Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Asian Journal of Plant Science and Research, 2014, 4(1):28-35

ISSN : 2249-7412
CODEN (USA): AJPSKY

Comparative study of extraction methods and properties of non edible oils for biodiesel production

Sirangala Thimappa Girisha*, Krishnappa Ravikumar, Bangalore Radhakrishna Mrunalini and Venkatachalapathy Girish

Department of Microbiology and Biotechnology, Jnanabharathi campus, Bangalore University, Bangalore

---

ABSTRACT

Due to the limited availability of fossil fuel and environmental problems, considerable attention has been given to biodiesel production as an alternative to petro diesel. In this connection the non-edible seeds of Caryota urens, Bassia longifolia and Saraca asoca were analysed for their oil properties and fatty acids. Four different methods are tried for oil extraction among them physical method is more suited compared to Bligh and Dyer, Folch et al. and Chemical solvent extraction method and among the three seeds B. longifolia yields more oil (40mg/g) compared to S. asoca (35gm/g) and C. urens (25gm/g) and the physico-chemical properties of the oil from B. longifolia seeds was found to be acid value 6.5mg KOH/g, Iodine value 114g/100g and viscosity mm^2 /sec – 38.2 which perform better for biodiesel production compared to the other two and among fatty acids oleic acid is also higher in B. longifolia (69%); hence B. longifolia is a better non-edible oil seed for biodiesel production than C. urens and S. asoca.

Key words: Non edible oil, fatty acids, biodiesel

INTRODUCTION

Today 86% of the world energy consumption and almost 100% of the energy needed in the transportation sector is met by fossil fuel [1]. The production and consumption of fossil fuels have caused the environmental damage by increasing the CO_2 concentration in the atmosphere [2]. A rapid increase in biodiesel production capacity and governmental mandates for alternative fuel usage around the world in the last several years has necessitated the development of alternative biodiesel feedstocks [3]. In USA and Europe their surplus edible oil is being used as feed stock for the production of biodiesel [4]. It is believed that large-scale production of biodiesel from edible oils may bring global imbalance to the food supply hence the environmentalists started to debate on the negative impact of biodiesel production from edible oil [5]. Hence, as a solution for the competition with food versus fuel crisis, the non-edible vegetable oils are found to be suitable for biodiesel production under the experimental conditions [6].

Biomass Energy grown for production of transport fuels is obtained from the naturally growing plants and there are a number of plants which produce seeds containing high percent of oil that could be used as biofuel. Fortunately, India with its rich biodiversity has more than 150 species of plants yielding oil which could be commercially exploited for production of energy. Biofuels is gaining immense importance and awareness across the globe because it is providing energy security and employment to rural community and improved income generation. The advantages of non-edible plants in biofuel production are that they can be cultivated along the borders and bunds of crop lands / habitations / road sides/ ravines and the unutilized barren lands where access is available to the farming community with access to land resources, decentralization for every household to grow the non-edible oil seed

plants in the farm vicinity, on borders, bunds, backyards, ravines, where people have access to monitor and collect the produce easily facilitates in the easy procurement of oil seed.

As proposed in the present study, the production of biodiesel from non edible sources has a greater advantage as their characterisation is very much necessary towards achieving and improving the quality of the renewable fuel. Extensive work has been done on the transesterification of non edible oils; however, no significant work has been done on, Collection of different non-edible seeds from different regions across Karnataka, Optimization of the extraction process for oil separation and oil characterization. Since, the studied seeds are available throughout the year there is no difficulty in procuring the seeds. Hence, the non edible plant seeds collected for the present study are *Caryota urens* (Arecaceae), *Bassia longifolia* (Sapotaceae) and *Saraca asoca* (Caesalpiniaceae).

MATERIALS AND METHODS

The non edible oils used in this study are produced locally in Bangalore. The seeds were collected from University of Agricultural Sciences, GKVK campus, Bangalore. The chemicals used in the experiments are procured from Himedia chemicals, Mumbai. The standards were procured from Sigma-Aldrich Chemical Co Ltd.

Extraction of oil: From three non edible oil seed (*Caryota urens*, *Bassia longifolia* and *Saraca asoca*) the oil is extracted.

Physical Method: Seeds were expelled by using oil expeller and the resulted oil, seed meal are separated to study the effect of different solvents on the extraction process, the common methods like Bligh and Dyer Method, Folch et al. method and Chemical solvent extraction were followed and the resultant oil were analyzed for their physicochemical and fatty acid percentage.

Bligh and Dyer Method: The seed sample of about 1 gram from each plant were taken and grinded using a mortar and pestle, later directly added to the glass tube and smashed with glass rod. With the addition of 20mL of (2:1 ratio) Methanol: Chloroform, again smashed with glass rod. The above mixture is vortexed for 20 minutes. Then, 10 ml chloroform, 18 ml water was added and mixed well. The upper layer (aqueous phase) containing some polar lipid species like acyl-CoA is discarded. The lower layer (organic phase contains TAGs, membrane lipids and other neutral lipids) is collected and transferred to a new tube. Then, the lipid extract is dried under N_2 and dissolved in 2ml 1:1 chloroform: methanol [7].

Folch et al. method: About 1gram of seed samples were homogenized with 20mL of chloroform/methanol (2/1) to a final volume 20 times the volume of the tissue sample (1 g in 20 ml of solvent mixture). After dispersion, the whole mixture is agitated for 15-20 min in an orbital shaker at room temperature. The homogenate is either filtrated or centrifuged to recover the liquid phase. The solvent is washed with 0.2 volumes (4 ml for 20 ml) of water or 0.9% NaCl solution. After vortexing for 10-30 seconds, the mixture is centrifuged at low speed (2000 rpm) to separate the two phases. The upper phase is removed by siphoning and kept it to analyze gangliosides or small organic polar molecules. The interface is rinsed one or two times with methanol/water (1/1) without mixing the whole preparation. After centrifugation and siphoning of the upper phase, the lower chloroform phase containing lipids is evaporated under vacuum in a rotary evaporator [8].

Chemical solvent extraction method: Total oil content can be extracted using chemicals such as benzene, ether or hexane. Soxhlet extraction is an extraction method that uses chemical solvents. Oils from the seeds are extracted through repeated washing or percolation with an organic solvent such as hexane or petroleum ether under reflux in special glassware (extraction chamber).

Determination of the physic- chemical properties of the Oil: AOAC standard methods were used to determine the physical and chemical properties of the oil like Acid value, Density/ Specific gravity, Iodine value, Saponification value and Viscosity [9].

Determination of fatty acid composition: Analysis of Fatty acids was carried out on Gas Chromatograph [10]. The GC was equipped with Flame Ionization Detector (FID) and stainless steel column, dimension 10 X 1/8, packed with 5 % DEGS-PS. The column was conditioned at 180^0C about 2 hours for attaining thermal stability before use. About 10µL of sample dissolved in hexane was loaded onto the column. The operating condition was programmed

at oven temperature 150^{0}C (hold time 5min) with increasing rate 8^{0}C/min to190^{0}C (hold time 0 min), 2^0 C/min to 200^0 C (hold time 10min), injection temperature 250^{0}C and detector temperature 250^0 C. Nitrogen was used as a carrier gas with flow rate of 20 ml/min.

The concentration of individual fatty acids in the test samples were determined by comparing the peaks obtained from the GC analysis with the peaks of authentic standards and n-heptane was used as an internal standard. All tests were performed in triplicate; the experimental design was completely randomized with three replicates. All data were expressed as mean values ± SE. The comparison between the mean values were tested using Duncan's new multiple range test and the ANOVA was also performed to find out the LSD (p = 0.05).

RESULTS AND DISCUSSION

The oil contents of *Caryota urens*, *Bassia longifolia* and *Saraca asoca* show that their use for biodiesel production would be highly economical and had agreeably high oil content and the physical state of all the oils was liquid at room temperature.

Table 1: Oil properties and types of fatty acids in *Caryota urens* (Arecaceae)

Physical Method (mg/g)	Bligh and Dyer Method (mg/g)	Folch et al. method (mg/g)	Chemical solvent extraction method (mg/g)	Physical and Chemical Properties	Fatty acid Percentage (GC analysis)
25	20.0	22.0	21.0	Acid value (mg KOH/g) 8.0 Density/ Specific gravity (g/cm^3) 0.924 Iodine value (g/100 g) 107 Saponification value (mg KOH/g) 185.5 Viscosity (mm^2/sec) 40.2	Arachidic - 2 Behenic -2 Eicosenoic - 1 Lignoceric - 3 Linoleic- 15 Linolenic - 2 Oleic - 66 Palmitic -6 Stearic - 3.0

Values are the means ±SE of three replicates each. Data were subjected to analysis of variance and compared for significance according to DMRT (P=0.05).

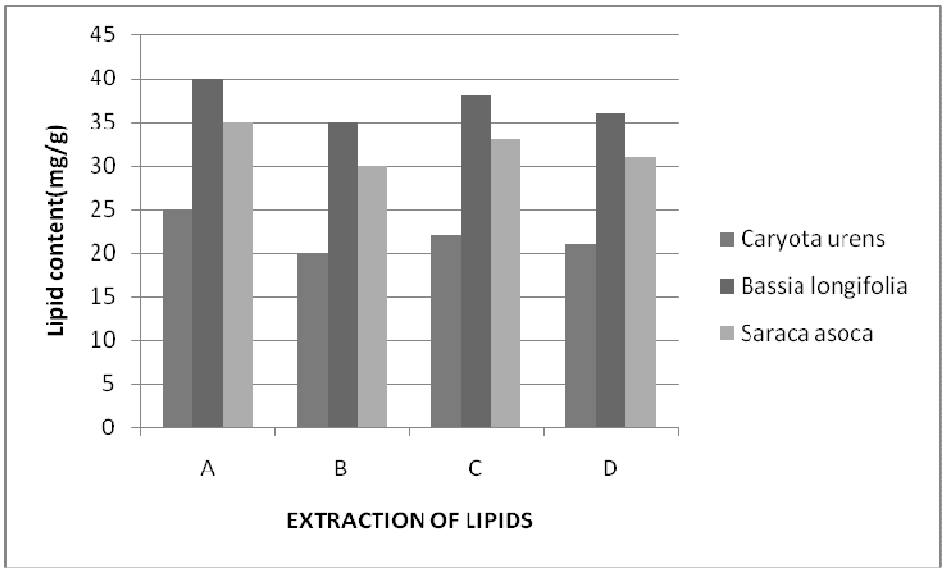

Figure 1: Comparison of the lipid content from different methods
A: Physical Method, B: Bligh and Dyer Method, C: Folch et al. method, D: Chemical solvent extraction method

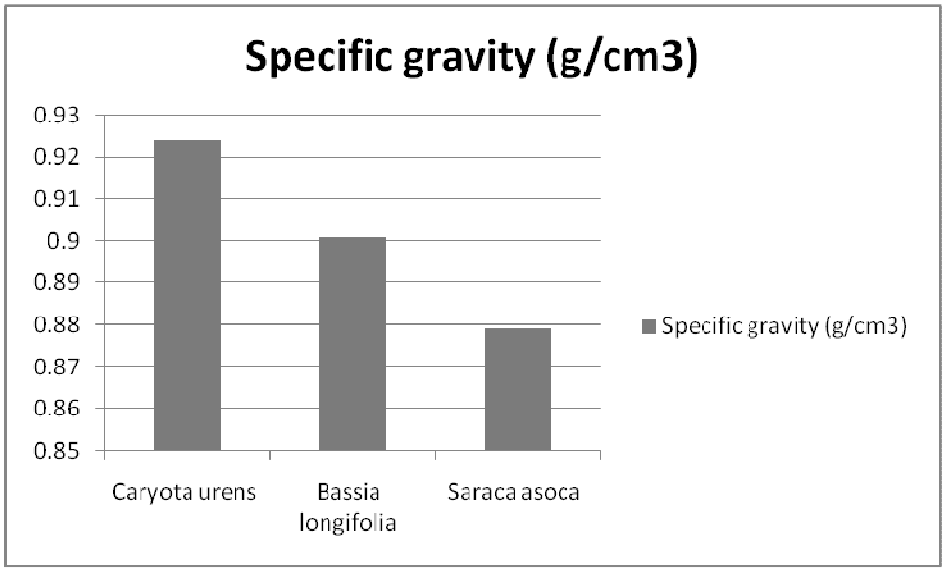

Figure 2: Comparison of the extracted lipid's Specific gravity

In *C. urens* the oil extraction by physical method is 25 mg/g, by Bligh and Dyer method 20mg/g, by Folch et al. method 22 mg/g and in Hexane extraction 21 mg/g (Table 1, Figure 1). The oil was yellowish in colour and had a specific gravity of 0.924 g/cm^3. Density and other gravities are important parameters for diesel fuel injection systems the values must be maintained within tolerable limits to allow optimal air to fuel ratios for complete combustion. High-density biodiesel or its blend can lead to incomplete combustion and particulate matter emissions [11] (Table 1, Figure 2).

The acid value was found to be 8.0 mg KOH/g which is an acceptable range for further use, acid value measures the presence of corrosive free fatty acids and oxidation products, this is actually an important variable in considering the quality of oil because the lower the free fatty acid, the better the quality of oil, the acceptable limit for edible oils is ≤10 [12] (Table 1).

Iodine value is 107 I_2g/100g; High iodine value indicates high unsaturation of fats and oils [13]. Oils with iodine value above 125 are classified as drying oils; those with iodine value 110–140 are classified as semidrying oils [14]. The higher I_2 value indicates that the oil has high unsaturated fatty acids which are reflected in the GC analysis (Table 1).

Table 2: Oil properties and types of fatty acids in *Bassia longifolia* (Sapotaceae)

Physical Method (mg/g)	Bligh and Dyer Method (mg/g)	Folch et al. Method (mg/g)	Chemical solvent extraction method (mg/g)	Physical and Chemical Properties	Fatty acid Percentage (GC analysis)
40	35.0	38.0	36.0	Acid value (mg KOH/g) 6.5 Density/Specific gravity (g/cm^3) 0.901 Iodine value (g/100 g) 114 Saponification value (mg KOH/g) 182 Viscosity (mm^2/sec) 38.2	Arachidic - 1 Behenic - 2 Eicosenoic - 5 Lignoceric - 2 Linoleic- 10 Linolenic - 2 Oleic - 69 Palmitic - 4 Stearic - 5

Values are the means ±SE of three replicates each. Data were subjected to analysis of variance and compared for significance according to DMRT (P=0.05).

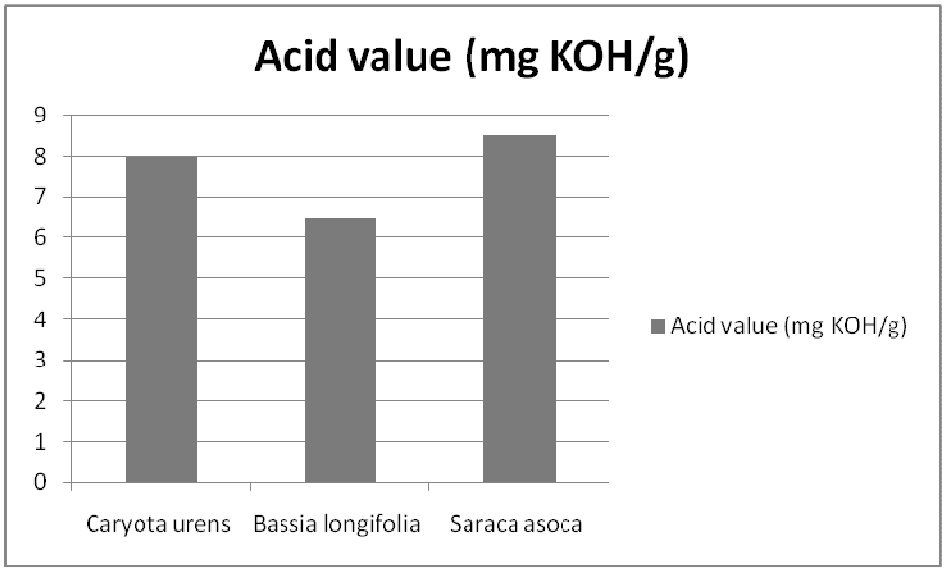

Figure 3: Comparison of the extracted lipid's Acid Values

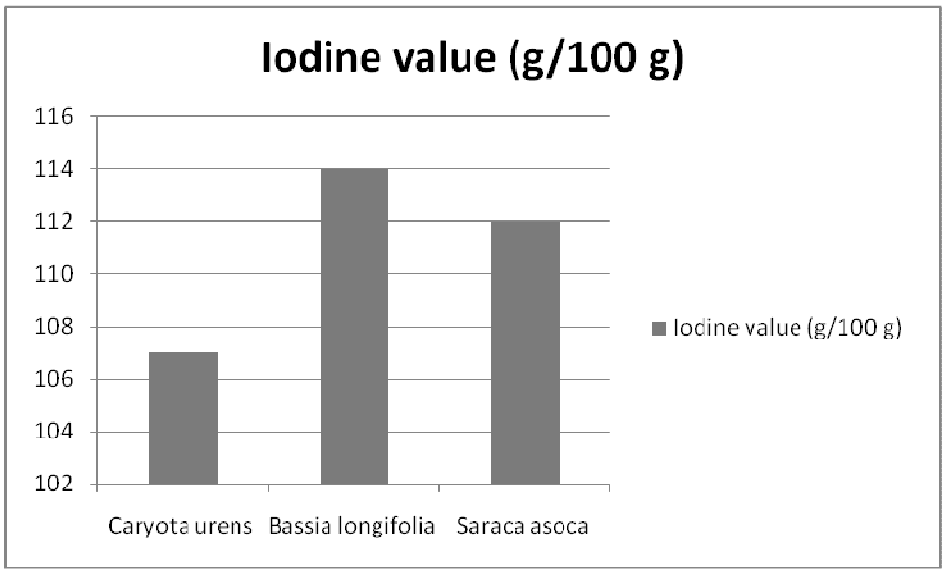

Figure 4: Comparison of the extracted lipid's Iodine Value

The saponification value is 185.5mgKOH/g; this was lower than other oil such as Jathropa (196mgKOH/g), Karanja oil (186mgKOH/g) and Mahua (190mgKOH/g). However, this saponification value falls just below the range expected of some non edible oils reported by [15]. The low saponification is an indication of the oil which may not be suitable for detergent (Table 1).

The viscosity was determined to be 40.2 mm^2/Sec. High viscosity is the major problem preventing the use of vegetable oils and animal fats directly in diesel engines as it affects the flow of fuel and spray characteristics [16] (Table 1) and the fatty acid percent varied from Arachidic – 2, Behenic -2, Eicosenoic – 1, Lignoceric – 3, Linoleic-15, Linolenic – 2, Oleic – 66, Palmitic -6 and Stearic - 3.0 (Table 1).

The oil extraction from the seeds of *B. longifolia* was also done by four different methods the oil yield by the Physical method was found to be 40 mg/g, 35mg/g by Bligh and Dyer method, 38mg/g by Folch et al. method and 36 mg/g by hexane extraction (Table 2). Although no major differences were observed in Physical methods and

extraction with solvent seems more efficient and economical, this is in conformity with the observations of [17]. The oil was pale yellow colour and the specific gravity is 0.901 g/cm^3 (Table 2).

Table 3: Oil properties and types of fatty acids in *Saraca asoca* (Caesalpiniaceae)

Physical Method (mg/g)	Bligh and Dyer Method (mg/g)	Folch et al. method (mg/g)	Chemical solvent extraction method (mg/g)	Physical and Chemical Properties	Fattyacid Percentage (GC analysis)
35	30.0	33.0	31.0	Acid value (mg KOH/g) 8.5 Density/ Specific gravity (g/cm^3) 0.879 Iodine value (g/100 g) 112 Saponification value (mg KOH/g) 182 Viscosity (mm^2/sec) 41.2	Arachidic - 3 Behenic - 2 Eicosenoic - 3 Lignoceric - 4 Linoleic- 8 Linolenic - 4 Oleic - 60 Palmitic - 10 Stearic - 6

Values are the means ±SE of three replicates each. Data were subjected to analysis of variance and compared for significance according to DMRT (P=0.05).

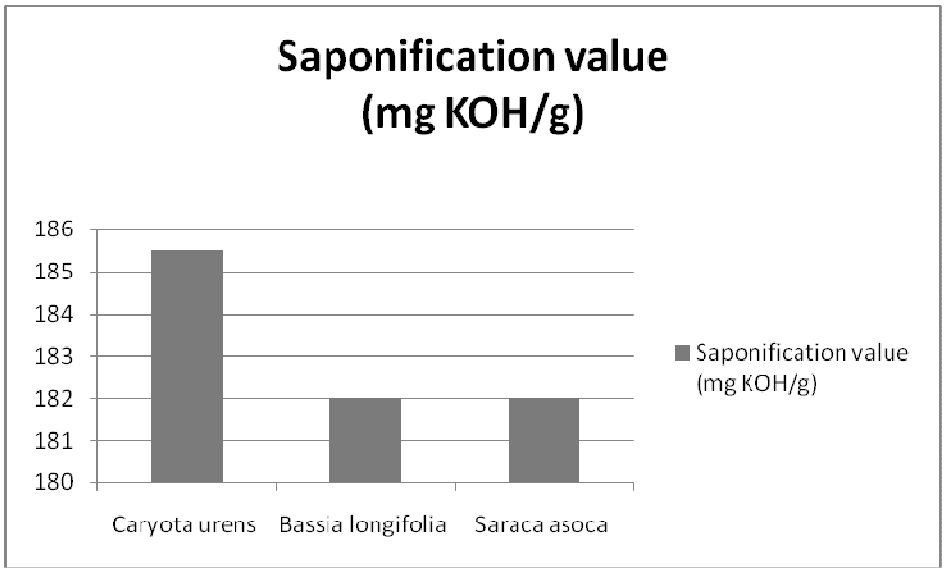

Figure 5: Comparison of the extracted lipid's Saponification values

The acid value was found to be 6.5 mg KOH/g which is an acceptable range for further use (Table 2 Figure 3).The I_2 value is 114g/100g; hence the oil composed a high concentration of unsaturated fatty acids which is evident in the GC analysis (Table 2 Figure 4).

The saponification value of *B longifolia* is 182 mg KOH/g; the lower value is an indication that the oil property may not support other usage (Table 2). The viscosity is 38.2mm^2/Sec (Table 2).The fatty acid percent varied from Arachidic – 1, Behenic – 2, Eicosenoic – 5, Lignoceric - 2, Linoleic- 10, Linolenic - 2, Oleic – 69, Palmitic – 4 and Stearic – 5 (Table 2). The high percentage of Oleic acid combined with the other properties mentioned above suggests that the oil makes a good biodiesel.

Similarly the oil extraction from the seeds of *S. asoca* was found to be 35 mg/g, 30mg/g, 33mg/g and 31 mg/g and the oil extracted was dark coloured (Table 3). The specific gravity is 0.879g/cm^3 (Table 3). The acid value was found to be 8.5 mg KOH/g (Table 3) which is an acceptable range for further use. The Iodine value is 112 I_2/100g (Table 3). Saponification value 186 mg KOH/g (Table 3 Figure 5) and the viscosity 41.2 mm^2/Sec (Table 3 Figure 6). The fatty acid percentage was Arachidic – 3, Behenic – 2, Eicosenoic – 3, Lignoceric - 4, Linoleic- 8, Linolenic - 4,Oleic – 60, Palmitic – 10 and Stearic – 6 (Table-3).

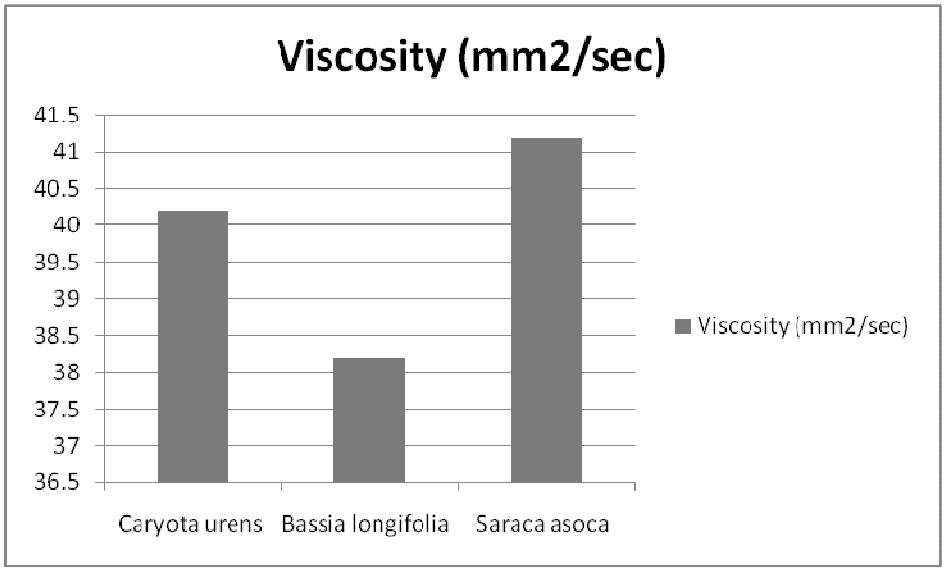

Figure 6: Comparison of the extracted lipid's Viscosity

CONCLUSION

Among the three seeds *B. longifolia* was found to be the most oil yielding non-edible oil seed than the other two and was found to be a better choice with respect to the total oil content, acid value, iodine value and viscosity where the oil was extracted by Physical method, *S. asoca* comparatively yielded oil with lower density and lesser saponification values and in *C. urens*, the oil characteristic values are not as significant compared to the other two seeds. The fatty acid content varied in all the three oil seeds, in which the oleic acid content was significant for oil quality which was higher in *B. longifolia* making it a good resource for biodiesel production, hence, among the selected three non-edible oil seeds *B.longifolia* has been proved to be a better resource for oil production than *S. asoca*, which is better than *C. urens*.

Acknowledgements
The authors are thankful to Bangalore University for providing infrastructural facility.

REFERENCES

[1] Dorian JP, Franssen HT, Simbeck DR, *Energy Policy*, **2006**, 34, 1984.
[2] Peter Westermann, Betina Jorgensen, Lene Lange, Birgitte K. Ahring, Claus H. Christensen, *International Journal of Hydrogen Energy*, **2007** 32,4135.
[3] Bryan R. Moser, *In Vitro Cell. Dev. Biol. Plant*, **2009**, 45,229.
[4] Ramadhs AS, Jayaraj S, *Renewable Energy*, **2004**, 29, 727.
[5] Butler RA, Why is oil palm replacing tropical rainforests? Why are biofuels fueling deforestation?, **2006**, http://news.mongabay.com/2006/0425-oil_palm.html.
[6] Meher L, Kulkarni M, Dalai A, *Eur J of Lipid Sci Technol*, **2006**, 108, 389.
[7] Bligh EG, Dyer WJ, *Can.J.Biochem.Physiol*, **1959**, 37,911.
[8] Folch J, Lees M, Sloane Stanley GH, *J Biol Chem*, **1957**, 226:497.
[9] AOAC, Official methods of analysis if the analysis of the Association of Official Analytical Chemists 15th Edition. Association of Official Analytical Chemists. Washington DC. **1990**.
[10] Chowdhury K, Banu LA, Khan S, Latif A, *Bangladesh J. Sci. Ind. Res*, **2007**, 42, 311.
[11] Galadima A, Garba ZN, Ibrahim BM, *Inter J of Pure App Sci*, **2008**, 2, 138.
[12] Balley AE, Industrial Oil and Fat Product, John Wiley-Interscience, New York, NY, USA, 3rd edition. **1982**.
[13] Knothe G, *Journal of the American Oil Chemists' Society*, **2002**, 79(9), 847.
[14] Cynthia Nkolika Ibeto, Chukwuma Obiajulu Benedict Okoye, Akuzuo Uwaoma Ofoefule, Comparative Study of the Physicochemical Characterization of Some Oils as Potential Feedstock for Biodiesel Production, **2012**.

[15] Singh RK, Padhi SK , *Natural Product Radiance*, **2009**, 8, 27.
[16] Hossain AK, Davies PA, *Renewable Energy*, **2010**, 35, 1.
[17] Ajiwe VSE, Umerie SC, Okeke CA, Oburota VN, *Biores Technol*, **1984**, 47, 85.