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Full Length Article

Direct *Calophyllum* oil extraction and resin separation with a binary solvent of *n*-hexane and methanol mixture

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This study investigated the use of a mixture of *n*-hexane and methanol as a binary solvent for the direct oil extraction and resin separation from *Calophyllum* seeds, in a single step. Optimal oil and resin yields and physicochemical properties were determined by identifying the best extraction conditions. The solvent mixture tested extracted oil and resin effectively from *Calophyllum* seeds, and separated resin from oil. Extraction conditions affected oil and resin yields and their physicochemical properties, with the *n*-hexane-to-methanol ratio being the most critical factor. Oil yield improved as *n*-hexane-to-methanol ratio increased from 0.5:1 to 2:1, and resin yield increased as methanol-to-*n*-hexane ratio increased from 0.5:1 to 2:1. Physicochemical properties of oil and resin, particularly for acid value and impurity content, improved as the *n*-hexane-to-methanol ratio decreased from 2:1 to 0.5:1. The best oil (51% with more than 95% triglycerides) and resin (18% with more than 5% polyphenols) yields were obtained with *n*-hexane-to-methanol ratios of 2:1 and 0.5:1, respectively, at a temperature of 50 °C, with an extraction time of 5 h. The best values for physicochemical property of oil were a density of 0.885 g/cm^3, a viscosity of 26.0 mPa.s, an acid value of 13 mg KOH/g, an iodine value of 127 g/100 g, an unsaponifiable content of 1.5%, a moisture content of 0.8% and an ash content of 0.04%.

1. Introduction

Calophyllum inophyllum is a versatile plant with multiple uses, including ship and furniture fabrication, as windbreaks, to reduce abrasion, to protect coastal demarcation, and as a source of plant oil and a second-generation biodiesel feedstock [1–10]. This plant is widely available in South-East Asia, Australia and India [11]. Today the land under cultivated *Calophyllum inophyllum* is increasing, because this crop may provide a new mode of agricultural development that does not compete with land uses for food production. *Calophyllum inophyllum* grows best in sandy soils, but it also grows well in rocky, clayey and calcareous soils.

The seed of *Calophyllum* is a good second-generation biodiesel feedstock, due to its higher oil content and productivity than other oilseeds such as soybean, sunflower, rapeseed, groundnut, coconut, jatropha, cotton, castor, or rubber [10–12]. The transesterification of *Calophyllum* oil results in a biodiesel with characteristics very similar to those of mineral diesel and with better lubrication capacity that can be stored and handled safely, due to its high flash point. Furthermore, the oil extracted from *Calophyllum inophyllum* seeds is rich in phospholipids and unsaponifiable compounds [12,13], and has antioxidant, anti-aging, UV-protective, antiradical, and therapeutic properties [14]. This oil also has biological and osteogenic activities, due to its high calophyllolide content [15]. However, *Calophyllum* seeds are lethal to humans and animals if ingested, because they contain a poisonous resin [16]. This resin is present in the oil at concentrations of about 10–30% [17]. This resin has high proanthocyanidin polymer content, with excellent antiviral activity [18], together with high contents of xanthone and its derivatives [19].

Calophyllum seeds have also been shown to contain many other chemical compounds, including inophyllum A-E, calophyllic acid, coumarins, phenols, polyphenols, triterpenoids, and flavonoids [16,19–23]. As a result, the oil produced from *Calophyllum* seeds is widely used as a traditional medicine and in cosmetics, and as a lamp oil.

Before its use as a raw material for industry and as a biodiesel feedstock, *Calophyllum* oil must be refined (i.e. degummed and neutralized), to eliminate free fatty acids and remove the gums and resin

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from the oil [11,24]. These processes unfortunately consume large amounts of water and chemicals, with large neutral oil losses (more than 30%). By contrast, *Calophyllum* oil has been successfully refined using a mixture of *n*-hexane and methanol (v/v, 3:1) to remove a large amount of resin from the oil, with very low losses of neutral oil (less than 2%) [25].

Calophyllum oil is widely extracted by mechanical pressing with a screw press [4–7]. This process results only small amounts of oil (less than 35% of dry seed mass), and the oil obtained is dark green, highly viscous and very acidic because it also contains the resin extracted from *Calophyllum* seeds. It is very difficult to separate this resin from the oil by filtration. The extraction of *Calophyllum* oil with *n*-hexane can improve its yield (more than 50%) and quality [5–7]. This method for oil extraction from *Calophyllum* seeds is very effective, simple and rapid. Due to its non-polar nature, *n*-hexane is an effective solvent for oil extraction [26]. However, polar solvents, such as methanol, are also effective for the extraction of both oil [27] and resin [16,25].

This study therefore decided to perform a comprehensive investigation of oil extraction and resin separation processes for *Calophyllum* seed using a binary solvent (i.e. a mixture of polar and non-polar solvents), with the aim of obtaining higher yields of purer oil and resin. The study thus examined the oil extraction and resin separation from *Calophyllum* seeds in a single step, in a mixture of *n*-hexane and methanol. The effects of *n*-hexane-to-methanol ratio, extraction time and temperature were analyzed to identify the most favorable extraction conditions giving the highest yields and the best physicochemical properties for oil and resin.

2. Materials and methods

2.1. Materials

Calophyllum fruits were provided by the Forest Research and Development Center (KHDTK Carita, Indonesia). *n*-Hexane and methanol with a purity of more than 98% were supplied by BRATACO Chemical Ltd (Indonesia). All the chemicals and analysis solvents used were of pure analytical grade and were obtained from Merck and Sigma-Aldrich (France and Indonesia).

2.2. Experimental procedure for oil extraction and resin separation

The shell of the *Calophyllum* was manually removed from the seed. The seed had a moisture content of 37.1 ± 0.2% (French standard NF V 03-903). For oil extraction, the seeds were dried in a ventilated oven at 60–70 °C for 48–72 h to reduce their moisture content to 5–6%.

Dried seeds (100 g) in 100 mL of methanol were ground in an electric blender for 5 min. 200–400 mL of *n*-hexane and 100–300 mL of extra methanol, corresponding to an *n*-hexane-to-total methanol ratio (v/v) of 2:4–4:2 (or 0.5:1–2:1) were then added to this seed-methanol mixture. In all experiments, the total volume of *n*-hexane and methanol in the system was fixed to be 600 mL. The seed-to-total solvent ratio (w/v, expressed in g/mL) was thus 1:6. Extraction was performed in a 2000 mL three-necked flask equipped with a hot plate magnetic stirrer and a reflux system. The effect of extraction temperature (40–50 °C) and extraction time (5–7 h) was assessed in this study. The stirring speed was fixed at 800 rpm.

The mixture was cooled to room temperature when the extraction was complete. The filtrate was then separated from the cake by filtration with a vacuum filter. The filtrate was left overnight, to allow the filtrate to separate into two layers. The upper layer was yellow and consisted of a mixture of *n*-hexane and oil. The lower layer was the methanol and resin fraction, and was dark green in color. The *n*-hexane and methanol were recovered from the corresponding two layers of filtrate containing the oil and the resin, respectively, by evaporation in a rotary evaporator. The oil and resin were then dried at 105 °C for 1 h.

The dried oil and resin were weighed, and their yields were calculated as follows:

$$\text{Crude oil yield} (\%\,\text{dry matter basis}) = \frac{\text{Mass of crude oil after drying}(g)}{\text{Mass of dried seeds}(g)} \times 100$$

$$\text{Crude resin yield} (\%\,\text{dry matter basis}) = \frac{\text{Mass of crude resin after drying}(g)}{\text{Mass of dried seeds}(g)} \times 100$$

All experiments were performed twice, and the mean and standard deviation were calculated for oil and resin yields. The influence of the extraction conditions (i.e. *n*-hexane-to-methanol ratio, extraction time and temperature) on oil and resin yields and their physicochemical properties was investigated in an experiment with a randomized factorial design, by analysis of variance (ANOVA, *F*-test, with $p = 0.05$ as the significance threshold).

2.3. Analytical methods

Moisture, protein and ash contents were determined in accordance with French standards NF V 03-903, NF V 18-100 and NF V 03-322, respectively, and crude fiber content was determined in accordance with Indonesian standard SNI 01-2891-1992. Resin content was determined using Soxhlet method with pure methanol as solvent (French standard NF V 03-908). Oil content and fatty acid composition were determined in accordance with French standard NF V 03-908 and by gas chromatography (GC), respectively, as previously described [28].

The quality of the oil obtained in the extraction processes was assessed by analyzing acid value (NF T 60-204 standard), iodine value (AOCS-Cd 1d-92 standard), peroxide value (SNI 3748-2009 standard), unsaponifiable content (AOCS Da 11-42 standard), moisture and ash contents (SNI 01-2891-1992 standard), density (AOAC 920.212 standard) and dynamic viscosity. The dynamic viscosity, expressed in mPa.s, at 40 °C was measured with an Anton Paar (Austria) MCR 302 modular compact rheometer equipped with CP50-2 cone-plate geometry (49.981 mm in diameter, with an angle of 1.998°). For the fatty acid content of the extracted oil and its glyceride fraction were analyzed by gas chromatography (GC), as previously described [28].

The resin quality was assessed by determining its acid value (NF T 60-204 standard) and its total polyphenol content. Adapted Folin-Ciocalteu method [29] was used with gallic acid as a standard for the determination of total polyphenol content. The gallic acid standard or the resin sample (1 mL) was mixed with Folin-Ciocalteu reagent (0.5 mL) and 20% sodium carbonate solution (1 mL), and the volume was made up to 10 mL with water. The mixture was incubated for 10 min at 70 °C, and absorbance at 700 nm was then measured at 25 °C. Each sample was analyzed in duplicate. The results are expressed as gallic acid equivalents (%, wt) and correspond to the total polyphenol content of the resin.

3. Results and discussion

3.1. Physicochemical properties of Calophyllum seeds

The *Calophyllum* seeds had an oil content of 54.0 ± 2.6% (dry matter basis), consistent with the values (40–73%) obtained by other researchers [10–12]. The oil obtained by extraction with *n*-hexane alone consisted of palmitic (14.7 ± 0.2%), palmitoleic (0.2 ± 0.0%), stearic (15.7 ± 0.3%), oleic (37.6 ± 0.2%), linoleic (30.4 ± 0.3%), linolenic (0.2 ± 0.0%), arachidic (0.8 ± 0.0%), eicosenoic (0.1 ± 0.0%) and behenic (0.2 ± 0.0%) acids. Unsaturated fatty acids, such as oleic acid and linoleic acid, predominated, as in other published studies of *Calophyllum* oil [8,10–12,30]. However, the oil also contained saturated fatty acids, in the form of palmitic acid and stearic acid. The *Calophyllum* seeds had also a resin content of 28.8 ± 0.8% (dry matter basis), consistent with the values 10–30% obtained by other
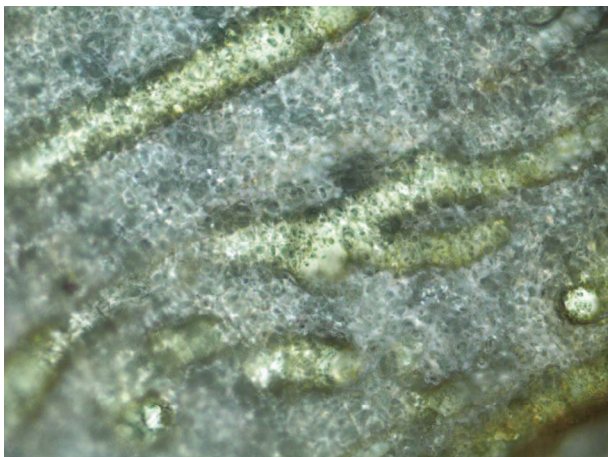
Fig. 1. Light micrographs of oil and resin within the cells of *Calophyllum* seeds (10 × magnification).

researcher [17].

Micrographs of *Calophyllum* seeds (Fig. 1) showed that the oil and resin were present within cells. Extraction in solvent should increase the fluidity of the oil and resin, facilitating their release throughout the fibrous matrix and their movement towards the surface in the presence of the solvent mixture. In addition, the solvent system can rupture the cell membranes, simultaneously dissolving the substances initially present within the intact cells [21]. It was difficult to extract *Calophyllum* oil with a screw press [4–7], due to its high fluidity and viscosity. Furthermore, the efficiency of oil extraction depends heavily on the friction between the material and the surface of the inside of the barrel and the screw [31]. Fibers, such as rice husk, are often added to the seeds to increase friction, and therefore, improve oil yield [11].

3.2. Oil extraction and resin separation from Calophyllum seeds

The mean moisture content of the *Calophyllum* seeds used in this study was $5.60 \pm 0.04\%$. Moisture content must be kept low, because the presence of water may lead to decreasing the dissolution of oil in the solvent due to the higher polarity of water than of triglycerides, and decreasing the mass transfer of triglycerides [32].

Oil is extracted from oilseeds essentially for the recovery of triglycerides, and a single non-polar solvent such as *n*-hexane is an excellent solvent for recovering triglycerides from various oilseeds because most triglycerides are non-polar molecules with long hydrocarbon chains. In addition, the oil was highly miscible in *n*-hexane. However, with *Calophyllum* seeds, the aim is to recover both oil and resin. Solvent and solute solubility properties determine the performance of the solvent

and are principally dependent on polarity, internal energy, the molar volume of solvent and its relationship to the solutes [26]. The resin of *Calophyllum* seeds is soluble in alcohol [16] such as methanol and ethanol [25], and is thus a polar component. Given the contrasting solubility properties of triglycerides and resin, it would be very difficult to recover them simultaneously with a single solvent. The use of a mixture of polar and non-polar solvents may be an option to overcome this problem. Binary solvent systems such as that used here can effectively simultaneously recover triglycerides and resin from *Calophyllum* seeds. Furthermore, as these two solvents are relatively immiscible, it was possible to separate the resin from the triglycerides directly after extraction, by simple decantation.

The simultaneous oil extraction and resin separation from *Calophyllum* seeds in a binary solvent resulted in high yields and good physicochemical properties for both oil and resin. The mixture of these two solvents made it possible to extract oil in *n*-hexane and resin in methanol from the seeds and to separate them in a single step. This synergistic effect between *n*-hexane and methanol on oil and resin extraction performance may be explained by their values of Hildebrand solubility parameter (δ, i.e. a numerical value that indicates the relative solvency behavior of a specific solvent [33]). δ values for *n*-hexane and oil (vegetable oil such as linseed oil, olive oil, castor oil, pine oil) are relatively similar, i.e. respectively $14.9~\text{MPa}^{1/2}$ and $14–19~\text{MPa}^{1/2}$. On the other hand, δ values for methanol and resin are also relatively close, i.e. $29.7~\text{MPa}^{1/2}$ and $21–29~\text{MPa}^{1/2}$ (phenols in general), respectively. Based on the concept of "like dissolves like", substances with the same solubility behaviors will dissolve in each other and vice versa [33]. Furthermore, resin and minor components (i.e. gums, phosphatides, sterols, phenols, polyphenols, ketones, etc.) were not efficiently extracted in *n*-hexane because these compounds were not very soluble in non-polar solvents [34]. In this study, the ratios of *n*-hexane-to-methanol added to the seeds were diverse, ranging from 2:1 to 4:1 (volume/weight, expressed in mL/g) for each experiment. Increasing the proportion of *n*-hexane in the solvent mixture improved triglycerides extraction. Conversely, the resin yield increased with the higher proportion of methanol in the solvent mixture. This may occur because the δ value of *n*-hexane and methanol mixture increases from $19.8~\text{MPa}^{1/2}$ to $24.8~\text{MPa}^{1/2}$ as *n*-hexane-to-methanol ratio decreases from 2:1 to 0.5:1.

This study used a randomized factorial experimental design to investigate in detail the influence of *n*-hexane-to-methanol ratio, extraction time and temperature on oil and resin yields. The ratio of *Calophyllum* seeds-to-total solvent (i.e. *n*-hexane plus methanol) was set to 1:6 (weight/volume, expressed in g/mL). However, both the seeds-to-*n*-hexane ratio and the seeds-to-methanol ratio were varied (from 1:2 to 1:4), implying that the *n*-hexane-to-methanol ratio also varied (from 0.5:1 to 2:1). Oil yield was influenced by the three extraction condition variables investigated: *n*-hexane-to-methanol ratio, extraction time and temperature (Table 1). However, an analysis of variance (*F*-test with

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

n-Hexane-to-methanol ratio (v/v)	Temperature (°C)	Time (h)	Crude oil yield (wt%)	Crude resin yield (wt%)	Oil density (g/cm³)	Oil viscosity at 40 °C (mPa.s)	Iodine value of oil (g iodine/100 g)	Acid value of oil (mg KOH/g)	Acid value of resin (mg KOH/g)
0.5:1	40	5	38.0 ± 1.0	15.7 ± 1.4	0.902 ± 0.002	24.6 ± 0.2	121.0 ± 3.2	10.1 ± 0.1	181.8 ± 0.8
0.5:1	40	7	36.3 ± 1.2	14.6 ± 0.3	0.880 ± 0.031	24.9 ± 3.4	136.4 ± 0.2	8.8 ± 0.1	175.1 ± 0.2
0.5:1	50	5	38.4 ± 1.2	17.7 ± 0.4	0.891 ± 0.045	23.4 ± 4.8	137.6 ± 4.8	7.8 ± 0.2	173.4 ± 1.2
0.5:1	50	7	34.1 ± 1.6	16.6 ± 1.0	0.881 ± 0.033	23.5 ± 1.0	127.1 ± 0.3	9.4 ± 0.7	167.7 ± 0.8
1:1	40	5	46.0 ± 0.1	12.4 ± 0.5	0.923 ± 0.004	20.2 ± 3.4	129.5 ± 0.8	10.5 ± 0.4	184.4 ± 5.1
1:1	40	7	43.0 ± 0.5	13.5 ± 0.2	0.910 ± 0.017	23.0 ± 1.1	123.8 ± 1.0	10.4 ± 0.1	175.0 ± 4.0
1:1	50	5	43.9 ± 0.8	14.0 ± 0.7	0.914 ± 0.018	21.7 ± 0.5	133.9 ± 0.3	9.5 ± 0.3	173.8 ± 0.3
1:1	50	7	45.1 ± 0.6	13.5 ± 0.5	0.860 ± 0.002	23.7 ± 1.3	138.1 ± 1.4	12.6 ± 0.1	159.6 ± 1.5
2:1	40	5	49.9 ± 0.1	10.0 ± 0.6	0.927 ± 0.000	25.6 ± 1.7	131.5 ± 0.7	16.3 ± 0.1	173.4 ± 1.2
2:1	40	7	49.7 ± 1.2	10.7 ± 0.7	0.894 ± 0.045	25.6 ± 0.8	132.2 ± 0.2	15.8 ± 0.8	194.7 ± 2.4
2:1	50	5	50.6 ± 0.6	10.2 ± 0.1	0.885 ± 0.030	26.0 ± 2.0	126.8 ± 3.7	13.0 ± 0.4	179.4 ± 2.4
2:1	50	7	49.0 ± 0.3	10.3 ± 0.0	0.884 ± 0.031	24.1 ± 4.2	125.1 ± 1.3	18.5 ± 0.2	191.2 ± 2.4

Table 2
F value for an analysis of variance (ANOVA) for the extraction yield and physicochemical properties of crude *Calophyllum* oil and resin.

Source of variation	F value for oil yield	F value for resin yield	F value for oil density	F value for oil viscosity	F value for iodine value of oil	F value for acid value of oil	F value for acid value of resin	F value for TAG content	F value for impurity content (DAG, MAG, and FA)	F value at $p = 0.05$
n-Hexane-to-methanol ratio (A)	424.97*	161.32*	0.51	3.30	2.75	739.05*	50.50*	46.45*	46.45*	3.89
Temperature (B)	0.77	10.95*	3.46	0.05	7.63*	1.73	9.46*	0.01	0.01	4.75
Extraction time (C)	19.14*	0.22	4.05	0.26	0.22	86.41*	0.00	5.76*	5.76*	4.75
Interaction AB	0.63	4.79*	0.50	0.50	26.98*	7.80*	21.49*	0.06	0.06	3.89
Interaction AC	3.65	3.37	0.27	0.90	1.48	19.55*	65.24*	2.10	2.10	3.89
Interaction BC	0.01	1.92	0.00	0.24	12.83*	172.86*	27.59*	0.33	0.33	4.75
Interaction ABC	8.09*	0.73	1.02	0.06	37.62*	10.55*	3.93*	3.10	3.10	3.89

* Significant.

Table 3
Effect of operating conditions on the glyceride and fatty acid contents of *Calophyllum* oil.

n-Hexane-to-methanol ratio (v/v)	Temperature (°C)	Time (h)	TAG (wt%)	Impurities (DAG, MAG, and FA) (wt%)	Palmitic acid (C16:0) (wt%)	Stearic acid (C18:0) (wt%)	Oleic acid (C18:1n-9) (wt%)	Linoleic acid (C18:2) (wt%)	Other fatty acids (i.e. palmitoleic, arachidic, linolenic, eicosenoic, and behenic acids) (wt%)
0.5:1	40	5	97.7 ± 0.1	2.3 ± 0.1	14.8 ± 0.0	15.8 ± 0.0	38.2 ± 0.1	29.6 ± 0.1	1.6 ± 0.0
0.5:1	40	7	96.4 ± 0.5	3.6 ± 0.5	14.7 ± 0.0	15.6 ± 0.2	37.5 ± 0.9	30.6 ± 0.7	1.6 ± 0.0
0.5:1	50	5	97.1 ± 0.1	2.9 ± 0.1	14.6 ± 0.1	15.9 ± 0.2	37.2 ± 0.3	30.8 ± 0.6	1.6 ± 0.0
0.5:1	50	7	96.9 ± 0.0	3.1 ± 0.0	14.6 ± 0.3	15.7 ± 0.0	38.5 ± 0.5	29.6 ± 0.2	1.5 ± 0.0
1:1	40	5	96.1 ± 0.0	3.9 ± 0.0	14.6 ± 0.2	15.9 ± 0.0	37.1 ± 0.3	30.9 ± 0.6	1.6 ± 0.0
1:1	40	7	96.5 ± 1.0	3.5 ± 1.0	14.6 ± 0.1	16.1 ± 0.3	37.9 ± 0.4	29.9 ± 0.6	1.6 ± 0.0
1:1	50	5	96.6 ± 0.4	3.4 ± 0.4	14.9 ± 0.0	15.8 ± 0.0	37.9 ± 0.0	29.8 ± 0.0	1.6 ± 0.0
1:1	50	7	96.2 ± 0.2	3.8 ± 0.2	14.7 ± 0.0	15.7 ± 0.0	36.5 ± 0.2	31.5 ± 0.3	1.6 ± 0.0
2:1	40	5	95.5 ± 0.2	4.5 ± 0.2	14.5 ± 0.6	16.0 ± 0.4	37.5 ± 0.7	30.5 ± 0.5	1.6 ± 0.0
2:1	40	7	95.0 ± 0.0	5.0 ± 0.0	14.9 ± 0.0	15.5 ± 0.0	37.8 ± 0.4	30.1 ± 0.3	1.6 ± 0.0
2:1	50	5	95.3 ± 0.3	4.7 ± 0.3	14.7 ± 0.3	15.8 ± 0.2	37.5 ± 0.4	30.3 ± 0.3	1.6 ± 0.1
2:1	50	7	95.2 ± 0.1	4.8 ± 0.1	14.7 ± 0.1	15.4 ± 0.0	37.5 ± 0.4	30.9 ± 0.2	1.6 ± 0.0

TAG, triglycerides; DAG, diglycerides; MAG, monoglycerides; FA, free fatty acids.

$p = 0.05$ as the significance threshold) showed that *n*-hexane-to-methanol ratio and extraction time influenced oil yield more strongly than temperature (Table 2). For each of the operating conditions tested, oil yield increased as the *n*-hexane-to-methanol ratio increased from 0.5:1 to 2:1. However, the triglycerides content of the oil extracted decreased slightly with this increase in the *n*-hexane-to-methanol ratio (Table 3). Furthermore, analysis of variance showed that the *n*-hexane-to-methanol ratio had a significant effect on the triglycerides content of the oil, which was also influenced by extraction time (Table 2).

Extraction time had an effect on oil yield, with higher oil yields for longer extraction times. However, increasing extraction time from 5 h to 7 h decreased oil yield, indicating that the system had already reached optimum at 5 h. The trend of this declining oil yield after 5 h may be linked to the possible formation of volatile degradation products which may lead to a reduction in the amount of oil extracted, as reported by Ramluckan et al. [35]. Increasing extraction time from 5 h to 7 h also slightly decreased the triglycerides fraction of the oil, indicating that conditions were optimal for an extraction time of 5 h. Longer extraction times probably resulted in triglycerides degradation, through hydrolysis and/or oxidation, into other glycerides (i.e. diglycerides, and monoglycerides) or even free fatty acids, as reported by Lee et al. [34]. Support for this hypothesis was provided by the increase in acid value and impurity content of the oil when extraction time was increased from 5 h to 7 h, particularly at an extraction temperature of 50 °C (Table 1). Oil yield and the triglycerides content of the oil remained constant with increases in temperature from 40 to 50 °C, indicating that optimal conditions were attained at 40 °C. Thus, oil can be efficiently extracted in *n*-hexane over relatively short times and at low temperatures, which should help to limit the costs associated with this process.

The best oil yield (i.e. 51% of dry seed mass, with a triglycerides content of more than 95%) was obtained with an *n*-hexane-to-methanol ratio of 2:1, a temperature of 50 °C and an extraction time of 5 h. In these conditions, the extracted resin yield was 10% of dry seed mass. For comparison, the best oil yield obtained in previous studies (51–54% of dry seed mass) [5,6] was similar, and was obtained under the following extraction conditions: extraction time of 16–48 h, seed-to-*n*-hexane ratio of 1:3, in the absence of methanol (*n*-hexane-to-methanol ratio of 3:0). In this study, the best oil yield was obtained with a much shorter extraction time (only 5 h, versus 16–48 h) but with a higher seed-to-*n*-hexane ratio (1:4 versus 1:3) and a higher seed-to-methanol ratio (1:2 versus 1:0), corresponding to the use of twice as much solvent. However, this surplus solvent could easily be recovered and reused. The oil yield obtained here was much higher than that obtained with a screw press [4], at 51% versus 33%. Thus, solvent-mediated oil extraction from *Calophyllum* seeds was much more efficient than oil extraction with a mechanical press, consistent with the findings of previous studies [5,6].

The *n*-hexane-to-methanol ratio and temperature also had significant effects on resin yield (Table 2). Higher resin yields were obtained in all the operating conditions tested, when the *n*-hexane-to-methanol ratio was decreased from 2:1 to 0.5:1 (Table 1). In addition, a slight increase in resin yield was also observed when temperature was increased from 40 to 50 °C, particular at the higher methanol-to-*n*-hexane ratio (i.e. 2:1). Higher solute solubility, through increases in solvability and mass transfer rate, and decreases in the viscosity and surface tension of the solvent, can be achieved at higher temperatures [21]. Resin yield remained fairly constant when extraction time was increased from 5 h to 7 h, indicated that the optimal extraction time was 5 h. The best resin yield (18%) was obtained with an *n*-hexane-to-

methanol ratio of 0.5:1, a temperature of 50 °C and an extraction time of 5 h. In these extraction conditions, the extracted oil yield was only 38%.

To comprehensively examine the effect of extraction conditions on performance of oil and resin extraction from *Calophyllum* seeds, it is necessary to investigate the liquid-liquid phase equilibrium (LLE) for the system of oil, resin, *n*-hexane and methanol using UNIFAC or ASOG method in next study. This is important for economic evaluation, and to design and model the oil and resin production from *Calophyllum* seeds.

For all extraction conditions tested, the extracted oil was of good quality, because its triglycerides fraction content remained high, at over 95% (Table 3). The oil thus contained few impurities (including diglycerides, monoglycerides, and free fatty acids), accounting for less than 5% of its content. The density (0.860–0.927 g/cm^3), viscosity (20–26 mPa.s), iodine value (121–138 g iodine/100 g), and acid value (less than 19 mg KOH/g) obtained were also satisfactory (Table 1). Furthermore, the extracted oil was rich in oleic (C18:1n-9) and linoleic (C18:2) acids, which accounted for 36.5–38.5% and 29.6–31.5% of its content, respectively (Table 3). It also had low moisture (less than 1%), ash (less than 0.05%) and unsaponifiable (less than 1.6%) contents, and it had a peroxide value of zero (Table 4). A comparison of this oil with that obtained by mechanical pressing [12] showed it had a lower unsaponifiable content (less than 1.6% versus 2.0%), and it was more stable against oxidation (peroxide value of 0 µg/g versus 1.45 µg/g). These characteristics should facilitate subsequent refining.

An analysis of variance for physicochemical properties of oil revealed a significant effect of extraction conditions, particularly for the acid and iodine values, and for triglycerides and impurity contents (Table 2). An increase in both the acid value (from 7.8 to 18.5 mg KOH/g) and impurity content (from 2.3 to 5.0%) was observed when *n*-hexane-to-methanol ratio was increased from 0.5:1 to 2:1 and extraction time was increased from 5 h to 7 h (Tables 1 and 3). Conversely, the triglycerides content of the extracted oil decreased with these increases

in *n*-hexane-to-methanol ratio and extraction time, from 97.7 to 95.0% (Table 3). Extraction temperature had no significant effect on acid value, or on the triglycerides or impurity content of the oil, but significantly affected iodine value (Table 2). Indeed, higher extraction temperatures were associated with a higher iodine content of the oil, particularly for *n*-hexane-to-methanol ratios of 0.5:1 and 1:1. This was probably due to an increase in the amount of unsaturated fatty acids, especially linoleic acid, with the increase of temperature (Table 3). Furthermore, the extraction conditions had no effect on either the density or the viscosity of the extracted oil. Indeed, both these variables remained fairly constant (0.860–0.927 g/cm^3 and 20–26 mPa.s) with increasing *n*-hexane-to-methanol ratio, temperature and extraction time (Table 1).

The oil obtained here was of better quality than that obtained in a previous study [7]: it had a lower acid value (less than 19 mg KOH/g versus 24 mg KOH/g), lower density (less than 0.93 g/cm^3 versus 0.94 g/cm^3) and much lower viscosity at 40 °C ($\leq$26 mPa.s versus 42 mPa.s). It was also of better quality than the oil produced by mechanical pressing [24,36], with an acid value less than 19 mg KOH/g versus 35–54 mg KOH/g, a density less than 0.93 g/cm^3 versus 0.93–0.95 g/cm^3, a viscosity $\leq$26 mPa.s versus 51 mPa.s, an ash content less than 0.05% versus 0.26–0.31%, and zero peroxide value versus 36–38 µg/g. Furthermore, its quality was relatively similar to the oil refined by degumming and neutralization, especially for density and ash content, and for peroxide value (0 µg/g versus 7–22 µg/g) and viscosity ($\leq$26 mPa.s versus 47 mPa.s) it was better [24]. The oil obtained by binary solvent extraction thus contained fewer impurities, including resin and free fatty acids in particular. The application of *Calophyllum* oil produced in this study as biodiesel feedstock is thus more favorable. This is supported by the suitability of *Calophyllum* oil characteristics as a biodiesel feedstock, i.e. high calorific value (9392 cal/g), low cloud point (13 °C), high flash point (234 °C), low water and sediment content ($<$ 0.05%), low phosphor content (0 mg/kg), low sulfated ash content ($<$ 0.014%) and low sulfur content ($<$ 0.03%) [24].

For all the extraction conditions tested, the extracted resin was of good quality. The resin extracted from *Calophyllum* seeds is comparable to myrrh [16]. Indeed, its acid value was systematically very high, at 160 mg KOH/g of resin or more (Table 1). An analysis of variance showed that extraction temperature and *n*-hexane-to-methanol ratio had a significant effect on resin acid value (Table 2). By contrast, extraction time had absolutely no effect on this parameter. Increases in the *n*-hexane-to-methanol ratio tended to increase the acid value of the resin, but this value decreased with increasing extraction temperature. Higher acid values at high *n*-hexane-to-methanol ratio were also observed for the extracted oil. The solvent may extract more free fatty acids, such as palmitic, stearic, oleic and linoleic acids, as a large volume was added. It may also dissolve other substances, such as calophyllic, benzoic and oxibenzoic acids [16]. Moreover, the extracted resin had high polyphenols content (i.e. 4.3–5.5%) (Table 4). This property would be advantageous for the use of the resin as an antioxidant.

The cakes (i.e. the solid raffinates) obtained were rich in carbohydrates (40 ± 5% of dry matter), fiber (25 ± 4% of dry matter), and proteins (14 ± 1% of dry matter). The chemical composition of the cake might hinder its direct exploitation, but it could be transformed into agromaterials, through the production, by hot pressing, of binderless boards for use in furniture, in particular [37–40].

4. Conclusion

A mixture of *n*-hexane and methanol effectively extracted oil and resin from *Calophyllum inophyllum* seeds in a single step, with simultaneous separation of the two extracted fractions. A decrease in *n*-hexane-to-methanol ratio increased resin yield, whereas higher *n*-hexane to methanol ratios were required to increase oil yield. The best oil and

Table 4
Physicochemical properties of the *Calophyllum* oil and resin obtained under the optimal extraction conditions (50 °C and 5 h).

Parameter	Unit	*n*-Hexane-to-methanol ratio		
		0.5:1	1:1	2:1
OIL				
Density	g/cm^3	0.891	0.914	0.885
Viscosity at 40 °C	mPa.s	23.4	21.7	26.0
Acid value	mg KOH/g	7.8	9.5	13.0
Iodine value	g iodine/100 g	137.6	133.9	126.8
Peroxide value	meq/kg	0	0	0
Unsaponifiable content	%, wt	0.75	1.19	1.52
Water content	%, wt	0.32	0.87	0.77
Ash content	%, wt	0.04	0.04	0.04
Glyceride composition:	%, wt			
Triglycerides (TAG)		97.1	96.6	95.3
Diglycerides (DAG)		2.1	1.9	2.6
Monoglycerides (MAG)		0.2	0.3	0.3
Free fatty acids (FA)		0.6	1.2	1.8
Fatty acid composition:	%, wt			
Palmitic acid (C16:0)		14.6	14.9	14.7
Palmitoleic acid (C16:1)		0.2	0.2	0.2
Stearic acid (C18:0)		15.9	15.8	15.8
Oleic acid (C18:1n-9)		37.2	37.9	37.5
Linoleic acid (C18:2)		30.8	29.8	30.3
Linolenic acid (C18:3)		0.2	0.2	0.2
Arachidic acid (C20:0)		0.8	0.8	0.8
Eicosenoic acid (C20:1)		0.1	0.1	0.1
Behenic acid (C22:0)		0.2	0.2	0.2
RESIN				
Acid value	mg KOH/g	173.4	173.8	179.4
Total polyphenol content (gallic acid equivalents)	%, wt	5.0	4.3	5.5

resin yields (i.e. 51% and 18% of dry seed weight, respectively) were obtained at 50 °C, with an extraction time of 5 h, with *n*-hexane-to-methanol ratios of 2:1 and 0.5:1, respectively. The oil had high trigly-cerides content (i.e. more than 95%), and the resin contained more than 5% polyphenols. The process described here thus appears to be feasible for the extraction and simultaneous separation of oil and resin from *Calophyllum inophyllum* seeds. It may also be considered efficient in terms of the extraction yield achieved.

Acknowledgments

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