PHYSICO-CHEMICAL PROPERTIES OF THE OILS AND FAT FROM *CROTALARIA CLEOMIFOLIA* SEED

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ABSTRACT

The seeds of *C. cleomifolia* (locally known as *kacang hantu*) collected along Simpang Pulai - Berinchang road, Cameron Highlands, was defatted with hexane and the resulting oil was analyzed for their physico-chemical properties such as acid value (4.3), iodine value (85) and peroxide value (0.6). Using the gas chromatography (GC) method, linoleic acid (57.59%) and oleic acid (28.60%) were detected as major polyunsaturated fatty acids while palmitic acid (5.07%) and behenic acid (1.63%) were major saturated fatty acids found in the *kacang hantu* seed oil. Polyunsaturated triacylglycerol (TAG) in *kacang hantu* seed oil were PLL (18.04%) followed by POL + SLL (11.92%), OOL (7.04%) and PLLn (6.31%).

Keywords: *Leguminosae, Crotalaria cleomifolia, kacang hantu, fatty acid, PUFA.*

INTRODUCTION

Seed oil consists of fatty acids which have high commercial value in food industries, pharmaceuticals, lubricants, cosmetics and others. *Crotalaria cleomifolia* seed oil has the potential to be explored in order to get an alternative source of polyunsaturated fatty acid such as linoleic acid and linolenic acid which are *essential fatty acids* since they cannot be synthesized by our body and has to be taken instead from dietary supplements (Lawson 1985). *C. cleomifolia* (Leguminosae) locally known as *kacang hantu* or *gegiring* is a shrub that can grow until 4 m high, yellow flowered and branches. The plant was firstly introduced in Malaysia in order to improve the quality of farm land (Burkill 1967). In Malaysia there are 20 *Crotalaria* species of which ten is endemic to Peninsular Malaysia mostly found in Perak, Selangor, Pahang, Melaka,

Negeri Sembilan and Johor. In Asia, some of *Crotalaria* species are used as herbal tea and vegetables (Huxtable 1989). Previous studies have shown that the oil extracted from the seeds of *C. striata syn. C. mucronata* (Hosamani and Ramesh 2001) contained some interesting acids such as cylopropanoic fatty acids; ricinoleic, malvalic, sterculic, and normal fatty acids; myristic, palmitic, oleic, linoleic and linolenic.

MATERIALS AND METHOD

Plant material: Seeds of *C. cleomifolia* (voucher specimen UKMB 29771 deposited in the Herbarium, UKM Bangi) were collected from Simpang Pulai-Berinchang road, Cameron Highlands, Pahang. *Extraction*: *C. cleomifolia* seeds (1340 g) were extracted using hexane at room temperature for 2 days (2 times). The extract was filtered and the solvent evaporated to give the crude oil which was stored in the freezer prior to analysis. *Physico-chemical analysis*: (a) Acid value (AV): Acid value of seed oil was determined based on PORIM 1995 method. (b) Iodine value (IV): Iodine value was determined based on PORIM 1995 method. (c) Peroxide value: Peroxide value was determined based on PORIM 1995 method. (d) Fatty acid composition: Fatty acid composition was determined by gas chromatography (GC) and GCxGC-MS (ToF). Before injecting sample into GCxGC-MS(ToF), the sample should undergo GC in order to get the whole chromatogram profiles. GC: About 0.1ml of the oil was converted to fatty acid methyl esters (FAME) by using NaOMe (1M) in 1ml hexane. 1µl of FAME layer was injected into Gas Chromatography Shimadzu 17A-FID equipped with capillary column BPX-70 (30m x 250µm x 0.25µm), using isothermal temperature at 120ºC in 0 min, 245ºC in 57 minutes, detector temperature at 260ºC and flow rate at 0.3mL/min. Nitrogen gas was used as a carrier gas. Then, using the same sample about 0.5 µL of FAME layer was injected into GCxGC-MS(ToF). In this study, measurements and peak identifications were preliminarily made with 1D-GC, and followed by a LECO Pergasus GCxGC-MS(ToF) system. The GCxGC-MS(ToF) system consists of an Agilent 6890 gas chromatograph equipped with a LECO dual jet thermal modulator between the primary and secondary columns and a LECO Pegasus IV Time-of-Flight Mass Spectrometer (TOFMS) as a detector. The primary column is 30.0m x 250µm ID x 0.10µm df RTx-5MS (non-polar column), maximum temperature 360ºC and the secondary column is 1.0m x 100µm ID x 0.10µm df RTx-17 (polar column), maximum temperature 300ºC and housed in the GC oven. The carrier gas was helium at constant rate of 1.0mL/min. for the entire run. Transfer line temperature to MS(ToF) was 250ºC. (e) TAG composition: Triacylglycerol composition was determined by High Performance Liquid Chromatography (HPLC). Separation method was based on equivalent carbon number (ECN) in TAG sample by using reverse phase liquid chromatography equipped with a refractive index detector.

TAG composition was determined by comparing the retention time profile of standard TAG (Ooi & Salimon 2006) and reported as percentage of peak area (PORIM 1995). About 0.5g of sample was dissolved in acetone:acetonitrile (63.5:36.5), from which 20µl of sample was injected into HPLC (Waters 1515) equipped with a capillary column (Spherisorb C18 ; 150mm x 4.8mm x 5µm) and flow rate at 1mL/min. Mobile phase used was acetone:acetonitrile (63.5:36.5) and time needed about 60min.

RESULTS AND DISCUSSION

C. cleomifolia seeds yielded 5.2% of the oil (magenta colour) which is comparable to the amount produced by *C. striata* seeds (5.2%) in a previous study (Hosamani & Ramesh 2001). The oil has an iodine value of 85-95, which is comparable to castor seed oil (Akpan et al. 2006). Oils with an iodine value less than 100 is classified as non drying oil, which has a wide variety of industrial uses; for preparing soaps and cleansers, cosmetics, lubricants, leather dressings, and candles. As a comparison *Elateriospermum tapos* seed oil (106) (Ooi & Salimon 2006), corn oil (103-128), cotton seed oil (99-119) and mustard seed oil (92-125) (Gunstone et al. 1994) are more suitable for use in the food industries. A low peroxide value (0-6) shows that the oil is stable to relative oxidation, all measured values are summarised in Table 1.

The GCxGC-MS(ToF) software was used to define all the peaks in the raw GCxGC chromatograms. The typical chromatogram obtained for the *C. cleomifolia* seed oil and selective identification of the peaks done by the GC x GC-MS(ToF) Wiley data base library. Major fatty acids present in *C. cleomifolia* seed oil are linoleic acid, oleic acid, linolenic acid, palmitic acid and behenic acid. *C. cleomifolia* seed oil contains a high percentage of polyunsaturated fatty acid (PUFA) and monoenes. Linoleic acid ($C_{18:2}$), is the dominant PUFA (57.59%). This value is similar to sunflower, cottonseed, soybean, corn, poppy seed, sesame and *Capcicum* seed oil (Formo et al. 1979). Due to high linoleic acid content, *C. cleomifolia* seed oil is suitable for industrial purposes, notably for preparing varnish (Gecgel et al. 2007). Then, followed by oleic acid ($C_{18:1}$) 28.6% and linolenic acid ($C_{18:3}$) 5.53%. Major saturated fatty acids present in *C. cleomifolia* seed oil are palmitic acid ($C_{16:0}$) 5.07%, followed by behenic acid ($C_{22:0}$) 1.63%. Minor polyunsaturated fatty acids are cis-9, cis-15-octadecadienoic acid ($C_{18:2}$) 0.03% and erucic acid ($C_{22:1}$) 0.02%. Besides, some minor amount of saturated fatty acids were detected; stearic acid ($C_{18:0}$) 0.45%, followed by 15-methyl palmitic acid ($C_{17:0}$) 0.40%, cyclopropaneoctanoic acid, 2-hexyl ($C_{17:0}$) 0.32%, arachidic acid ($C_{20:0}$) 0.27% and heneicosanoic acid ($C_{21:0}$) 0.09%. Both of 15-methyl palmitic acid and cyclopropaneoctanoic acid, 2-hexyl have been reported previously by Hansen et al. 1955 and Rakotomanga et al. 2005 respectively. The five fatty acids

(linoleic, oleic, linolenic, palmitic and behenic acid) comprise more than 98% of the total of fatty acid methyl esters (FAMEs) in *C. cleomifolia* seed oil The proportion of unsaturated and saturated fatty acids were calculated 92% and 8% (see Table 2).

TAG present in *C. cleomifolia* seed oil was analyzed by reverse phase High Performance Liquid Chromatography (HPLC), whereby mechanism of the separation involves long chain and degree of unsaturated fatty acid (Gutierrez dan Barron 1995). Due to the limitation on available standard TAG, triacylglycerols of *C. cleomifolia* seed oil was identified by comparing with the retention time profile of triacylglycerols standards (soybean oil) using the same parameters. Major peaks of TAG in *C. cleomifolia* seed oil was identified as polyunsaturated TAG; PLL (18.04%) followed by POL + SLL (11.92%), OOL (7.04%) and PLLn (6.31%) as shown in Table 3.

Table 1. Analytical values shown by *C. cleomifolia* seed oil

Seed oil content	5.3%
Acid value	4.3
Iodine value	85.0
Peroxide value	0.6

Table 2. Fatty acid composition in *C. cleomifolia* seed oil

Fatty acids (saturated)	**Composition (%)**	**Fatty acids** (polyunsaturated)	**Composition (%)**
Palmitic acid	5.07	Oleic acid	28.60
15-methyl palmitic acid	0.40	Linoleic acid	57.59
Cyclopropaneoctanoic acid, 2-hexyl	0.32	cis-9, cis-15-octadecadienoic acid	0.03
Stearic acid	0.45	Linolenic acid	5.53
Arachidic acid	0.27	Erucic acid	0.02
Heneicosanoic acid	0.09		
Behenic acid	1.63		

Table 3. TAG composition in *C. cleomifolia* seed oil

TAG	Retention Time (min)	Relative Composition (%)
PLLn	18.190	6.31
PLL	20.841	18.04
LnOO	22.155	2.35
OOL	24.755	7.04
POL+SLL	25.684	11.92

Ln: - Linolenic acid, L: Linoleic acid, O: Oleic acid, P: Palmitic acid, S: Stearic acid

CONCLUSION

The physico-chemical properties of *C. cleomifolia* seed oil has potential to be exploited further for various applications or as a source for unsaturated fatty acids especially linoleic acid.

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