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Full Length Research Paper

Extraction and analysis of *Jatropha curcas* L. seed oil

Patel Shivani[1]*, Patel Khushbu[1], Nilkanth Faldu[1], Vasudev Thakkar[2] and R. B. Shubramanian[2]

[1]Department of Biotechnology, Shree M. & N. Virani Science College, Rajkot, India-360005.
[2]BRD School of Sciences, Sardar Patel University, Vallabh Vidhyanagar, India.

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***Jatropha curcas* L. is a multipurpose shrub with a variety of applications and enormous economic potentials for its seed oil, which can be converted into biodiesel- an alternative to petro-diesel. It aims to overcome energy crisis problem and also to reduce environmental changes. The fact that the oil of *J. curcas* cannot be used for nutritional purposes without detoxification makes its use as an energy source for fuel production very attractive. The present study deals with oil extraction by various methods and its physico-chemical analysis. In this study, extraction of Jatropha oil from seeds was optimized using organic solvents. The effects of parameters on the oil extraction namely type of organic solvents and different techniques were also investigated to optimize the processing conditions for achieving maximum oil yield. The acid value and antioxidant property of the oil were also investigated. The maximum oil yield was obtained by using Soxhlet extraction method and hexane as a solvent. Petroleum ether was found to be the best solvent; though its yield is lesser when compared with hexane and isopropanol. This is because it gives pure and colorless oil, whereas hexane gives faint yellow color oil. The acid value of oil was found to be 2.24 ± 0.01 mg KOH/g. The scavenging activity of leaf and oil extract was found to be 29.92 ± 4.72 and 19.94 ± 1.39%, respectively. The oil can also be used as a source of antioxidants.**

Key words: Biodiesel fuel, oil, Soxhlet extraction, antioxidants.

INTRODUCTION

In recent times, the world has been confronted with an energy crisis due to depletion of resources and increased environmental problems. This situation has led to the search for an alternative fuel, which should be not only sustainable, but also environmental friendly (Barnwal and Sharma, 2005). *Jatropha curcas* is considered to be the best sustainable source. It is a multipurpose, drought resistant shrub belonging to the family of Euphorbiaceae. The seeds of *J. curcas* are considered to be the good source of lipids. They contain approximately 20 to 39% of oil and this makes their use as an energy source for fuel production very attractive. In addition to this, the oil from its seeds has been found useful for manufacture of candles and soap, in cosmetic industry and also as for medicinal purposes (Akbar et al., 2009; Gubitz et al., 1999). Various methods for recovering (extracting) this oil from the seeds have been investigated. Conventional method such as solvent extraction is the most widely used technique, owing to their high efficiency in oil recovery (90 to 98%). But the major disadvantage in using solvent extraction technique is its high energy input and toxicity of solvent. This has lead to the development of enzyme-based techniques (Sharma et al., 2002).

Aqueous enzymatic oil extraction (AEOE) is a promising technique for extraction of oil from Jatropha seeds. The presence of certain enzymes during extraction enhances oil recovery by breaking cell walls and oil bodies. As plant cell walls have a complex structure, different enzymatic preparation is required to break up the cell wall. This process is eco-friendly and does not produce volatile organic compounds as atmospheric pollutants. The major disadvantage associated with AEOE is the long process time necessary for the enzymes to liberate oil bodies. Another factor is the use of enzymes which are not commercially available (Gupta et al., 2005). This prevents the use of this method

*Corresponding author. E-mail: admin@bt.vsc.edu.in or smpatel@vsc.edu.in. Tel: +919825394037 or (0281) 2562681 Ext. 2233. Fax: (0281) 2563952.

in regular day to day life.

The physico-chemical properties of the oil are very important for its future use. For biodiesel production, the amount of free fatty acids in the oil should be less than 1%, if not, these FFAs react with the alkaline catalyst during transesterification to produce soaps instead of esters (Akbar et al., 2009). In addition, the amount of free fatty acid present is a useful parameter which gives an indication about the age and extent of deterioration of oil. Reactive oxygen species (ROS) or free radicals are an entire class of highly reactive molecules derived from the metabolism of oxygen. They cause oxidation of biomolecules which leads to cell injury and death. Their deteriorative effects can be diminished by natural antioxidants available in foods (Aqil et al., 2006). Antioxidants retard lipid peroxidation, which is one of major processes producing deterioration of food during processing and storage. Thus, there is an increased quest to obtain natural antioxidants with broad spectrum.

Thus, the present study deals with extraction of oil by use of solvent extraction and enzymatic extraction techniques. The parameters taken into consideration were the type of organic solvent and type of separation/extraction technique. The physico-chemical property of the oil was also analyzed.

MATERIALS AND METHODS

J. curcas seeds were collected from the nursery of Shree M. & N. Virani Science College, Rajkot, India. Damaged seeds were discarded and a healthy and clean seeds were selected. The seeds were dried for sometime before use. All other chemicals and solvents used were of analytical grade.

Oil extraction

Solvent extraction techniques

J. curcas L. seeds were cracked and the shells were carefully removed. The kernels thus obtained were used for oil extraction. The kernels (1 g) were grounded by using mechanical method (mortar and pestle). The oil was extracted by using different organic solvents (20 ml) such as petroleum ether, hexane and isopropanol. The extracted lipid was obtained by different techniques such as filtration, centrifugation and separating funnel; in order to get rid of the solid from solvent before the solvents was removed. Extracted seed oil was stored in freezer at -20°C for subsequent physicochemical analysis.

Aqueous enzymatic oil extraction (AEOE) from seed kernels

J. seeds (5 g) were soaked in water for 2 h. The soaked seeds were ground (without addition of water to the soaked seeds) to a thick paste by using mortar and pestle. This paste was then dispersed in distilled water at 1:2 (w/v) paste to water ratio followed by gentle stirring with a magnetic stirrer. Cellulase (250 mg) (Himedia 15FTU/ml) was added before the pH of the slurry was adjusted to 4 by adding appropriate amount of 0.5 N HCl or NaOH. The enzyme mixture was then incubated overnight at 40°C with constant shaking at 100 rpm. The upper oil phase was collected by centrifugation at 10,000 x g for 20 min. A control (aqueous oil extraction, AOE) was also carried out for the earlier mentioned extraction during which no enzyme was added (Sharma et al., 2002).

Soxhlet extraction

The method described by Akbar et al. (2009) with slight modification was used. The seed kernels (3 g) were grounded using a mechanical method and defatted in a soxhlet apparatus. The extraction was carried out by using three different solvents such as hexane, isopropanol and petroleum ether. The process continued for 6 h. Solvent was removed by vacuum evaporation and exposure to heat in a drying oven at 50°C. The amount of oil recovered was calculated as percentage of total oil present in *J. curcas* seed kernels. Each extraction was run in triplicate and the final value is the average of all.

Acid value, %FFA

The acid value of the oil was determined by dissolving 5 g oil sample in 25 ml fat solvent (95% ethanol: ether; 1:1). The earlier mentioned solution was titrated with 0.1 N KOH using phenolphthalein as an indicator. The titration was carried out until faint pink colour appears. Note that the volume of KOH carried out similar experiment for blank which do not contain any sample. Acid value is calculated as per the standard formula given by Sadashivam et al. biochemical methods, II edition.

Antioxidant assay

Preparation of extract

Fresh leaves of *J. curcas* were collected from the nursery of Shree M. & N. Virani Science College, Rajkot. The extract of leaves and oil was prepared by using methanol and the extract was filtered. The solvent present in the filtrate was evaporated by using rotary evaporator. The amount of dried extract was measured and then used for antioxidant assay.

DPPH (1,1- diphenyl picryl hydrazyl) free radical scavenging activity

The method described by Coruh et al. (2007) was used. A 0.05 mg/ml of DPPH (1,1- diphenyl picryl hydrazyl) methanol solution which absorbs at 517 nm was used. The extract solution of 1 mg/ml concentration was prepared by dissolving the dried extract of leaf and seeds in methanol and 0.1 ml of solution from extract were added to 1.4 ml of DPPH solution. The absorbance at 517 nm was recorded after 5 min of incubation at room temperature. Radical scavenging capacity of the extract was calculated as the % DPPH radical scavenging effect which is:

DPPH scavenging effect (%) = [(A0- A1) / A0 x 100]

Where, A0 is the absorbance of control with methanol and A1 is the absorbance of sample.

RESULTS

Oil extraction

The amount of oil recovered was calculated as % of total

Table 1. Extraction of oil by use of various solvents.

Organic solvent	Extraction method (% yield)			
	Separating funnel	Centrifugation	Filtration	Soxhlet extraction
Petroleum ether	28.6 ± 4.16	48.3 ± 0.57	10.3 ± 4.0	53.66± 1.05
Isopropanol	19.66 ± 2.3	53.33 ± 1.52	15.66 ± 4.0	59.66 ± 2.5
Hexane	36 ± 9.53	52.66 ± 4.04	20 ± 6.2	78.66 ± 0.9

Table 2. Free radical scavenging activity (DPPH method).

Solvent used	DPPH scavenging (%)	
	Leaf extract	Seed oil
Methanol	29.92 ± 4.72	19.94±1.39

oil present in *J. curcas* seed kernels. Three different solvents were used to evaluate their efficiency. The oil extraction capabilities of petroleum ether, hexane and isopropanol are shown in Table 1.

Table 1 shows the comparative data of oil extracted by use of various solvents and extraction method. The extraction yield with petroleum ether was found in range of 10 to 53%. Similarly, the extraction yield with hexane was found to be in the range of 20 to 78% and with isopropanol was found to be near 15 to 59%. The best result is obtained by using hexane and soxhlet extraction method. Use of aqueous oil extraction (AOE), as control was found to yield 21.4% oil. The same data that is, AOE yield in the range of 17 to 21% was reported by other authors (Gupta et al., 2005). In AEOE method, the enzymes used such as cellulase and pectinase failed to extract large amount of oil. Their yield is very less (in the range of 2%) when compared with that described by other authors (Gupta et al., 2005).

Physico-chemical property of oil

The physical property that is, colour of the oil extracted by different organic solvents was also studied. All solvents except hexane yields colorless oil and without impurities. Hexane yield faint yellow colour oil. The chemical property that is %FFA content of the oil was found to be 2.24% (mg KOH/g). The free radical scavenging activity, expressed in percentage inhibition of the leaf and oil extract of *J. curcas* is shown in Table 2.

DISCUSSION

Figure 1 shows the comparison between different techniques of oil extraction and solvents used. The results reveal that extraction by Soxhlet method yield oil in the range of 53 to 78%. This value was taken as 100% recovery of oil, while calculating the oil recovery by other methods. Soxhlet extraction is better when compared with other methods, because the process is continuous and there is complete oil recovery.

Among the solvents, hexane is considered as the best solvent because its yield is maximum when compared with other solvents (Sepidar et al., 2009). But, the oil recovered by hexane and isopropanol is slightly yellow in colour. This might cause problem in using oil for further biodiesel production. Thus, it is better to use petroleum ether for efficient biodiesel production. In AEOE, enzymes are used to facilitate the release of oil from oil bodies enmeshed in protein and cellulosic/hemicellulosic networks. Use of only cellulase and pectinase did not improve the yield of oil. For better oil yield, additional enzyme preparation such as Protizyme, Pectinex Ultra SP-L, Promozyme, etc. are required (Gupta et al., 2005). Experimental result showed that Jatropha oil seeds have FFA content 2.24%. The FFA and moisture contents have significant effects on the transesterification of glycerides with alcohol using catalyst. The high FFA content (> 1% w/w) will happen to soap formation and the separation of products will be exceedingly difficult and as a result, it has low yield of biodiesel product. A two-step process was investigated for feedstock having the high FFA content (Akbar et al., 2009).

DPPH is a free radical which accepts an electron or hydrogen radical to become a stable molecule. It is reported that the decrease in the absorbance of DPPH radical is due to the reaction between antioxidant molecules and radicals, resulting in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from purple to light purple. The result reveals that leaf of *J. curcas* contains more amounts of indigenous antioxidants. Hence, it shows highest antioxidant property.

Conclusion

The soxhlet extraction method gave higher oil yields than

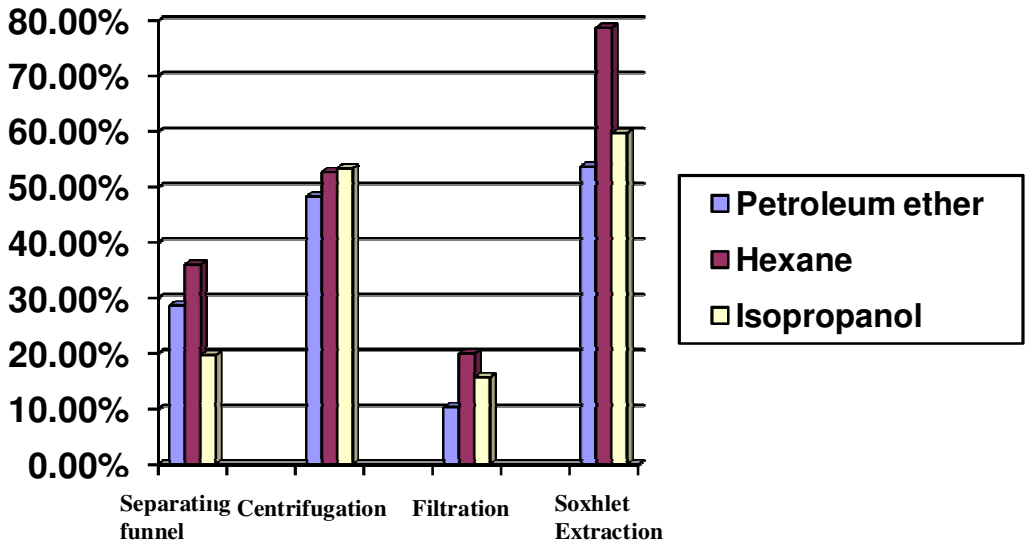

Figure 1. Effects of solvents and extraction technique in % oil yield from *Jatropha* seed kernels.

other extraction methods. This was probably due to continuous extraction for approximately 6 h. The titration method for FFA determination was faster, efficient and sensitive.

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