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

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ABSTRACT

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:

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

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

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