</i> -value								
	oil-resin yield	oil yield	separated oil percentage	oil density	oil viscosity	iodine value of oil	acid value of oil	acid value of resin	at <i>P</i> = 0.05
Alcohol type (<i>A</i>)	0.00	5.02	3.26	7.36*	5.19	0.05	13.80*	2.15	5.32
<i>n</i> -Hexane to alcohol ratio (<i>B</i>)	1.26	92.72*	43.86*	30.64*	1.45	1.77	14.27*	10.12*	4.07
Interaction of <i>AB</i>	5.41*	12.80*	0.40	0.82	0.40	1.26	0.38	4.79*	4.07

*significant

Table 4. The operating conditions effect on the phytochemical content and its performance as being antibacterial

Alcohol type	<i>n</i> -Hexane to alcohol ratio (v/v)	Steroid	Other phytochemical compounds (flavonoid, alkaloid, tannin, saponin, quinone, triterpenoid)	<i>Escherichia coli</i> , <i>Salmonella</i>	<i>Staphylococcus aureus</i>
Ethanol	1.0:1	+ve	–ve	–ve	+ve
	1.3:1	+ve	–ve	–ve	+ve
	1.8:1	+ve	–ve	–ve	+ve
	2.5:1	+ve	–ve	–ve	+ve
Methanol	1.0:1	+ve	–ve	–ve	+ve
	1.3:1	+ve	–ve	–ve	+ve
	1.8:1	+ve	–ve	–ve	+ve
	2.5:1	+ve	–ve	–ve	+ve

+ve – positive, –ve – negative

was better. The oil obtained in this study was comprised of inferior impurities, mainly free fatty acids and resin. Its utilisation for biodiesel production is, hence, more favourable. The suitability of the *Calophyllum* oil properties as a biodiesel feedstock has been proven by many researchers (JAIN et al. 2018). In addition, the content of coumarin and xanthone in the *Calophyllum* oil demonstrated the antioxidant properties to protect against oxidation (KILHAM 2004).

The analysis of the variance (*F*-test at *P* = 0.05) showed the effect of the extraction conditions on the oil quality, mainly the density and the acid value, was significant (Table 3). Both augmented as the *n*-hexane-to-alcohol ratio increased from 1:1 to 2.5:1 (Table 2) for each alcohol type tested, but the acid value and density of the oil extracted by the mixture of *n*-hexane and ethanol were higher than those extracted by the mixture of *n*-hexane and methanol.

The separated resin had a very high acid value, i.e., at least 104 mg KOH·g⁻¹ (Table 2), for every extraction condition tested. The analysis of the variance

showed the *n*-hexane-to-alcohol ratio significantly affected the acid value of the resin (Table 3). Its augmentation enhanced it. Free fatty acids (such as palmitic, stearic, oleic and linoleic acids) and other substances (such as calophyllic, benzoic and oxibenzoic acids) may be more dissolved in the solvent as its quantity added was high.

Table 4 presented the phytochemical compounds (i.e., alkaloid, flavonoid, saponin, tannin, quinone, steroid, triterpenoid) and the performance of the oil as an antibacterial against *Escherichia coli*, *Salmonella* and *Staphylococcus aureus*. The oil was negative for all the phytochemical compounds analysed, except steroid. The steroid contained in the *Calophyllum* oil was generally canophyllic acid, canophyllol and canophyllal (DWECK, MEADOWS 2002). On the other hand, the resin was also negative for all the phytochemical compounds analysed.

Furthermore, the oil had an inhibitory activity against *Staphylococcus aureus* with a diameter of inhibition ≥ 6 mm, but it was negative against *Escherichia coli* and *Salmonella*, as reported in a previous study (YIMDJIO et al. 2004; ADEWUYI et al.

2014). The *Calophyllum* oil demonstrated significant antibacterial activity due to its content of the phytochemical agents, i.e., friedelin, canophyllol, canophyllic acid and inophynone (KILHAM 2004).

CONCLUSION

The binary solvent of *n*-hexane mixed with alcohol has efficaciously extracted oil-resin from the *Calophyllum inophyllum* seeds and purified it in one step. The influence of the *n*-hexane-to-alcohol ratio was the most important on the oil yield and quality, and an increase in the *n*-hexane-to-alcohol ratio improved the oil yield and quality. The best oil yield of 59.5 and 58.9% was, respectively, obtained with ethanol and methanol at the *n*-hexane to alcohol ratio of 2.5:1 and 40°C for 5 hours. The percentage of the oil separated from the resin on the optimal process condition was 89.3% with ethanol and 88.1% with methanol. The separated oil had a good quality and inferior impurities, plus it had an inhibitory activity against *Staphylococcus aureus*. This extraction process, therefore, appears as practical, the oil-resin from the *Calophyllum inophyllum* seeds being simultaneously extracted and purified. In addition, it is also efficient from the extraction yield point of view.

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