Fatty acid composition and total lipid content of the seed oil of *Leucaena leucocephala* (Lam) de Wit

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Fatty acid composition and total lipid content of the seed oil of *Leucaena leucocephala* (Lam) de Wit

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Abstract. The common name for L. leucocephala are Lamtoro or Petai Cina (Indonesian), White lead tree (English) is one of the fastest growing leguminous trees in semi arid area. It is planted as a shade tree for cash crops, soil fertility improvement and a variety of other purposes. The seed oil of L. leucocephala (Lam) de Wit was investigated for its fatty acid composition and total lipid content. The main aims of the work were to determine the total lipid content and the fatty acid composition of L. leucocephala. The oil was obtained by n-hexane soxhlet extraction from the pulverized dried seeds of L. leucocephala, followed by the separation process of the oil by using silica gel column chromatography with different solvents then classified into different lipid classes (neutral lipids /NL, glycolipids/GL, and phospholipids/PL). The result showed that linoleic acid and ricinoleic acid dominated NL and GL whereas palmitic acid dominated in PL. NL, GL and PL fractions content unsaturated fatty acid 86.81%, 89.34% and 34.75% respectively. PL fraction was dominated by saturated fatty acid (65.25%).
Keywords: Lipid content, vegetable oil, fatty acid, *L leucocephala*

1. Introduction

Soybean and palm oil are the important sources of plant oil especially in China and Indonesia with the large production. In this case the question arises in the other rich natural resources. There is actually a lot of oil seeds that can be used as the core of the world vegetable oil production.

Among the 17 selected Asian countries, represent more than half of the world population, i.e. 3.9 billion people out of total of 7.35 billion as per July 2015. It is able to predict that the need of oils and fat are closely related to the population [1]. To cover the world's plant oil needs which increase every year is predicted reach more than 200 million of ton at 2020. In Indonesia palm oil is the most important sources of vegetable oil, although the people know that palm plantation destroyed tropical forest in many places. Not only destroy the soil but also destroy the whole environmental , for example spend water reserves, destroy living natural reserves, of course it will affect the climate, so it is important for Indonesia to find new alternative sources plants [2].

The fatty acid composition of the oil is very important for the oil. According to Bhatnagar *et al.* [3] the industrial value of plant oils depends on the constituent of their fatty acid composition. *L. leucocephala* is small evergreen medicinal tree belonging to the family Leguminosae. This species can grow fast in drought-prone and semi-arid areas [4]. Common name for *L. leucocephala* is Lamtoro or petai cina (Indonesia), Ipil-ipil in the Philippines, Yinhue in China and petai Belalang in Malaysia. The legume size is 10–17cm long containing 15–25 seeds each [5]. This plant is very important because almost all parts of the tree can be used for the human beings and animal. In Indonesia the leaves is used as animal feed, the unripe seeds and young leaves are used as human food, whereas the wood of the

stem is used as furniture materials. *L. leucocephala* leaves and seed contain lipid, carbohydrate and protein. Seeds contain 28.2 % protein, 45.80 % total carbohydrates and fat content 6.05 % [6]. The seed oil could be used in engineering as a novel biodevice useful in biomembrane modelling of drugs and xenobiotics [7], this oil also has the potential to be a bio inhibitor of corrosion of mild steel and copper. The product obtained from the plant is natural and eco-friendly [8].

The seed gum of *L. leucocephala* can be used as a binder in tablet formulation. Other than that this plant also have some benefits because some parts of this plant was reported to show medicinal activity as controller stomach diseases, to contraception and abortion [9]. Mimosine, an amino acid inside the seed of *L. leucocephala* showed anticancer activity. Another studies reported about the seed oil of *L. leucocephala*, the seeds content a dark green to brown oil containing 26-29% saturated acids and 71-73% unsaturated acids [10]. Except sterol this oil also contains glycolipid, hydrocarbon and carotenoids [11]. *L. leucocephala* seed oil has antibacterial activity against both Gram-positive and Gram-Negative bacteria but no activity against fungi [12]. The present study had been connected to determine the fatty acid composition and total lipid content of the seed oil of *L.leucocephala*for possible future commercial uses and to fulfil the human being need.

Lipids are distinguished into 2 groups hydrolysable and unhydrolysable lipids. Hydrolysable lipids (saponifiable lipids) content neutral lipid (simple lipids), this lipid showed as a hydrophobic molecule without any charged, ester from one molecule of glycerol plus three molecules of fatty acids. Another group is complex lipids, these lipids have more than 2 dominant components, glycolipids and phospholipids. Glycolipids is a lipid which contain fatty acid, sphingosine and oligosaccharide, whereas phospholipids have phosphoric acid bound to nitrogenous base.

2. Material and Methods

2.1. Plant Material

The dried seed pods used in this work were obtained from the plant *L. leucocephala* (Lam) De Wit growing around Salatiga area. The seeds were indirect sun dried for 2 days and pulverized by using grinder.

All chemicals and reagents used in this study were of analytical grade were purchased from E Merck (Germany). TLC plates (20x20 cm) were procured from Sigma- Aldrich, Germany.

2.2. Plant Extraction Methods

The seeds powdered (100 g) were extracted for 9 hours by soxhlet apparatus using n-hexane as a solvent. Crude extract was obtained after the solvent was evaporated. The oil was dried by nitrogen streaming and stored at -10°C for further analysis.

2.3. Analysis of selected physicochemical properties of seed oil

Physicochemical parameter of water content, acid value, peroxide value and saponification value were used according to the official method Standard National Indonesia (SNI 01-3555-1998)

2.4. Analysis of Fatty Acid Composition of the Oils

Analysis of fatty acid composition of the oils, was preceded with methyl esterification process to transform fatty acids content to their methylesthers. Then determination of fatty acid composition was analysed by GC-MS Shimadzu QP 2010 S. The injector, column and detector temperatures were set at 70°C, 280°C and 310°C respectively. The apparatus was completed with a flame ionization detector (FID) capillary column AGILENTTJ%W DB- (30 m x 0.25mm). The flow rate of helium as a carrier gas with split ratio 73. 0 was set as 3,0 mL/min. The fatty acids were identified with reference to the retention times of standard fatty acid methyl ester performed base on similarity indexes. The Mass Spectrum of every peak from the chromatogram was compared to data base.

2.5. Separation of Lipid Classes and Fatty Acid Distribution [13]

Separation process of the oil into different lipid classes (neutral lipids, glycolipids, and phospholipids) was done by using silica gel column chromatography with different solvents. Neutral lipids (NL), glycolipids (GL), and phospholipids (PL) were eluted using chloroform, acetone and methanol, respectively. Then the lipid fraction were screened by TLC, for components identification was used n-hexane: diethyl ether: acetic acid (60:40:1, v/v/v) as developing solvent for neutral lipids, whereas 25% chloroform: methanol: ammonia solution (65:25:4, v/v/v/) for glycolipids and phospholipids. For lipid detection, the plates were sprayed with different reagent such as 40% sulphuric acid for the marking of all lipid, for the marking of GL with a-naphthol/sulphuric acid and for the marking of PL with the molybdate-blue reagent [14].

3. Result and Discussion

3.1. Total Lipid content of Leucaena leucocephala Seed Oil

By adopting soxhlet method for 9 hours, the total oil of *Leucaena leucocephala* seed obtained approxmately3.82 ± 0.15%, this result was lower than (7.2 %) and Badal [6] (6.05%). The difference of oil contents depends on cultivar, soil condition, location, extraction method and type of solvent [15]. The colour of the crude oil was greenish brown, this result similar with the report of Sethi and Kulkarni [4] that the colour of *L. leucocephala* seed oil was green to brown. Total lipid of *L. leucocephala* seed oil show that saturated fatty acid content is amount 25.18% w/w and unsaturated fatty acid content is 74.82% w/w. These finding are similar with those value reported by Sethi and Kulkarni [4] that *L. leucocephala* seed oil content 26-29% saturated fatty acid and 71-73% unsaturated fatty acid.

The oil was separated into neutral lipid (NL), glycolipid (GL) and phospholipid (PL). The dominant fatty acids in NL and GL were linoleic acid (unsaturated fatty acid) and follow by ricinoleic acid (unsaturated fatty acid) whereas the most dominant fatty acid in PL was palmitic acid (saturated fatty acid) and follow by linoleic acid.

3.2. Fatty Acid Composition of L. leucocephala seed oil

Fractionation of *L. leucocephala* seed oil gave three fractions were Neutral Lipid (NL), Glycolipid (GL) and Phospholipid (PL). From the fractionation were obtained neutral lipid, glycolipid and phospholipid as shown at Fig. 1.

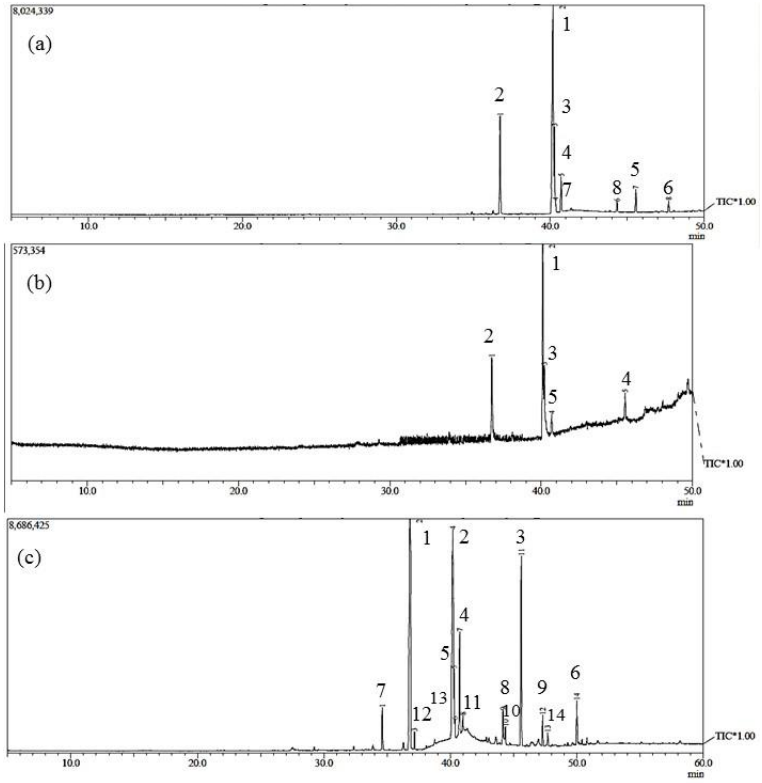

Figure 1. Chromatogram GC-MS of *Leucaena leucocephala* seed oil. (a) Neutral Lipid Fraction, (b) Glycolipid Fraction and (c) Phospholipid Fraction.

Every peaks of each spectrum were compared to the Wiley data base and the similarity of the spectrum was checked and used to decide what the compound is. The profile of Fatty Acid Composition of the three fractions have been identified (**Table 1**)

Fifteen fatty acids, one amide and two unidentified compounds were identified from *L. leucocephala* seed oil. In which linoleic acid was the predominant fatty acid 57,46% in NL and 50.43% in GL, while palmitic acid 30.68% in PL. Follow by ricinnoleic acid 16.1% in NL, 23% in GL, and linoleic acid 27.39% in PL. Elaidic acid (15.91%) was detected only in GL fraction, oleic acid (12.64%) in NL and palmitic acid only in PL (30.68%).

Eight fatty acids were found in NL, four among them were unsaturated fatty acid (86.81%). Five fatty acids were found in GL (89.34%), three among them were unsaturated fatty acids, whereas in PL were found fourteen fatty acids include 2 compounds were not able to determine, and only 4 unsaturated fatty acids inside (34.75 %). Further on NL and GL were dominated by unsaturated fatty acids whereas PL was dominated by saturated fatty acids.

Table 1. Fatty Acid composition of Leucaena seed Oil.

Fatty Acids	MW	Molecule	Content (%)		
			NL	GL	PL
Linoleic Acid C18:2	280	$C_{18}H_{32}O_2$	57.46	50.43	27.39
Ricinoleic acid C18:1	298	$C_{18}H_{34}O_3$	16.71	23.00	-
Oleic acid C18:1	282	$C_{18}H_{34}O_2$	12.64	-	-
Stearic acid C18:0	284	$C_{18}H_{36}O_2$	5.57	4.38	7.14
Dioctyl adipic acid C 22:0	370	$C_{22}H_{42}O_4$	3.60	6.28	15.16
Docosanoic acid C21:0	326	$C_{21}H_{42}O_2$	1.47	-	0.8
14-octadecenoic acid C18:1	282	$C_{18}H_{34}O_2$	1.32	-	-
Eicosanoic acid C 19:0	298	$C_{19}H_{38}O_2$	1.22	-	1.56
Elaidic acid C 18:1	282	$C_{18}H_{34}O_2$	-	15.91	-
Palmitic acid C16:0	256	$C_{16}H_{32}O_2$	-	-	30.68
Petroselinic acid C18:1	282	$C_{18}H_{34}O_2$	-	-	3.58
Ethyl Hexadienoic acid C8:2	174	$C_8H_{14}O_4$	-	-	2.93
n-octyl-palmitic acid C22:0	340	$C_{22}H_{44}O_2$	-	-	1.92
11-octadecenoic acid C18:1	282	$C_{18}H_{34}O_2$		-	0.85
Unidentified 1	-	-	-	-	3.1
Unidentified 2	-	-	-	-	2.65
Cocomonoetanolamide	217	$C_{13}H_{27}O_2N$	-	-	1.18
3(3,5-ditert- butyl -4-hydroxy phenyl) -propionic acid	278	$C_{17}H_{26}O_3$	-	-	1.06
Total			99.99	100	100
Unsaturated fatty acid			86.81	89.34	34.75
Saturated fatty acid			10.18	10.66	65.25

NL: Neutral Lipid GL: Glycolipid PL: Phospholipid

According to Badal [6] and Hakimi *et al.* [16] *L. leucocephala* seed oil show the similar result, the oil was dominated by linoleic acid (65.4% , 52.8%, 50.7% respectively). But there are different compounds in *L. leucocephala* seed oil from Badal [6], Hakimi *et al.* [16] and this research. Ricinoleic acid was found and dominant in this research (NL and GL) but this compound was not found in Badal [6] and Hakimi *et al.* [16] (from Malaysia). Vegetable oils have a characteristic fatty acid composition that is very important for product authentication. But on the fact, fatty acid composition not only depends on species but also on region, degree of ripeness, harvesting and processing conditions and climate. The difference of growth location of the plant material influenced to the oil content [15].

L. leucocephala seed oil have a thermal stability up to 255.47°C and some absorbance in the UV-B and UV-C ranges , this data inform that the seed oil can be used as material cosmetics or pharmaceutical preparation [17].

4. Conclusion

In conclusion the fatty acids of *L. leucocephala* seed oil was separated in Neutral Lipid, Glycolipid and Phospholipid. Linoleic acid and Ricinoleic acid dominated NL (57.46% & 16.71%) and GL (50.43% &

23%)whereas palmitic acid and linoleic acid dominated in PL (30.68% 27.39%). NL, GL and PL fractions content unsaturated fatty acid 86.81%, 89.34%and 34.75% respectively. PL fraction was dominated by saturated fatty acid (65.25%).

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