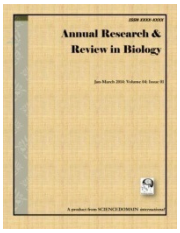

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Variability of Oil Content, Fatty Acid Composition and Karanjin Content in *Pongamia pinnata* and Its Relationship with Biodiesel Quality

S. Panpraneecharoen[1], C. Khamchum[1,2], P. Vaithanomsat[2,3], M. Thanasombat[3] and V. Punsuvon[1,2,4*]

[1]Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand.
[2]Center of Excellence-oil Palm, Kasetsart University, Bangkok, Thailand.
[3]Kasetsart Agricultural and Agro-Industrial Product Improvement Institute (KAPI), Kasetsart University, Bangkok, Thailand.
[4]Center for Advanced Studied in Tropical Natural Resource, NRU-KU, Kasetsart University, Bangkok, Thailand.

Authors' contributions

This work was carried out in collaboration between all authors. Authors SP and CK designed the study, wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: To study the variability of oil content, fatty acid composition, karanjin and total carotene content in *Pongamia pinnata* (*P. pinnata*) and its relationship with biodiesel quality
Place and Duration of Study: Department of Chemistry, Faculty of Science, Kasetsart University, Bangkok, Thailand, from January 2011 to May 2013.
Methodology: The *P. pinnata* oil contents were extracted by Soxhlet apparatus and examined for fatty acid composition of the oil using gas chromatography (GC).The saponification number (SN) and iodine value (IV) were determined as described in ASTM D5558 and AOCS official method 1c-85.The cetane number (CN) of each of the FAMEs was estimated from SN and IV values. The karanjin content was determined using HPLC with UV-Vis detector. The total carotene content was determined using

**Corresponding author: Email: fscivit@ku.ac.th;*

spectrophotometer.
Results: The variability of oil content was observed in 45 tree accessions of *P. pinnata* collected from three provinces in the southern of Thailand. The oil content was varied from 26.65 to 33.12 % (wt). Out of these, the fatty acid profiles of 20 accessions with varying seed oil content were examined. Large variations in the fatty acid content (% wt) were observed in stearic (C_{18}), oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acid with values of 3.88-13.84 % (wt), 37.15-47.42 % (wt) and 16.61-22.23 % (wt), respectively. Less variation in the fatty acid content (% wt) was observed for palmitic (C_{16}), linolenic ($C_{18:3}$) and behenic (C_{20}) acids with values of 12.82-17.63 % (wt), 0.00-5.88 % (wt) and 6.30-12.87 % (wt), respectively. The saponification number (SN), iodine value (IV) and cetane number (CN) of fatty acid methyl esters or biodiesel of oils varied from 196.81 to 207.69, 61.66 to 90.07 and 52.72 to 59.41, respectively. The fatty acid composition, IV and CN were used to predict the quality of fatty acid methyl esters. The fatty acid methyl esters of oil of *P. Pinnata* accessions RB3, RS11, RS15 (from Ranong), PK2(from Phungnga) and KB1 (from Krabi) were found to be the most promising (CN > 52.72) as biodiesel. Karanjin varying from 1.26 to 1.49 % (wt) was also found in these five selected samples. The low karanjin content enhances the quality of the biodiesel that support by purity of methyl ester obtained after transesterification.
Conclusion: All *P. pinnata* produced a high oil content greater than 30 % (wt), with CN>51 and IV<120, thus it meet the specific requirements of international biodiesel standards. The *P. pinnata* with low karanjin content can be ideal for biodiesel, too.

Keywords: Pongamia pinnata; fatty acid composition; oil content; biodiesel; karanjin content.

1. INTRODUCTION

P. pinnata belongs to the family Leguminaceae. It is a medium sized glabrous tree that generally attains a height of about 18 m and a trunk diameter>50 cm. It can grow under a wide range of agroclimatic conditions and it is a common sight around coastal areas, riverbanks, tidal forests and roadsides. It can grow on soil types ranging from stony to sandy to clay, including verticals. It is highly tolerant of salinity and can be propagated either by seeds or by root suckers [1]. The fruits are harvested during February-April, and have high non-edible seed oil content [2]. After 5-7 years of growth it bears fruits containing one to two kidney-shaped brownish-red kernels [3]. *P. pinnata* oil is eco-friendly, biodegradable and has been identified as one of the best alternatives to petrochemical [4]. The seed kernel containing 27-40 % oil contains mainly oleic acid ($C_{18:1}$; 44.5-71.3 %) and linoleic acid ($C_{18:2}$; 10.8-18.3 %) along with minor quantities of palmitic acid ($C_{16:0}$; 3.7-7.8 %) and stearic acid ($C_{18:0}$; 5.5-6.0 %) [5]. *P. pinnata* oil consists of karanjin, karanjone and diketone pongamol [6]. Karanjin is an active principal component responsible for the curative effects of the oil in skin disease. Seed extract inhibits the growth of the herpes simplex virus and also possesses hypoglycemic, antioxidative, antiulcerogenic, anti-inflammatory and analysis properties. Karanjin possess pesticidal and insecticidal activity [7]. The chemical structure of karanjin is shown in Fig. 1. Freshly extracted oil is yellowish orange to brown in color having a disagreeable odor and a bitter taste. The presence of toxic compounds makes *P. pinnata* oil inedible [8].

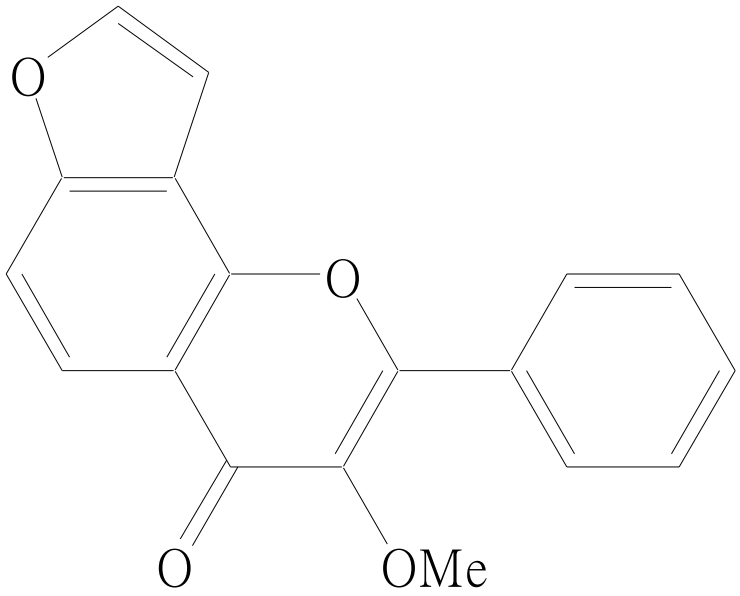

Fig. 1. Chemical structure of karanjin

P. pinnata oil is regarded as a potential fuel substitute as it contains 16-22 carbon atoms per molecule. Attempts have been made to convert *P. pinnata* oil to methyl esters [1,4]. With the growing interest in the seed oil of *P. pinnata*, the need for establishing plantations has been realized. In this context, the present study was undertaken to assess the variability of some of the important biodiesel qualities as a prelude in the selection process for more efficient biodiesel yield. In addition, the karanjin content was also analyzed in the selected samples for enhancing the added value of the *P. pinnata* oil. With these objectives, the survey, collection and characterization of *P. pinnata* (a native plant in southern Thailand) was undertaken to assess the variability of various economically important parameters for the development of this species as a profitable crop for biodiesel production in Thailand in the near future.

The CN is a dimensionless descriptor of the ignition quality of a diesel fuel. It is a prime indicator of biodiesel quality. The higher the CN, the shorter is the ignition time. The CN increases with the length of the branch carbon of the FAME component [9]. Standards for CN have been established worldwide. American Society for Testing and Materials (ASTM D6751) for biodiesel fuel requires a minimum CN of 47. The European standard (EN 14214) requires a minimum CN of 51 [10].

2. MATERIALS AND METHODS

2.1 Collection of Samples

Exploration trips were conducted during January and February, 2011 to various districts of Ranong, Phungnga and Krabi provinces and 45 accessions were collected. Representative samples consisting of 2-3 kg of pods from all sides of the selected tree were collected. Pods were stored at room temperature. One kilogram of pods was randomly picked from each lot, threshed and each final sample was randomly drawn from this material for seed oil content

determination. The accessions code RB and RS were allocated to the samples from Ranong province. The codes PK and KB represented the samples from Phungnga and Krabi provinces, respectively.

2.2 Oil Content Determination

The seed oil contents were extracted using hexane as solvent in Soxhlet apparatus (Buchi, Switzerland) on a dry weight basis. The dried seeds were sampled in triplicate.

2.3 Fatty Acid Composition Using Gas Chromatography

The 20 accessions covering the ranges of oil content were examined for fatty acid composition of the oil using gas chromatography (GC). Briefly, seed oils were methylated with boron trifluoride in methanol followed with NaOH/methanol treatment to form methyl esters as described by the AOAC 969.3 official method. The fatty acid methyl esters were identified using GC equipment with a chromatography analysis section using the capillary column DB-WAX (30 m x 0.25 mm ID, 0.25 μm). The oven temperature was set from 80 to 250ºC at 10ºC/min and held for 2 min. The injector temperature was maintained at 250ºC and the detector temperature was set at 250ºC throughout the experiment. A flame ionization detector (FID) was used for the analysis. The carrier gas flow was helium. The fatty acid methyl esters (FAME) were analyzed by using an internal standard solution and by comparing the retention time and quantification performed by the area normalization method.

2.4 Analysis of *P. pinnata* Oil for Quality

The saponification number (SN) and iodine value (IV) were determined as described in ASTM D5558 and AOCS official method 1c-85. The cetane number (CN) of each of the FAMEs was estimated from the following equation [11].

$$CN = 46.3 + \frac{5458}{SN} - 0.225 \times IV$$

2.5 Karanjin Content Analysis

P. pinnata seed oil (5 g) was subjected to liquid-liquid extraction with methanol in the ratio 1:2 (wt/v). Extraction was repeated four times. All methanol fractions were pooled and concentrated to 25 mL. The extract was determined for karanjin using an HPLC instrument with UV-Vis detector. The analysis was carried out using a Phenomenon Luna C18, 250 x 4.6 mm x 5 μm column and karanjin was detected at 300 nm. The mobile phase consisted of methanol, water and acetic acid in the ratio of 85:13.5:1.5 [7]. The flow rate was 1 mL/min. The oven temperature was set at 40ºC.

2.6 Total Carotene Content Analysis

Total carotene content of *P. pinnata*oil was carried out according to Kaur et al. [12]. Briefly explained, the *P. pinnata* oil 0.2 g was accurately weighted into a 25 cm^3 volumetric flask and made up volume with hexane. The absorbance of the solution at 446 nm was determined using a spectrophotometer (PG Instruments, England). Result is given as mg/kg of oil. The carotene content was calculated by the following equation:

$$\frac{25 \times 383 (a_s - a_b)}{100 W}$$

Where $a_s$is the absorbance of the sample, a_b is the absorbance of the cuvette error (blank) and W is the weight of the sample in gram.

2.7 Biodiesel Production

*P. pinnata*oil had free fatty acid (FFA) content 11.90 % that necessitated acid pretreatment to reduce FFA less than 2 % (100 g oil treated with 9:1 methanol to oil molar ratio, 1.0 g conc. H_2SO_4 for 2 h at 60ºC). After the acid pretreatment, the FFA content was reduced to 1.5%. This pretreated *P. Pinnata* oil was used for transesterification reaction.

The transestrification reaction was carried out in a 500 ml round bottom three-necked flask under reflux condenser and using a hotplate for control temperature of reaction. The other neck was fitted with a thermometer. Magnetic stirring rate was about 750 rpm at 60ºC for 1 h of reaction time. Methanol was used as alcohol and potassium hydroxide (KOH) was used as catalyst for transesterification. Molar ratio between methanol and oil was 11:1 for transesterification. The catalyst amount was selected as 1.5 %wt. After completed the reaction, the excess methanol was distilled off under vacuum evaporator and glycerin layer was separated in a separation funnel. The fatty acid methyl ester layer was washed with warm water and then removed excess water by heating at 105ºC. The biodiesel was investigated the fatty acid methyl ester (FAME) content by gas chromatography (Hewlett-Packard, Agilent Technologies 6890) followed EN 14103 standard.

3. RESULTS AND DISCUSSION

3.1 Oil Content

The *P. pinnata* trees sampled had a long period of pod and seed development starting from fertilization of the flower and ending with maturity of the seed. *P. pinnata* trees started flowering during March–May and fruits setting occurred during June–July. The initial stage of fruit development was slow. Following fruit set, the pod appeared flat initially. The fruit envelope attained its maximum size during August–September. The pods appeared green in color from June–January and changed to half brown and then became brown from January–February. Based on the observations, the half brown and brown pods of 45 trees were picked. The seeds were taken out of the pods for oil content analysis. The half brown pods seed and oil are shown in Fig. 2.

Oil yield is the most important factor which affects the overall commercial success of *P. pinnata* cultivation and its use as an energy crop. The frequency distribution of 45 tree accessions of *P. pinnata* for seed oil content is presented in Table1.

Table 1 shows that 73 % of the accessions exhibited oil content ranging from 25.00 to 30.00 % by weight, while 27 % exhibited higher oil content in the range of 30.10 to 33.12 % by weight.

The 20 *P. pinnata* accessions selected for fatty acid analysis covered the whole range of oil content from a minimum of 25.67 % in KB4 (from Krabi) to a maximum of 33.12 % in PK2 (from Phungnga). The variability in the oil content might be due to the variation in the

species or different ecological conditions or differences in the maturation stage of the seeds. The five accessions with very high oil content (>30 %) coded RB3, RS11, RS15 (from Ranong), PK2 (from Phungnga) and KB1 (from Krabi) will be valuable for the selection and development of a high oil yielding crop for biodiesel production.

Fig. 2. *P. pinnata* pods, seeds and oil

Table 1. Frequency distribution of 45 tree accessions of *P. pinnata* for seed oil content

Characteristic	Range (%wt)	Number of accessions
Seed oil	25.00-30.00	33
	30.10-33.12	12

3.2 Fatty Acid Composition

The quality of oil is a function of its fatty acid composition. The palmitic, stearic, oleic, linoleic, linolenic and behenic fatty acid compositions varied widely in the 20 accessions selected for this study as shown in Table 2.

The major fatty acid was oleic acid ($C_{18:1}$) in a concentration range of 37.15-47.42 %, followed by linoleic acid ($C_{18:2}$) with 10.61-22.23 %, palmitic acid ($C_{16:0}$) with 12.82-17.63 %, stearic acid ($C_{18:0}$) with 3.88-13.84 %, behenic acid ($C_{20:0}$) with 6.30-12.87 % and linolenic acid ($C_{18:3}$) with 0-5.88 %. The results of the large differences in fatty acid concentration were determined as 9.96 %, 10.27 %, 11.62 % for stearic, oleic and linoleic acids, respectively. In addition, the results for the less different fatty acid concentrations were 4.81, 5.88 and 6.57 % forpalmitic, linolenic and behenic acids, respectively.

A comparison of the fatty acid composition in our work with that of Mukta et al. (2009), who studied the variability of Indian *P. pinnata* oil, showed that Indian *P. pinnata*had a higher range in oleic acid (46.66-13.25 %) and linoleic acid (12.02-32.58 %) than in our study. The reason was Indian *P. pinnata* oil contained only four major fatty acids (palmitic, stearic, oleic and linoleic acid) but the Thai *P. pinnata* oil contained six major fatty acids (palmitic, stearic, oleic, linoleic, linolenic and behenic acid). The total amount of fatty acids in both studies may be more equivalent if the oleic and linoleic components were included from our study.

The oleic/linoleic acid (O/L) ratio and the iodine value indicate stability and shelf life, respectively. When the O/L ratio and iodine values in the current study were compared with Indian *P. pinnata* oil [13], the O/L ratio of both oils was nearly the same, which indicated that the Thai and Indian oils had the same stability. The Indian oil had a higher iodine value than the Thai oil, indicating that the Thai oil had a longer shelf life than the Indian oil. The O/L ratio among the various *P. pinnata* accessions varied from 1.72 to 4.17. Accession PK5 exhibited a high O/L ratio and a low iodine value (Table 2), which indicated higher stability and longer shelf life.

The saponification number (SN) is an indicator of the molecular weight of the fatty acid or the chain length of the fatty acid in the oil. Our Thai *P. pinnata* oil had a higher SN value (196.81-207.69) than the Indian oil (183.30-200.91) [13]. This result indicated that the Thai oil consisted of long chain fatty acids such as linolenic ($C_{18:2}$) and behenic ($C_{22:0}$) acids but that the Indian oil lacked both these acids as mentioned earlier in the fatty acid composition analysis.

3.3 Biodiesel Quality

The SN and IV values were determined following ASTM D5558 and AOCS 1c-85. The CN values of the fatty acid methyl esters of the oil were empirically determined. The values for SN, IV and CN varied from 196.81 to 207.69, 61.66 to 90.07 and 52.72 to 59.41, respectively (Table 2). The CN indicates the ability of a fuel to ignite quickly after being injected; a higher value indicates a better ignition quality of the fuel [9]. As noted earlier, biodiesel standards of USA and European organizations have set this value as 47 and 51, respectively. The IV is the degree of unsaturation with an increase in the CN resulting in a decrease in the IV which indicates that the degree of unsaturation decreases. This saturation will lead to the solidification of fatty acid methyl esters at higher temperatures. In addition, the European organization standard mentioned earlier set the IV value at below 120. In the present investigation, the values for the two standards (IV and CN) fell within these limits for 20 of the accessions studied. Among the FAMEs of *P. pinnata*, all accessions had a CN value higher than 51, indicating that all the accessions studied satisfied the CN requirement for both the USA and European standards mentioned earlier. The IV of 20 accessions (Table 2) ranged from 61.66 to 90.07 and thus also met the standards' specifications for the IV. In addition to these parameters, the concentration of linolenic acid containing three bonds in FAMEs should not exceed the limit of 12% in accordance with the European standard mentioned earlier. *P. pinnata* oil could produce fatty acid methyl esters most suitable for use as biodiesel since it met the major specifications for biodieselstandards of USA and European organization [14].

3.4 Karanjin Content

The HPLC chromatogram of the karanjin standard and the karanjin in the oil extracted from five promising seed sources is shown in Fig. 3.

The karanjin peak in oil appeared at 3.45 min of retention time, which was the same retention time as for the karanjin standard peak. The quantification of the average karanjin contents for code numbers RB3, RS11, RS15, PK2 and KB1 were 1.28 %, 1.49 %, 1.46 %, 1.29 % and 1.26 %, respectively. The results for the karanjin content were found to be slightly different among the five accessions as they originated from different provinces. When the karanjin content results of our study were compared with the Indian *P. pinnata* [2], the Indian *P. pinnata* had higher karanjin content (3.26-5.33%) than our Thai *P. pinnata*

(1.26-1.49 %). This kind of large variation in karanjin content may be attributed to the geographical location. In addition, extraction of karanjin from oil before biodiesel production can enhance the values of the *P. pinnata* oil since karanjin has many uses in the fields of plant protection and medicine.

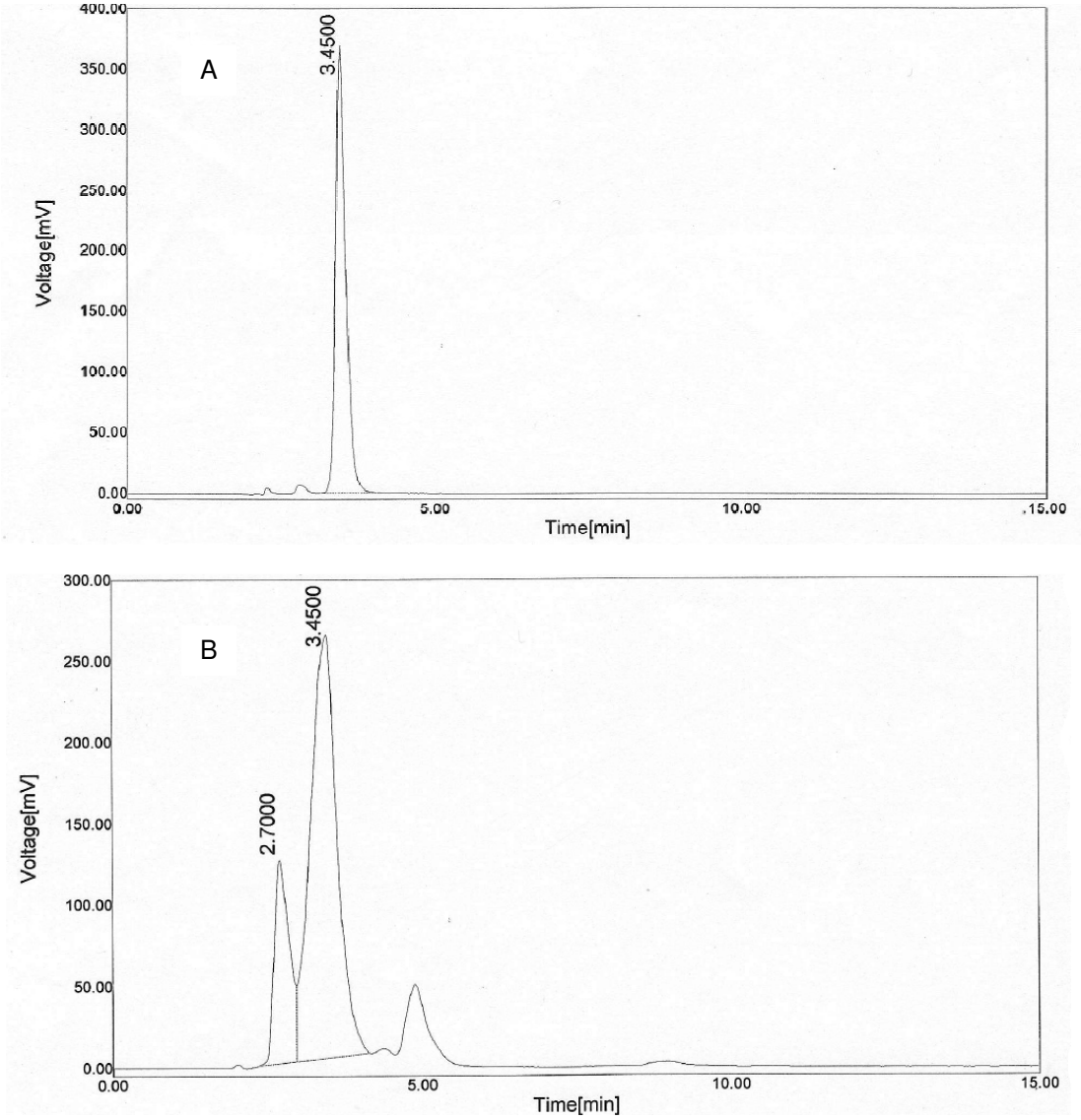

Fig. 3. HPLC chromatogram of karanjin standard (A); karanjin in oil extracted from seed (B)

Table 2. Variability for oil content, fatty acid composition and biodiesel quality in 20 accessions on *P. pinnata* oil

Code no	Seed oil content (%)	Fatty acid composition[a]						SN	IV	CN	O/L[b]
		Palmitic	Stearic	Oleic	Linoleic	Linolenic	Behenic				
RB1	26.65	13.01	5.32	47.42	14.99	4.49	9.12	207.65	83.75	53.74	3.16
RB2	26.83	13.31	13.28	37.15	18.14	4.23	6.30	205.99	83.45	54.02	2.05
RB3	30.48	14.09	8.71	44.36	16.27	3.36	7.25	198.56	71.95	57.60	2.73
RB4	27.97	15.39	13.84	40.86	12.21	1.91	7.42	202.27	61.66	59.41	3.35
RB5	26.89	16.67	5.16	48.15	12.47	2.17	8.27	213.69	71.89	55.67	3.86
RS11	30.51	12.95	5.61	42.04	20.98	3.69	8.65	206.85	88.41	52.79	2.00
RS12	27.35	13.34	5.06	42.97	20.43	4.07	8.48	203.35	87.72	53.40	2.10
RS13	27.81	15.35	4.89	43.64	16.43	2.29	10.24	198.95	81.02	55.50	2.66
RS14	30.09	13.45	5.87	47.34	18.18	2.50	7.64	207.69	78.41	54.94	2.60
RS15	31.31	12.82	6.32	46.22	18.89	2.83	8.55	199.29	83.40	54.92	2.45
PK1	30.07	13.59	4.67	40.84	21.52	3.26	9.89	195.40	83.62	55.42	1.89
PK2	33.12	12.59	4.71	40.19	19.84	3.90	11.56	204.69	85.71	53.68	2.03
PK3	28.75	14.06	6.34	41.41	19.67	2.42	9.98	201.82	81.96	54.90	2.11
PK4	32.53	13.70	3.88	38.33	22.23	3.66	11.32	195.04	84.77	55.21	1.72
PK5	29.19	17.63	6.18	44.20	10.61	-	12.87	202.04	67.73	58.08	4.17
KB1	30.70	13.75	5.28	43.39	18.56	4.71	10.70	204.52	90.07	52.72	2.34
KB2	29.28	13.37	5.03	45.40	17.54	4.24	10.80	206.23	79.96	54.77	2.59
KB3	30.29	13.93	4.88	44.53	21.24	4.27	7.92	196.81	85.12	54.88	2.09
KB4	26.67	15.69	4.99	45.26	15.56	2.82	11.58	203.58	80.24	55.06	2.91
KB5	28.40	15.62	4.49	42.24	16.19	5.88	11.03	201.15	76.52	56.22	2.61
Mean	29.24	14.22	8.53	43.30	17.60	3.69	9.48	202.78	80.37	55.15	2.57
SD	1.93	1.38	10.39	2.97	3.26	1.03	1.77	4.66	7.25	1.70	0.64
Range	26.65-33.12	12.82-17.63	3.88-13.84	37.15-47.42	10.61-22.23	0-5.88	6.30-12.87	196.81-207.69	61.66-90.07	52.72-59.41	1.72-4.17

[a]Row percentage may not add to 100% due to the non-inclusion of other constituents and [b]Oleic/Linoleic acid

3.5 Total Carotene Content

The carotene content in *P. pinnata* oil is 45.38 mg/kg that is lower value than carotene content in palmoil. The carotene content in the palm oil was 500-600 mg/kg [12]. The low content of carotene in *P. pinnata* oil is responsible for the yellow color oil that it is not orange color oil as the palm oil.

3.6 Biodiesel Production

The purity of FAME in biodiesel was analyzed by GC follow EN 14103 standard. The result showed chromatogram in Fig. 4 and FAME content in Table 3.

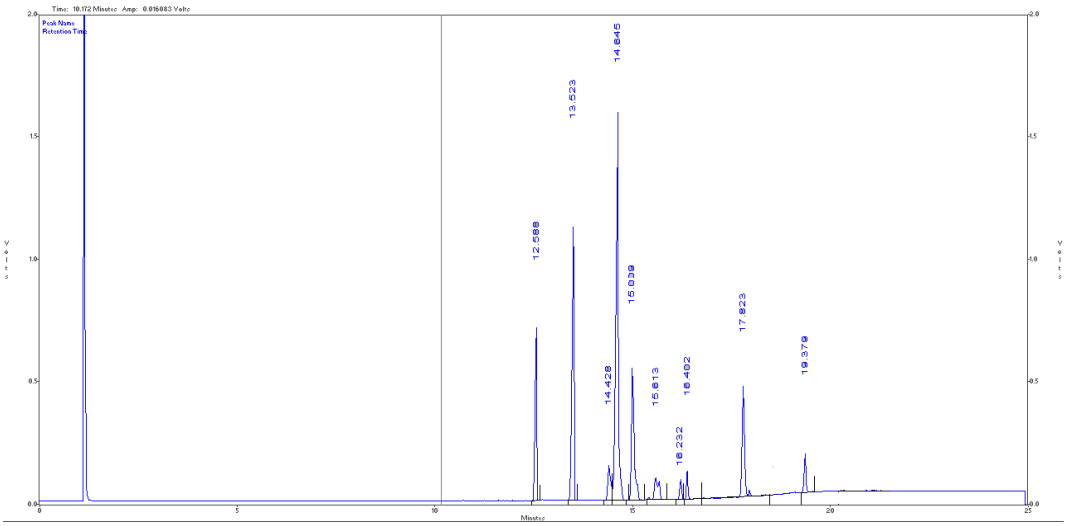

Fig. 4. GC chromatogram of *P. pinnata* biodiesel

Table 3. Fatty acid methyl ester content in P. pinnata biodiesel

Methyl esters	Retention time (min)	%
Methyl palmitate	12.588	9.572
Methyl heptadecanoate[a]	13.523	18.653
Methyl stearate	14.428	3.499
Methyl oleate	14.645	37.346
Methyl linoleate	15.009	12.917
Methyl linolenate	15.613	3.179
Methyl arachidate	16.232	1.272
Methyl behenate	16.402	1.676
Methyl erucate	17.823	8.986
Methyl lignocerate	19.379	2.900
Total methyl esters	-	100

[a]*Internal standard*

The purity of FAME calculated from methyl esters content in Table 3 was 98.13 % that met the requirement of EN 14103. The result of FAME suggested that high fuel quality methyl

ester was obtained from crude *P. Pinnata* oil. The result imply that the karanjin compound is not affect the quality of biodiesel.

4. CONCLUSION

The present study documented the variation in the oil content, fatty acid composition, saponification number, iodine value and cetane number of Thai *P. pinnata* oil from southern Thailand. *P. pinnata* coded as RB3, RS11, RS15 from Ranong, PK2 from Phungnga and KB1 from Krabi provinces all produced a high oil content greater than 30 % (wt), with CN>51 and IV<120, thus meeting the specific requirements of international biodiesel standards. The karanjin content of the five selected *P. pinnata* samples was in the range 1.26-1.49 % which was 3-5 times less than the karanjin content detected in the Indian *P. pinnata*. The *P. pinnata* with low karanjin content can be ideal for biodiesel.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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