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Optimisation of concurrent *Calophyllum* oil-resin extraction and separation

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Abstract: This research optimised the application of a hexane-methanol mixture as a binary solvent for the concurrent oil-resin extraction and separation from *Calophyllum* seeds on a pilot scale, in a direct stage. The optimum oil and resin yields were determined by optimising the extraction conditions using response surface methodology and a second order polynomial model. The extraction conditions affected the oil and resin yields, with the extraction time as the biggest influencing factor. Optimum oil (65%) and resin (16%) yields were predicted to be obtained at 5.2 h and 433 rpm. The model validation with these extraction conditions showed that the predicted results and actual oil (62%) and resin (15%) yields were in passable agreement. The oil was composed of 75.4% triglycerides with a density of 0.874 g·cm^{-3}, a viscosity of 26.4 mPa·s^{-1}, an acid value of 46.4 mg KOH·g^{-1}, an iodine value of 98.0 g iodine·100 g^{-1}, trace water and sediment contents, and zero ash content. The resin had a viscosity of 4 694.8 mPa·s^{-1}, a total phenolic content of a 4.51% gallic acid equivalent, an antioxidant activity of an 8.82 mg ascorbic acid equivalent·g^{-1}, and an acid value of 126.2 mg KOH·g^{-1}.

Keywords: binary; *n*-hexane; methanol; phenolic; antioxidant

Today, the cultivation of *Calophyllum* in Indonesia is well developed, as the plants thrive in sandy, rocky, clayey and calcareous soils. It acts as a windbreak, reducing abrasion and safeguarding the littoral demarcation, and its wood is used in ship building and furniture making. Oil extracted from *Calophyllum* seeds has also been massively used as a raw material for biodiesel since its productivity is high and the oil obtained has properties close to those of mineral diesel (Jain et al. 2018; Arumugam and Ponnusami 2019). *Calophyllum* oil has long been applied as a traditional medicine and used in cosmetics due to the useful phytochemical constituents it contains, such as coumarins, triterpenoids, alkaloids, calophyllolide, steroids, inophyllum, flavonoids, phenols, calophyllic acid, polyphenols, and xanthones (Dai and Mumper 2010; Léguillier et al. 2015). This oil has been proven to have anti-ageing, antioxidant, UV-protective,

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therapeutic and antiradical properties (Boucher 2000), and to also have biological (Adewuyi et al. 2014) and osteogenic activities (Liu et al. 2015).

In addition to the oil, *Calophyllum* seeds also contain a resin (Dweck and Meadows 2002), its content is represented by around 29% of the dry matter weight (Kartika et al. 2018). This resin is poisonous, but it contains useful chemical compounds such as xanthones and their derivatives (Kolb et al. 2011), 4–6% of polyphenols (Kartika et al. 2018), and flavonoids (Ginigini et al. 2019). It has shown excellent antiviral, anticancer, antimicrobial, anti-HIV, and anti-inflammatory activity.

Currently, the coincident extraction and purification method using a binary mixture of polar and nonpolar solvents efficiently extracts the oil and resin from *Calophyllum* seeds, and separates them in a direct stage (Kartika et al. 2018, 2019). The highest yields of oil (51%) and resin (18%) have been attained at 50 °C, 800 rpm and a 2:1 ratio of *n*-hexane to methanol over a duration of 5 h (Kartika et al. 2018). However, for the same time and stirring speed, a higher oil yield (59%) has recently been obtained at a higher *n*-hexane-to-methanol ratio (2.5 : 1) (Kartika et al. 2019). The percentage of the oil separated from the resin was more than 88%, and it was low in impurities. It also showed an inhibitory activity against *Staphylococcus aureus*, and the separated resin possessed 4–6% of the total phenolic content.

The research presented here was carried out to optimise the concurrent *Calophyllum* oil-resin extraction and separation on a pilot scale using a binary solvent made of a mixture of *n*-hexane and methanol. The optimisation of the extraction time and stirring speed was analysed using a response surface methodology and a second order polynomial model to maximise the oil and resin yields and to obtain the optimal physiochemical properties of the oil and resin.

MATERIAL AND METHODS

Material. *Calophyllum* dry fruits stored in a material warehouse for one year were obtained from KHDTK (Kawasan Hutan Dengan Tujuan Khusus) Carita, the Forest Research and Development Centre in Indonesia. BRATACO Chemical Ltd. (Indonesia) provided the technical methanol and *n*-hexane (98% purity). Pure analytical grade chemicals and solvents were supplied by Sigma-Aldrich and Merck (Indonesia).

Oil-resin extraction and separation procedure. Before being used for oil extraction, the *Calophyllum* seeds with a moisture content of 29.7 ± 0.04% (French standard NF V 03-903) were first cleaned of their shells. They were further dried to decrease their moisture content to less than 5% using a ventilated oven at 60–70 °C for 48–72 hours.

A mixture of dry seeds (1 kg) and methanol (1 L) was ground in a blender for 5–10 min. Extra methanol (1 L) and *n*-hexane (5 L), corresponding to total methanol-to-*n*-hexane ratio of 2 : 5 (v/v), were then poured into the mixture. A seed-to-total solvent ratio of 1 : 7 (w/v, expressed in $kg{\cdot}L^{-1}$) was thus used in all the experiments. A 10 L reactor equipped with a heater, an agitator and a reflux system was used to conduct all the experiments. Various extraction times (4–6 h) and stirring speeds (200–600 rpm) were investigated in this research to maximise the oil and resin yields, and the temperature was kept constant at 50 °C.

The mixture was cooled to room temperature after the complete extraction and was then filtered using a vacuum filter to separate the cake from the filtrate. The filtrate was left for several hours to separate into two layers: the *n*-hexane-oil fraction in the upper layer and the methanol-resin fraction in the lower layer. A rotary evaporator was used to recover the oil and resin by evaporation of the *n*-hexane and methanol. The oil and resin were subsequently dried for 1 h at 105 °C before being weighed. The oil yield, resin yield, extraction efficiency and separated resin percentage were then determined:

$$\mathrm{COY}(\%\ \mathrm{d.m.}) = \frac{\text{Mass of oil after drying}\,(\mathrm{kg})}{\text{Mass of dry seeds}\,(\mathrm{kg})} \times 100 \quad (1)$$

$$\mathrm{CRY}(\%\ \mathrm{d.m.}) = \frac{\text{Mass of resin after drying}\,(\mathrm{kg})}{\text{Mass of dry seeds}\,(\mathrm{kg})} \times 100 \quad (2)$$

$$\mathrm{EE}(\%\ \mathrm{d.m.}) = \frac{\text{Mass of oil after drying}\,(\mathrm{kg})}{\text{Mass of oil contained in seeds}\,(\mathrm{kg})} \times 100 \quad (3)$$

$$\mathrm{SRP}(\%\ \mathrm{d.m.}) = \frac{\text{Mass of resin after drying}\,(\mathrm{kg})}{\text{Total mass of oil and resin}\,(\mathrm{kg})} \times 100 \quad (4)$$

where: COY – crude oil yield; CRY – crude resin yield; EE – extraction efficiency; SRP – separated resub percentage; d.m. – dry matter.

The effect of the extraction conditions on the oil and resin yields and on their physiochemical properties was examined with a central composite experimental design, and the extraction conditions were

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optimised using response surface methodology and a second order polynomial model. The response surface methodology is "a collection of mathematical and statistical techniques that are useful for the modelling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimise the response" (Montgomery 2001). A central composite design is an approach of the response surface methodology for exploring the model, and it is an effective design for fitting the second order polynomial model. The extraction condition variables investigated in this study were the extraction time (4–6 h) and stirring speed (200–600 rpm). The appropriate range of these variables was determined on the basis of previous studies (Kartika et al. 2016, 2018, 2019). The central composite design gave a total of eleven experiments, and the central point experiment was conducted with three replications. Design-Expert software (trial version 12.0) was utilised for the data analysis and the accuracy assessment of the second order polynomial model was conducted by the *F*-test with $P = 0.05$ as a significance threshold.

Analytical methods. The French standards NF V 03-908, NF V 03-903, NF V 03-322 and NF V 18-100 were used to examine the oil, moisture, ash and protein contents, respectively. The quality of the oil obtained from each experiment was determined by examining the iodine (American standard AOCS-Cd 1d-92), saponification (Indonesian standard SNI 04-7182-2006) and acid (French standard NF T 60-204) values, ash content (SNI 01-2891-1992), density (AOAC 920.212), and dynamic viscosity. A modular compact rheometer (MCR 302, Anton Paar, Austria) with a CP50-2 cone-plate (49.981 mm diameter and 1.998° angle) was used to measure the dynamic viscosity at 25 and 40 °C. The compositions of the glycerides and free fatty acids of the oils were examined by gas chromatography (GC), as previously explained (Kartika et al. 2013).

The resin quality was determined by examining the viscosity at 25 °C with a modular compact rheometer, functional groups with a spectrometer (Spectrum 65 FT-IR, Perkin Elmer, UK), total phenolic content (Folin-Ciocalteu method) as described by Kartika et al. (2018), antioxidant activity (DPPH method) as explained by Nariya et al. (2013), and the acid value (NF T 60-204).

RESULTS AND DISCUSSION

***Calophyllum* seed properties**. For a moisture content of 2.6 ± 0.02%, the oil content of the seeds was 67.7 ± 1.03 dry matter basis % (db). This was higher than the 54–64.5 db.% used in the previous research (Kartika et al. 2018, 2019), possibly because the seeds aged during the storage of the *Calophyllum* fruits, so their oil content increased. The seeds were also rich in proteins (6.8 ± 0.4 db.%) and poor in ashes (1.7 ± 0.09 db.%).

Oil-resin extraction and their separation. The experimental results (Table 1) showed that the time and stirring speed both affected the oil yield, but, based on the variance analysis results (*F*-test with $P = 0.05$ as a significance threshold), the effect of the time was stronger than that of the stirring speed (Table 2). The oil yield improved as the time and stirring speed increased, and the optimum oil yield (65.2%) was reached at 5.2 h and 405 rpm (Figure 1). This enhancement was supported by an improvement in the extraction efficiency from 77 to 96%.

The improvement in the oil yield was limited to 5.2 h, after which it decreased continuously. This indicates that the system had reached the optimum condition at 5.2 h, and it had also reached equilibrium. Although the oil yield decreased after 5.2 h, its triglyceride content was relatively stable (about 77%), indicating that the time had no influence on the triglycerides (Table 2). This increment in time did not cause the triglycerides to degrade into diglycerides, monoglycerides or free fatty acids, in contrast to what was observed by Kartika et al. (2018). An explanation may be that the diglyceride, monoglyceride and free fatty acid contents of the oil obtained in this study were much higher than in the previous one (22–25 *vs* 2–5%). This may have affected the equilibrium of the triglyceride degradation, as reported by Lee et al. (2000).

Compared to the highest oil yield (51–54 db.%) observed in the previous research (Jahirul et al. 2015), the optimal oil yield in this study (65 db.%) was higher, and the time required to obtain it was 3–10 times shorter (5.2 h *vs* 16–48 h) but the *n*-hexane-to-seed ratio was 1.7 times higher (5 : 1 *vs* 3 : 1) and the solvent-to-seed ratio 2.3 times higher (7 : 1 *vs* 3 : 1). This excess solvent could be recuperated and recycled in the process (Kartika et al. 2013). The amount of oil extracted in this research was also higher than that extracted with a binary solvent over the same time and at the same temperature (65 *vs* 51%) (Kartika et al. 2018). The increment of the *n*-hexane-to-methanol ratio from 2 : 1 to 2.5 : 1 might have increased the oil yield due to a decrease in polarity, even though the stirring speed used was lower (400 *vs* 800 rpm).

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Table 1. Effect of the operating conditions on the extraction performance and physiochemical properties of the crude *Calophyllum* oil

Time (h)	Stirring speed (rpm)	Crude oil yield (wt. %)	Extraction efficiency (wt. %)	Density ($g \cdot cm^{-3}$)	Viscosity ($mPa \cdot s^{-1}$)		Iodine value (g iodine·100 g^{-1})	Acid value (mg KOH·g^{-1})	SV (mg KOH·g^{-1})	Ash content (wt. %)	TAG content (wt. %)	Impurities content (DAG, MAG, FA) (wt. %)	FA content (wt.%)
					at 25 °C	at 40 °C							
4	200	52.44	77.46	0.872 ± 0.002	56.8 ± 0.1	30.1 ± 0.1	98.4 ± 1.5	55.4 ± 0.6	247.6 ± 5.1	0.012	76.8 ± 0.3	23.2 ± 0.3	17.4 ± 0.1
6	200	56.55	83.53	0.865 ± 0.001	58.0 ± 0.2	26.5 ± 0.1	87.9 ± 0.0	49.4 ± 0.3	242.8 ± 2.7	0.002	76.7 ± 0.1	23.3 ± 0.1	16.5 ± 0.0
4	600	54.10	79.91	0.871 ± 0.000	57.5 ± 0.1	29.2 ± 0.1	88.1 ± 0.9	51.5 ± 1.4	262.5 ± 6.0	0.000	75.1 ± 0.1	24.9 ± 0.1	17.7 ± 0.2
6	600	54.84	81.01	0.884 ± 0.000	58.2 ± 0.1	31.0 ± 0.1	91.2 ± 0.3	53.9 ± 0.3	262.9 ± 9.6	0.010	77.5 ± 1.0	22.5 ± 1.0	16.3 ± 0.3
3.6	400	52.48	77.53	0.870 ± 0.000	59.3 ± 0.1	30.5 ± 0.1	98.0 ± 0.2	49.0 ± 0.5	234.3 ± 14.4	0.000	76.1 ± 0.0	23.9 ± 0.0	16.6 ± 0.3
6.4	400	60.23	88.97	0.887 ± 0.000	57.0 ± 0.1	33.4 ± 0.1	86.6 ± 0.3	51.4 ± 0.3	228.5 ± 2.7	0.005	76.5 ± 0.7	23.5 ± 0.7	16.9 ± 0.3
5	117	55.10	81.39	0.885 ± 0.000	59.3 ± 0.1	29.4 ± 0.1	85.9 ± 0.3	49.9 ± 0.9	239.8 ± 4.2	0.002	75.0 ± 0.1	25.0 ± 0.1	17.8 ± 0.2
5	683	57.45	84.85	0.869 ± 0.000	58.1 ± 0.1	27.7 ± 0.1	93.4 ± 1.0	54.8 ± 0.4	242.9 ± 2.2	0.002	77.0 ± 0.5	23.0 ± 0.5	16.7 ± 0.1
5	400	64.70	95.57	0.865 ± 0.001	53.1 ± 0.2	25.1 ± 0.1	94.1 ± 0.2	54.4 ± 0.2	252.8 ± 2.9	0.002	77.8 ± 0.3	22.2 ± 0.3	16.5 ± 0.3
5	400	64.72	95.61	0.887 ± 0.000	56.9 ± 0.1	32.0 ± 0.1	87.5 ± 0.3	54.4 ± 0.3	208.0 ± 7.1	0.000	77.3 ± 0.1	22.7 ± 0.1	16.6 ± 0.0
5	400	65.60	96.90	0.859 ± 0.000	42.6 ± 0.1	24.6 ± 0.1	90.0 ± 1.6	46.2 ± 0.5	228.8 ± 14.4	0.002	76.8 ± 0.6	23.2 ± 0.6	16.4 ± 0.1

SV – saponification value; TAG – triglycerides; DAG – diglycerides; MAG – monoglycerides; FA – free fatty acids; wt. – weight

Table 2. *P*-value of the ANOVA for the oil yield, resin yield and triglyceride content

Source of variation	*P*-value		
	oil yield	TAG content	resin yield
Model	0.0034*	0.2306	0.0300*
(*A*)	0.0188*	0.2164	0.0355*
(*B*)	0.5102	0.3969	0.0750
Interaction *AB*	0.3495	0.1420	0.2288
(A^2)	0.0009*	0.2277	0.0094*
(B^2)	0.0009*	0.1240	0.7686
Lack of fit	0.0588	0.2537	0.3244
R^2	0.95	0.67	0.87
Adjusted R^2	0.89	0.34	0.73

*Significant at $P = 0.05$; *A* – extraction time; *B* – stirring speed; A^2 – quadratic of extraction time; B^2 – quadratic of stirring speed; R^2 – coefficient of determination; TAG – triglycerides

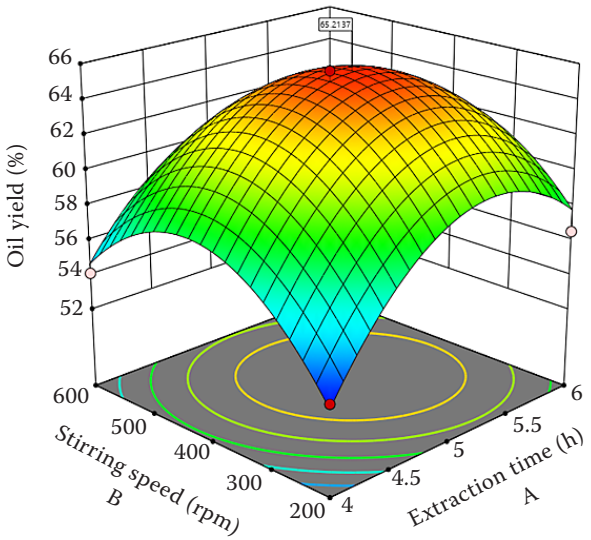

Figure 1. 3D response and contour of the extraction condition effect on the oil yield and its optimisation

$Y = 51.50A + 0.12B - 0.0042AB - 4.78A^2 - 0.00012B^2 - 93.13$; $R^2 = 0.95$

Compared to the highest oil yield obtained in a study by Indartono et al. (2019), the optimal oil yield in the present research is twice that achieved with a screw press (65 *vs.* 33%).

The time and stirring speed also affected the resin yield (Table 3), and the effect of the time was stronger than that of the stirring speed (based on the variance analysis result – *F*-test with $P = 0.05$) (Table 2). The resin yield was enhanced as the stirring speed and time increased, and the optimum

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Table 3. Effect of the operating conditions on the resin separation performance and its physiochemical properties

Time (h)	Stirring speed (rpm)	CRY (wt.%)	SRP (wt.%)	Viscosity at 25 °C (mPa.s)	Acid value (mg KOH·g^{-1})	Antioxidant activity (AA eq. mg·g^{-1})	Total phenolic content (wt.% GA eq.)	Wavenumber of functional groups (cm^{-1})				
								alkanes (C-H)	alkenes (C=C)	oxygen groups (C-O)	carbonyl groups (C=O)	aromatic
4	200	11.98	17.72	7 022.2 ± 23.4	153.4 ± 1.3	11.65 ± 0.35	5.18 ± 0.01	886, 1 376, 1 443, 2 853, 2 923	1 598	1 133, 1 157	1 706, 1 736	698, 1 598
6	200	14.29	18.13	5 283.2 ± 5.4	143.9 ± 1.5	13.94 ± 0.45	5.04 ± 0.02	886, 1 376, 1 443, 2 853, 2 923	1 598	1 133, 1 157	1 708, 1 736	698, 1 598
4	600	15.53	20.88	2 536.5 ± 15.5	112.4 ± 4.1	9.37 ± 0.06	5.09 ± 0.12	886, 1 376, 1 444, 2 853, 2 923	1 598	1 133, 1 159	1 706, 1 739	698, 1 598
6	600	15.42	20.50	3 805.8 ± 14.5	119.6 ± 0.2	9.15 ± 0.10	4.68 ± 0.16	887, 1 376, 1 444, 2 853, 2 923	1 598	1 134, 1 157	1 706, 1 736	698, 1 598
3.6	400	11.54	17.40	3 731.6 ± 27.8	156.1 ± 4.3	9.55 ± 0.40	4.90 ± 0.05	887, 1 376, 1 444, 2 853, 2 923	1 598	1 134, 1 157	1 706, 1 736	698, 1 598
6.4	400	15.03	19.17	3 056.8 ± 11.3	112.2 ± 2.1	12.96 ± 0.13	4.01 ± 0.19	887, 1 376, 1 444, 2 853, 2 923	1 598	1 133, 1 157	1 706, 1 736	698, 1 598
5	117	15.77	21.26	3938.1 ± 33.3	156.1 ± 0.5	9.44 ± 0.40	4.51 ± 0.06	887, 1 376, 1 444, 2 853, 2 923	1 598	1 133, 1 157	1 706, 1 736	698, 1 598
5	683	16.42	19.89	3 423.3 ± 36.9	125.3 ± 2.4	9.71 ± 0.24	4.53 ± 0.05	887, 1 376, 1 443, 2 853, 2 923	1 598	1 133, 1 157	1 706, 1 736	698, 1 598
5	400	16.66	20.04	5 046.1 ± 45.5	111.0 ± 1.9	11.77 ± 0.44	5.05 ± 0.11	886, 1 376, 1 443, 2 853, 2 923	1 598	1 133, 1 157	1 706, 1 736	698, 1 598
5	400	16.36	19.33	6 109.3 ± 9.6	120.6 ± 1.1	13.29 ± 0.75	4.55 ± 0.06	886, 1 376, 1 443, 2 854, 2 923	1 598	1 133, 1 157	1 706, 1 736	698, 1 598
5	400	15.38	18.99	2 515.1 ± 26.8	120.9 ± 2.7	10.43 ± 0.50	4.87 ± 0.05	887, 1 376, 1 443, 2 853, 2 923	1 598	1 133, 1 159	1 706, 1 739	698, 1 598

CRY – crude resin yield; SRP – separated resin percentage; AA – ascorbic acid; GA – gallic acid; eq. – equivalent; wt – weight

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resin yield (16.7%) was reached at 585 rpm and 5.2 hours (Figure 2). The resin yield decreased after 5.2 h, indicating that the optimal time and system equilibrium were at 5.2 hours. The optimal resin yield in this research was lower than the highest resin yield obtained in a previous study (18%) (Kartika et al. 2018). It was obtained over the same time with the same methanol-to-seed ratio, but with a lower stirring speed (400 *vs* 800 rpm) and methanol-to-*n*-hexane ratio (0.4 : 1 *vs* 0.5 : 1). The reduction in the methanol-to-*n*-hexane ratio from 0.5 : 1 to 0.4 : 1 may have decreased the resin yield due to a decrease in polarity. The percentage of resin separated in this study was relatively constant, i.e., about 19.39%, and the extraction conditions had no effect on it.

The optimisation of the oil yield with the resin yield as a constraint resulted in optimum oil and resin yields of 65.1 and 16.3%, respectively, and they were obtained at 5.2 h and 433 rpm. The optimum yields of the oil and resin obtained through this simultaneous optimisation were similar to those optimised individually (65.2 and 16.7%, respectively) and were obtained in the same time (5.2 h), but with a different stirring speed. This confirmed that the effect of the time on the oil and resin yields was more significant than the stirring speed. The experimental validation of this modelled optimum extraction condition resulted in actual oil and resin yields of 61.9 ± 1.2% and 14.6 ± 0.9%, respectively (Table 4). These results are

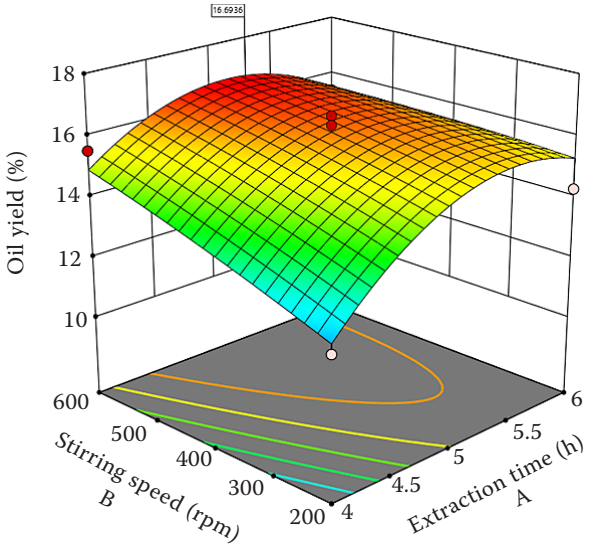

Figure 2. 3D response and contour of extraction condition effect on resin yield and its optimization

$Y = 17.31A + 0.021B - 0.0030AB - 1.52A^2 - 0.0000029B^2 - 34.25$; $R^2 = 0.87$

Table 4. Physiochemical properties of the *Calophyllum* oil and resin produced from the model validation of the optimal extraction conditions (5.2 h and 433 rpm)

Parameter	Unit	Value	
		actual	prediction
Oil			
Yield	wt (%)	61.9 ± 1.2	65.1
Density	g·cm^{-3}	0.874 ± 0.007	0.874
Viscosity (25 °C)	mPa·s^{-1}	48.5 ± 4.0	56.08
Viscosity (40 °C)	mPa·s^{-1}	26.4 ± 1.8	29.05
Acid value	mg KOH·g^{-1}	46.4 ± 3.7	51.8
Iodine value	g iodine·100 g^{-1}	98.0 ± 1.2	90.6
Saponification value	mg KOH·g^{-1}	247.7 ± 1.3	241.1
Ash content	wt. (%)	0	0
Water and sediment content	vol (%)	trace	trace
Triglycerides	wt. (%)	75.4 ± 1.5	77.5
Diglycerides	wt. (%)	6.7 ± 0.7	6.1
Monoglyceride	wt. (%)	0.4 ± 0.0	0.4
Free fatty acids	wt. (%)	17.6 ± 0/7	16.4
Resin			
Yield	wt. (%)	14.6 ± 0.9	16.3
Viscosity (25 °C)	mPa·s^{-1}	4 694.8 ± 15.5	4 073.5
Acid value	mg KOH·g^{-1}	126.2 ± 2.1	126.1
Antioxidant activity (ascorbic acid equivalent)	mg·g^{-1}	8.82 ± 0.46	11.08
Total phenolic content (gallic acid equivalent)	wt. (%)	4.51 ± 0.31	4.71

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quite close, i.e., a difference of less than 5% for the oil yield and 10% for the resin yield.

The quality of the extracted oil was quite good for all the extraction conditions. Its triglyceride content was 74–78%, but it contained quite high amounts of impurities (i.e., diglycerides, monoglycerides and free fatty acids) (Table 1). Compared to a previous study by Kartika et al. (2018), the triglyceride content of the oil obtained in this research was lower (74–78 *vs* 95–98%). This was probably because the triglycerides had been hydrolysed into free fatty acids during the storage of the *Calophyllum* fruits. It was proven that the free fatty acid content (16–18 *vs* < 2%) and acid value (46–56 mg KOH·g^{-1} *vs* 7–19 mg KOH·g^{-1}) of the oil obtained in this research were very high. The other physiochemical properties of the oil were quite satisfactory (Table 1). Its iodine value (90–99 g iodine·100 g^{-1} *vs* 121–138 g iodine·100 g^{-1}) and its viscosity at 40 °C (25–35 mPa·s^{-1} *vs* 21–26 mPa·s^{-1}) were respectively lower and higher than those obtained from the previous study (Kartika et al. 2018). This was probably because the triglycerides had oxidised during the storage of the *Calophyllum* fruits. The triglycerides with a lower iodine value generally showed higher viscosity, due to their higher percentage of saturated fatty acids.

The quality of the resin extracted in this study was good for all the extraction conditions (Table 3). It had a very high acid value (111–156 mg KOH·g^{-1}) because the solvent not only extracted the free fatty acids, but also dissolved other acids such as calophyllic, benzoic and oxibenzoic acids, as previously reported (Kartika et al. 2018). The total phenolic content and the antioxidant activity of the extracted resin was also quite high, i.e., a 4.0–5.2% gallic acid equivalent (GAE) and a 9–14 mg ascorbic acid equivalent·g^{-1}, respectively. This was very beneficial for its application as an antioxidant. In addition, its property of being very viscous (viscosity > 2 500 mPa·s^{-1} at 25 °C) would support that application.

Based on the results of the Fourier-transform infrared spectroscopy (FT-IR) analysis (Table 3), all the resins obtained from this research had the functional groups of C-H aliphatic stretching vibration (2 853–2 923 cm^{-1}), symmetric and asymmetric bending of the methyl groups (1 376–1 443 cm^{-1}) and vinylidenes (886–887 cm^{-1}), C=C stretching frequency of the alkene and aromatic band (1 598 cm^{-1}), C-O-C stretching vibration attached with the aliphatic and aromatic compounds (1 133–1 159 cm^{-1}), C=O stretching frequency of the acid (1 706 cm^{-1}) and ester (1 736 cm^{-1}), and ring aromatic compound (698 cm^{-1}), including the resin obtained from the model validation of the optimal extraction conditions. Such functional groups may be found in the calophyllic, benzoic and oxibenzoic acids (Dweck and Meadows 2002), polyphenols and phenols (Kartika et al. 2018), calophyllolide, coumarins, inophyllums, calanolides and tamanolides (Ginigini et al. 2019).

The actual quality of the oil and resin obtained from the model validation of the optimum extraction conditions was quite good and relatively close to their predicted values for the most part (Table 4). This indicated that the optimisation of the time and stirring speed to maximise the *Calophyllum* oil-resin extraction and their separation using a binary solvent was successful.

CONCLUSION

The binary solvent effectively extracted the oil and resin from the *Calophyllum inophyllum* seeds, and separated them in a direct stage. The extraction conditions were optimised on the yields of the oil and resin, with the extraction time being the biggest influential factor. The optimum for the oil (65 db.%) and resin (16 db.%) yields was obtained at 5.2 h and 433 rpm. The oil and resin were predicted to be of good quality with quite a high triglyceride content, total phenolic content and antioxidant activity. The actual yields of the oil (65%) and resin (15%) and the qualities obtained were in good agreement with their predicted values, confirming that the model of the optimal extraction conditions was valid. A workable process on a pilot scale and optimal operating conditions for the oil-resin extraction and separation was, thus, successfully achieved.

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REFERENCES

Adewuyi A., Fasusi O.H., Odorinde R.A. (2014): Antibacterial activities of acetonides prepared from the seed oils of *Calophyllum inophyllum* and *Pterocarpus osun*. Journal of Acute Medicine, 4: 75–80.

Arumugam A., Ponnusami V. (2019): Biodiesel production from *Calophyllum inophyllum* oil a potential non-edible feedstock: An overview. Renewable Energy, 131: 459–471.

Boucher C. (2000): *Calophyllum* oil extracted at ambient temperature has UV protecting, antiradical, antioxidant,

https://doi.org/10.17221/67/2020-RAE

anti aging and therapeutic properties. France Patent FR9907772A. Dec 22, 2000.

Dai J., Mumper R.J. (2010): Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules, 15: 7313–7352.

Dweck A.C., Meadows T. (2002): Tamanu (*Calophyllum inophyllum*)-The African, Asian, Polynesian and Pacific Panacea. International Journal of Cosmetic Science, 24: 1–8.

Ginigini J., Lecellier G.J., Nicolas M., Nour M., Hnawia E., Lebouvier N., Herbette G., Lockhart P., Raharivelomanana P. (2019): Chemodiversity of *Calophyllum inophyllum* L. oil bioactive components related to their specific geographical distribution in the South Pacific region. PeerJ, 7: e6896.

Indartono Y.S., Heriawan H., Kartika I.A. (2019): Innovative and flexible single screw press for the oil extraction of *Calophyllum* seeds. Research in Agricultural Engineering, 65: 91–97.

Jahirul M.I., Brown R.J., Senadeera W., Ashwath N., Rasul M.G., Rahman M.M., Hossain F.M., Moghaddam L., Islam M.A., O'Hara I.M. (2015): Physio-chemical assessment of beauty leaf (*Calophyllum inophyllum*) as second-generation biodiesel feedstock. Energy Reports, 1: 204–215.

Jain M., Chandrakant U., Orsat V., Raghavan V. (2018): A review on assessment of biodiesel production methodologies from *Calophyllum inophyllum* seed oil. Industrial Crops and Products, 114: 28–44.

Kartika A.I., Yani M., Ariono D., Evon P., Rigal L. (2013): Biodiesel production from jatropha seeds: Solvent extraction and in situ transesterification in a single step. Fuel, 106: 111–117.

Kartika I.A., Bernia O.T.O., Sailah I., Prakoso T., Purwanto Y.A. (2019): A binary solvent for the simultaneous *Calophyllum* oil-resin extraction and purification. Research in Agricultural Engineering, 65: 63–69.

Kartika A.I., Evon P., Cerny M., Suparno O., Hermawan D., Ariono D., Rigal L. (2016): Simultaneous solvent extraction and transesterification of jatropha oil for biodiesel production, and potential application of the obtained cakes for binderless particleboard. Fuel, 181: 870–877.

Kartika A.I., Cerny M., Vandenbossche V., Rigal L., Sablayrolles C., Vialle C, Suparno O., Ariono D., Evon Ph. (2018): Direct *Calophyllum* oil extraction and resin separation with a binary solvent of n-hexane and methanol mixture. Fuel, 221: 159–164.

Kolb J.P., Menasria F., Billard C., Meyer M., Azebaze A.G., Nkengfack A.E. (2011): Use of xanthone derivatives as a medicament for cancer. US Patent US20110263694A1. Oct 27, 2011.

Lee C.G., Seng C.E., Liew K.Y. (2000): Solvent efficiency for oil extraction from spent bleaching clay. Journal of the American Oil Chemists' Society, 77: 1219–1222.

Léguillier T., Lecsö-Bornet M., Lémus C., Rousseau-Ralliard D., Lebouvier N., Hnawia E., Nour M., Aalbersberg W., Ghazi K., Raharivelomanana P., Rat P. (2015): The wound healing and antibacterial activity of five ethno medical *Calophyllum inophyllum* oils: An alternative therapeutic strategy to treat infected wounds. PLoS ONE, 10: e0138602.

Liu W., Liu Y., Chen Z., Chiou W., Tsai Y., Chen C. (2015): Calophyllolide content in *Calophyllum inophyllum* at different stages of maturity and its osteogenic activity. Molecules, 20: 12314–12327.

Montgomery D.C. (2001): Design and Analysis of Experiments. 5th Ed. New York, John Wiley & Sons, Inc.

Nariya P.B., Bhalodia N.R., Shukla V.J., Acharya R., Nariya M.B. (2013): *In vitro* evaluation of antioxidant activity of *Cordia dichotoma* (Forst f.) bark. AYU An International Quarterly Journal of Research in Ayurveda, 34: 124–128.

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