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Lipid extraction from natural plant source of *Adenanthera pavonina* using mixed solvent by superheated extractor

Ramachandran Kasirajan, Sivakumar Pandian, Suganya Tamilarasan, and Renganathan Sahadevan†

Bio-Remediation Research Laboratory, Department of Chemical Engineering, Anna University, Chennai 600 025, India
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Abstract−The lipid content was extracted from the saga seed by superheated condition and soxhlet apparatus. The mixture of hexane, chloroform and methanol was utilized as a mixed solvent for these extraction operations. Different parameters such as different solvent, temperature, mean particle size and solvent flow have been examined. The optimized lipid extraction was achieved as 26.2 wt% by using superheated condition from the saga seed powder at 90 °C for 120 min. Then the fatty acids profile of the optimized *Adenanthera pavonina* oil were analyzed by gas chromatography. Unsaturated fatty acid was high as 83.7% compared with saturated fatty acid barely 15.4% by relative.

Keywords: *Adenanthera pavonina*, Saga Seed, Mixed Solvents and Solvents Superheated Extraction

INTRODUCTION

Plant lipids are of substantial economic importance. On the whole, the annual world invention of plant lipids (triacylglycerols) is just about 130 million tons by means of soybean oil making up more or less 50% in total. Cotton seed is 17%, peanut is 10%, sun flower is 9%, and rapeseed is 6% and other important sources of plant lipids in the midst of sesame seed, copra, palm, linseed and castor bean seed make up the remaining amount [1]. The plant *Adenanthera pavonina* is widely distributed in Asian countries of China, Bangladesh, and India and also available in African countries. *Adenanthera pavonina* belongs to the family *Leguminosae* and generally called as Saga tree or Red sandal wood tree; in Tamil it's called as Ani kundamani or Manjadi. Synonyms of *Adenanthera pavonina* are *Adenanthera polita*, *Adenanthera gersenii*, *Corallaria parvifolia*. The leaves and the bark of the plant are used as a treatment for chronic rheumatism, gout, haematuria, haematemesis and diarrhoea. A red powder made from the wood is used as an antiseptic paste [2,3].

Nutritional studies have demonstrated one quarter of the seed as oil with a high percentage of proteins [4]. Readily available studies published very little that examine chemicals accumulated in *Adenanthera pavonina* seeds. Those investigations led only to the isolation of a few protein flavonoids [5], fatty acids [6,7], triterpenoids [8], trypsin inhibitors [9], and carbohydrates [10]. Zarnowski et al. [12] investigated the comprehensive composition of lipids present in the oil of *Adenanthera pavonina* seeds. Moreover, an achievable use of this oil for a submicron oil-in-water lipid emulsion formulation was also described.

Supercritical carbon dioxide fluid extraction of crop seed oil as the latest and prevailing method has been reported and compared with alternative extraction methods. Abbasi et al. [13] applied five extraction methods including normal stirring, Soxhlet, microwave irradiation, ultrasonic irradiation using n-hexane and petroleum benzene as a solvent and supercritical CO_2 fluid extraction. Their results showed significant differences in the extraction yields with the lowest value for supercritical CO_2 fluid extraction. The fatty acids compositions for the first four methods were all nearly the same, but supercritical CO_2 fluid extraction method, carried out at different operating conditions, resulted to differences, especially in unsaturated fatty acid compositions. Liu et al. [14] used supercritical CO_2 extraction and n-hexane Soxhlet method and observed minor differences in the fatty acid composition of the oils extracted by the two methods.

Superheated solvent extraction is a modern intensifying extraction technique that combines temperature and pressure with liquid solvents to realize rapid and proficient extraction of lipids from several dissipated natural sources. The most important benefit over conventional solvent extraction methods conducted at atmospheric pressure is that pressurized solvents stay behind in the liquid state, still higher than their standard atmospheric pressure, allowing high temperature boiling point extraction [14].

To the best of our knowledge, superheated extraction (SHE) of *Adenanthera pavonina* seed oil using mixed solvent has not yet been examined. Our objective was to evaluate the effects of some operating conditions such as temperature, mean particle size and solvent flow rate on the extraction efficiency of saga seed oil using SHE method. The fatty acids profile of the seed oil was compared with those obtained by conventional techniques such as Soxhlet extraction.

MATERIALS AND METHODS

1. Materials

Adenanthera pavonina seeds were collected from Anna University Campus, Chennai. Methanol 99.9% purity and n-hexane 99% purity was purchased from Merck India Ltd. Chloroform (>99.9% purity) was purchased from SRL Chemicals India Ltd. These solvents were reused after preliminary distillation and there were no changes in purity of the recycled solvents.

†To whom correspondence should be addressed.
E-mail: rengsah@rediffmail.com

2. Methods

2-1. Preliminary Operation

Adenanthera pavonina seed was initially weighed, followed by moisture removal operation in a hot oven at 40 °C until it reached zero weight loss. 2.8 wt% of moisture removed from saga seed (moisture content of the fresh saga seed was analyzed by gravimetric method). Moisture removed was crushed and it was separated based on the size of the granular particle by sieving as per the ASTM standard.

2-2. Lipid Extraction by Soxhlet

The extraction setup generally consists of a three-necked round bottom flask (250 ml). The middle neck of the round-bottom flask was connected to a sample jar with a reflux condenser, a thermometer was positioned in one of the two side necks and the third neck was used for taking samples for the period of the extraction method. The flask was submerged in a heat controlled water bath [15]. 25 gm of crushed seed was taken in a jar and 300 mL of total solvents used per batch for 8 hr.

2-3. Lipid Extraction by Superheated Solvent Extractor and Quantification

Superheated extraction followed by separation setup schematic diagram is shown in Fig. 1. In each extraction operation 25 gm of crushed saga seed was taken into the extraction vessel. Length, diameter and thickness of the extraction vessel are 200 mm, 38.1 mm and 4 mm, respectively. All lines (6.35 mm) and vessels were fabricated by using stainless steel. 0.2 μm pore size filter cloth was used for both sides of the vessel to allow the solvents move freely. Appropriate temperature and flow rate of mixed solvent from storage vessel into the extractor through pre-heater was maintained constant by controlling temperature using electrical heating coil, and hot air oven was also used to maintain constant temperature, and by extraction vessel inlet needle valve which was adjusted manually [16]. The pressure was maintained by adjusting the extraction vessel outlet needle valve manually (pressure control valve). The solvents were pumped (Milton Roy-Electromagnetic dosing pump with flow of up to 2.14 lph and pressure up to 50 bar) through the pre-heater into the extraction vessel continuously maintaining constant temperature, pressure and flow rate. Afterwards, the extract was collected in a vessel equipped with flash column for the purpose of mixed solvent separation. These separated solvents were reused for next cyclic extraction operation. Trace amounts of solvent present in the oil were completely removed by simple distillation and reused.

Extract yield was quantified by the following expression:

$$Y_{oil} = \frac{W_i - W_r}{W_i} \times 100$$

where, 'Y_{oil}' is the extract yield or oil yield in terms of percentage, 'W_i' is initial weight of saga seed taken in extraction vessel, 'W_r' is the weight of raffinate. All units are represented in weight basis.

2-4. Lipid Characterization

Properties such as density, average molecular weight, iodine value, acid value, FFA content and saponification value of extracted *Adenanthera pavonina* oil were analyzed by using standard procedures [17,18]. We weighed about 5 g of saga seed oil and transferred it into individual 100 mL conical flasks. We weighed about 0.05 g of potassium hydroxide (1% by weight of the oil) and dissolved it in 1.44 g anhydrous methanol. The KOH was dissolved in methanol, with heat applied if required. The resulting solution was slowly transferred to the sample in a conical flask with stirring. After complete transfer of KOH solution in methanol the stirring was continued for 120 minutes to complete the reaction. After completion of the reaction time we pipetted out and transfered approximately 2-3 mL of reaction mixture to 5 mL test tube and kept it for phase separation. Separated FAME used for the fatty acid composition present in saga seed oil was analyzed by gas chromatography (GC) analysis. One gram of oil was taken and fatty acid composition analysis

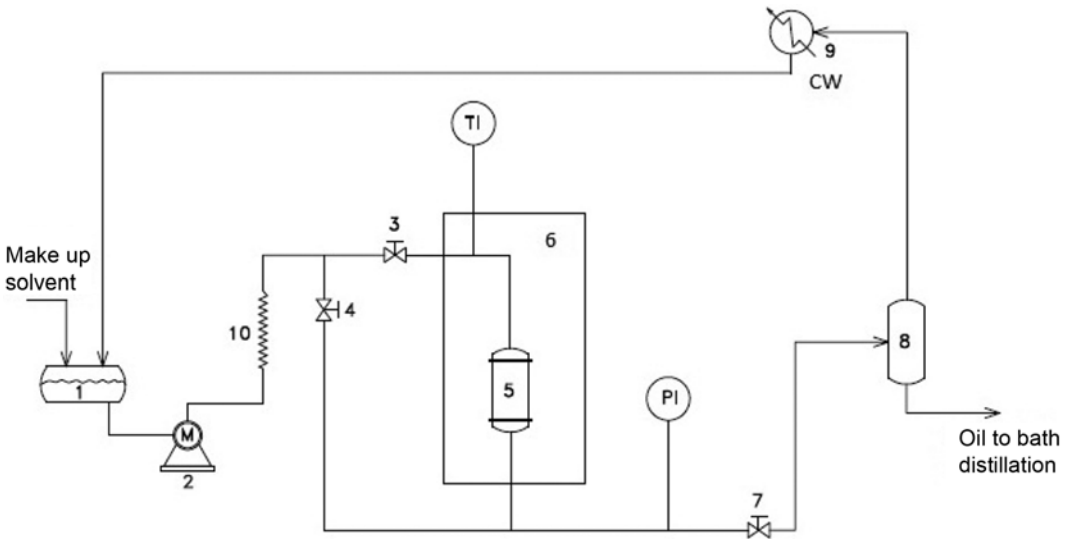

Fig. 1. Superheated mixed solvent extraction schematic diagram - set up.

1. Solvent storage vessel
2. High pressure pump
3. Inlet valve
4. Bypass valve
5. Extractor
6. Hot air oven
7. Outler valve
8. Flash column
9. Condensor
10. Heating coil

TI. Temperature indicator
PI. Pressure indicator
CW. Cooling water

was carried out by gas chromatograph, which consisted of CHEMIT GC 8610 flame ionization detector in the column BPX-70. Nitrogen and hydrogen were used as carrier gas and oxygen was used for detonation purpose. The data was composed with Winchrom software. Thin layer chromatography (TLC) was essentially used to separate and determine the concentration of different types of lipid groups present in the saga seed oil [1].

RESULTS AND DISCUSSION

The most important factors troubling superheated solvent extraction were particular to the selection of solvent, extraction temperature, solvent flow rate, mean size of the particle and extraction time, each of them at three different levels varying from 70 to 110 °C, 0.25 to 0.75 mm, and 0.5 to 1.5 L/hr, respectively with respect to different time period of up to 120 min. In the midst of an operational environment, that may distress the superheated solvent extraction efficiencies, for the reason that maintaining liquid state of extraction solvent and pressure is of slight magnitude. Subsequently, the extraction pressure was reserved stable and equivalent to 15 bars to guarantee methanol, chloroform and n-hexane being in liquid circumstances at the extraction temperatures.

1. Different Solvent System

Preference of the solvent system for extraction of oil from *Adenanthera pavonina* seed is an essential factor. Selection of the solvent for oil extraction at the preliminary action would permit cost-effective invention without further expense required for the purification of the product. Moreover, the preferred solvent must have good extraction ability and stumpy viscosity to augment the free transmission. Professional extraction requires the diffusion of fluid into the solid. An organic solvent has superior solubility with oil. Additionally, it has been used to humiliate the particle cell walls and to liquefy the oil to augment the oil yield. The effect of different solvent systems on saga seed oil extraction yield is shown in Table 1. Hexane is widely used for oil extraction for its low slippery residual effects, high stability, low boiling point and minimum corrosiveness. Different solvents such as hexane (H), chloroform (C), mixture of chloroform: methanol (C : M) and mixture of chloroform: methanol: hexane (C : M : H). This solvent system was taken in terms of volume ratio and was selected based on types of lipid present in the seed, such as polar and nonpolar lipids at 15 bar pressure, 70 °C temperature and 1 lph of solvent flow. From this investigation, the highest oil extraction yield was achieved as 25.3 wt% from *Adenanthera Pavonina* using the most valuable solvent mixture of 2 : 1 : 3 volume ratio of chloroform : methanol : hexane. From other studies, this mixed solvent system was used for oil extraction from *Adenanthera pavonina* seed.

Table 1. Effect on solvent different solvent system of Hexane (H), Chloroform (C), mixture of Chloroform : Methanol (C : M) and mixture of Chloroform : Methanol : Hexane (C : M : H)

S. No.	Solvents	Yield (wt%)
1	Chloroform (C)	19.4
2	Hexane (H)	20.9
3	Chloroform : Methanol (C : M)	20.3
4	Chloroform : Methanol : Hexane (C : M : H)	25.3

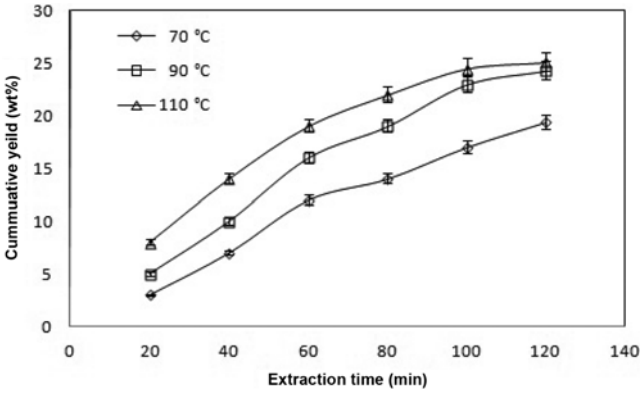

Fig. 2. Effect of temperature on oil extraction at 1.0 L/hr of solvent flow, 0.5 mm of mean particle size, 3 : 2 : 1 volume ratio of hexane, chloroform and methanol.

2. Effect of Temperature

At the primary movement, the most effective temperature for saga seed oil extraction was examined for the reason that the optimistic consequence of temperature on distribution of solutes in extraction processes, it may perhaps to carry out superheated solvent extraction process at the higher permeable temperature. The temperature effect was evaluated between 70 to 110 °C, mean particle size of 0.50 mm, solvent flow rate of 1 L/hr, for 2 hr of extraction time at 15 bar pressure. The extraction yield is shown in Fig. 2. From this figure, it is observed that the extraction efficiency increased generally with increasing temperature. The resulted collective yield values was obtained as 19.4, 24.3 and 25.1 wt% at 70, 90 and 110 °C, respectively. On the contrary to clear yellow saga seed oil at 70 and 90 °C, slight brownish color was acquired at 110 °C due to the effect of degradation of essential components at the superior temperatures. Additionally, extreme heating gave rise to uninvited creation of free fatty acids from triacylglycerols during the extraction operation [19]. Based on the mentioned specific statement to prevent any unsolicited changes in seed oil quality in a successful manner, further investigations were carried out at 90 °C.

3. Effect of Mean Size of the Particle

The mean particle size is an essential constraint for the extraction of oil from biomass. The smaller dimension of biomass leads to greater in the interfacial area between the solid and liquid. Hence, the increase in interfacial area enhances the oil extraction yield. The mean size of the seed particles was selected as 0.25, 0.5, and 0.75 mm. The consequence of mean size of the seed particles on the extraction of saga seed oil as collective effectiveness at 90 °C temperature, 15 bar pressure, 1 L/hr flow rate, and up to 120 min extraction time is shown in Fig. 3. The extraction efficiency was found to be 26.2, 23.8 and 19.3 wt% for 0.25, 0.50, and 0.75 mm, respectively.

Fig. 4 shows that the cumulative extraction yield progressively improved from 19.3 wt% to 26.2 wt% with decrease in mean particle size from 0.75 mm to 0.25 mm. With further reduction of the mean particle size, there was no any supplementary increase in oil extraction. This shows that the highest lipid extraction yield of 26.2 wt% was achieved with a seed mean particle size of 0.25 mm. It is

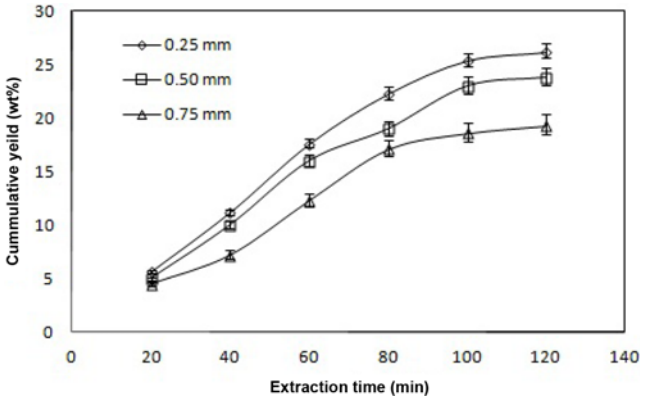

Fig. 3. Effect of mean particle size on oil extraction at 90 °C temperature, 1.0 L/hr of solvent flow, 3 : 2 : 1 volume ratio of hexane, chloroform and methanol.

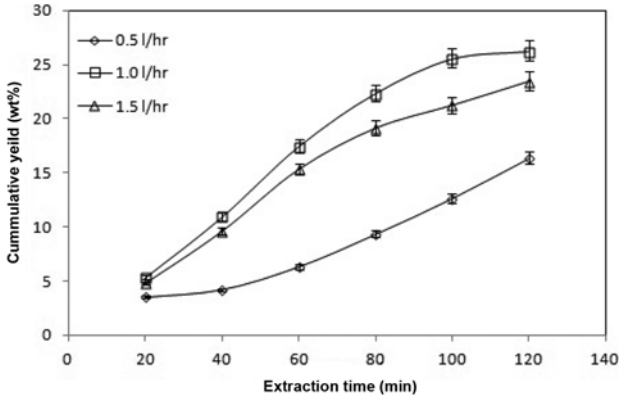

Fig. 4. Effect of solvent flow on oil extraction at 90 °C temperature, 0.25 mm of mean particle size, 3 : 2 : 1 volume ratio of hexane, chloroform and methanol.

well acknowledged that the rate of mixed solvent extraction is controlled by the mean particle size of the seed. The worse extraction rate is endorsed because of the higher mean particle size. However, the higher particle size creates complexity for the solvents to penetrate into the core of the seed to leach the lipid. Note that the particle size not only enhances the extraction rate, but also raises the lipid extraction yield. Han et al. [21] mentioned that the most important cause for escalating oil yield was due to shrinkage in particle size, which in turn enhances the specific surface area of the oilseed interacting with the solvent. For further investigation, to have higher efficiencies, the most favorable value for the mean particle size was decided as 0.25 mm.

4. Effect of Solvent Flow

The outcome of mixed solvent flow rate contained by 0.5-1.5 L/hr on extraction yield of *Adenanthera pavonina* seed oil at 90 °C temperature, 15 bar pressure, 0.25 mm particle size, and up to 120 min extraction time is shown in Fig. 4. The consequential values were 16.3, 26.2 and 23.4 wt% at 0.5, 1.0 and 1.5 L/hr, respectively. This investigation shows that the extraction rate of saga seed oil was escalating too fast from the flow rate of 0.5 to 1.0 L/hr. The extraction yield increased with increasing flow rate, because the mass of saga seed oil moves from the surface of the small solid particles into the solvent phase synchronized for the most part of the extraction process, and increasing the solvent flow rate resulted in faster mass transfer. Moreover, we observed that the increasing flow rate from 1.0 to 1.5 L/hr causes low yield due to the low diffusivity at higher solvent flow rate trend to reduce the contact time between solid and liquid. In solid-liquid interaction, the rate of diffusion is time dependent. As per the Knudsen diffusion statement, parameters such as time, temperature and pressure are mainly considered for diffusion in solids. The time duration needed to contact between solid and liquid at higher flow of solvent is less. However, the yield was found as less at 1.5 lph when compared with 1 lph of solvent flow. To avoid longer extraction times, slower extraction rate and immense amount of final extracts, 1.0 L/hr solvent flow rate was selected as the most favorable value.

5. Comparison with Conventional Method

The *Adenanthera pavonina* seed oil extraction yield was found

Table 2. Fatty acid composition identified in the oil of *Adenanthera pavonina*

Sl. No.	Fatty acids	Carbon atom	Formula	Relative %	
				SHE	Soxhlet
1.	Hexadecanoic acid	C16:0	$C_{16}H_{32}O_2$	7.2	6.9
2.	Heptadecanoic acid	C17:0	$C_{17}H_{34}O_2$	1.4	1.3
3.	Stearic acid	C18:0	$C_{18}H_{36}O_2$	2.1	1.9
4.	Oleic acid	C18:1 *Cis*	$C_{18}H_{34}O_2$	22.4	22.8
5.	Elaidic acid	C18:1 *Trans*	$C_{18}H_{34}O_2$	0.3	0.2
6.	Linoleic acid	C18:2	$C_{18}H_{32}O_2$	49.8	50.3
7.	cis-10-Nonadecenoic acid	C19:1	$C_{19}H_{36}O_2$	1.2	-
8.	Arachidic acid	C20:0	$C_{20}H_{40}O_2$	0.9	0.6
9.	Gadoleic acid	C20:1	$C_{20}H_{38}O_2$	6.4	5.8
10.	Behenic acid	C22:0	$C_{22}H_{44}O_2$	0.3	-
11.	Erucic acid	C22:1	$C_{22}H_{42}O_2$	1.9	1.6
12.	Lignoceric acid	C24:0	$C_{24}H_{48}O_2$	3.2	3.4
13.	Nervonic acid	C24:1	$C_{24}H_{46}O_2$	1.7	1.4
14.	Hexacosanoic acid	C26:0	$C_{26}H_{52}O_2$	0.3	-
15.	Unknown	-	-	0.8	3.6
	Total fatty acids			99.9%	99.8%

Table 3. Properties of oil extracted from *Adenanthera pavonina*

Properties	Units	Values
Molecular weight of fatty acids	g/mol	285.86
Molecular weight if triglyceride	g/mol	895.63
Density	g/cc	0.916
Acid value	mg of KOH/g	8.6
Iodine value	-	89.1
Saponification value	mg of KOH/g	182.3
Free fatty acid (FFA)	wt%	4.3
Saturate fatty acids (SFAs)	Relative %	15.4
Unsaturated fatty acids (USFAs)	Relative %	85.7

to be 26.2 wt% and 19.7 wt% for superheated mixed solvent extraction and Soxhlet extraction, respectively. The maximum value was achieved for the superheated mixed solvent extraction and the smallest value was for the Soxhlet extraction method. The result shows that superheated extraction is more efficient than Soxhlet extraction with respect to the quantity of oil extracted and time required for extraction. Therefore, superheated extraction for higher scale of oil extraction from *Adenanthera pavonina* seed will be pertinent.

6. Characterization of Saga Seed Oil

The composition of fatty acid was found (by gas chromatography) as linoleic acid 49.8%, oleic acid 22.4%, hexadecanoic acid 7.2%, gadoleic acid 6.4% and remains shown in Table 2 and quantified in terms of relative percentage. From this fatty acid profile it has been observed that the unsaturated fatty acids (USFAs) were high as 83.7% when compared with saturated fatty acids (SFAs) found as 15.4%. Properties of *Adenanthera pavonina* such as molecular weight, acid value, iodine value, saponification value, and free fatty acid (FFA) were and shown in Table 3. The natural lipid and phospholipids present in saga seed oil was 86.2 wt% and 13.8 wt%, respectively, analyzed by thin layer chromatography.

CONCLUSIONS

Oil extraction from *Adenanthera pavonina* seed biomass using superheated mixed solvent process demonstrated superior results when compared with other conventional techniques. The mixture of hexane, chloroform and methanol as 3 : 2 : 1 volume ratio attained higher oil yield. The maximum oil yield was obtained as 26.2% with most favorable conditions of 1.0 L/hr solvent flow, temperature of 90 °C at 15 bar pressure, mean particle size of 0.25 mm and 120 min extraction time. Natural lipid rich as 86.2% was present in extracted oil from *Adenanthera pavonina* seed. This saga seed oil was found to be among the most excellent sources for biofuels production due to the higher natural lipids present in this oil.

REFERENCES

1. S. M. Sam, L. A. Akonye, S. I. Mensah and G. J. Esenowo, *Don. Scientia Africana*, **7**(2), 35 (2008).
2. K. R. Kirtikar and B. D. Basu, *Indian medicinal plants*, Int. Book Distributors, 2nd Ed., Dehradun, India, **2** (1991).
3. A. Ghani, Medicinal Plants of Bangladesh (Chemical Constituents and Uses), Asiatic Society of Bangladesh (2003).
4. I. H. Burkill, *A dictionary of the economic products of the malay peninsula*, 2nd Ed., A H. Government of Malaysia and Singapore, Kuala Lumpur, Malaysia, **1** (1966).
5. A. Gennaro, L. Merlini and G. Nasini, *Phytochemistry*, **11**, 1515 (1972).
6. A. M. Balogun and B. L. Fetuga, *Food Chem*., **17**, 175 (1985).
7. S. Sotheeswaran, M. R. Sharif, R. A. Moreau and G. J. Piazza, *Food Chem*., **49**, 11 (1994).
8. N. Yadav, G. Misra and S. K. Nigam, *Plant Med*., **29**, 176 (1976).
9. J. M. Lam, K. H. Pwee, W. Q. Sun, Y. L. Chua and X. J. Wang, *Plant Sci*., **142**, 209 (1999).
10. R. A. Moreira, C. C. Castelo-Branco, A. C. O. Monteiro, R. O. Tavares and I. M. Beltramini, *Phytochemistry*, **47**, 1183 (1998).
11. R. Zarnowski and Y. Suzuki, *J. Food Compos. Anal*., 649 (2004).
12. H. Abbasi, K. Rezaei, Z. Emamdjomeh and S. M. E. Mousavi, *Eur. J. Lipid Sci. Technol*., **110**, 435 (2008).
13. S. Liu, F. Yang, C. Zhang, H. Ji, P. Hong and C. Deng, *J. Supercritical Fluids*, **48**, 9 (2009).
14. M. H. Eikani and F. Golmohammad, *J. Food Lipids*, **16**(4), 514 (2009).
15. T. Suganya and S. Renganathan, *Bioresour. Technol*., **107**, 319 (2012).
16. M. H. Eikani, F. Golmohammad and S. S. Homami, *Food and Bioproducts Processing*, **90**, 32 (2012).
17. AOCS (American Oil Chemical Society), *Official methods and recommended practices of AOCS*, 5th Ed., American Oil Chemical Society, Champaign, IL, USA (1998).
18. G. Vicente, M. Martinez and J. Aracil, *Bioresour. Technol*., **92**, 297 (2004).
19. J. M. Luque-Rodriguez, M. D. Luque De Castro and Preez-Juan, *Talanta*., **68**, 126 (2005).
20. X. Han, L. Cheng, R. Zhang and J. Bi, *J. Food Eng*., **92**, 370 (2009).