

**CHEMICAL ANALYSIS OF SEED AND OIL OF *JATROPHA CURCUS L*' A BIOFUEL PLANT
CULTIVATED IN HYDERABAD KARNATAKA REGION, NORTH KARNATAKA.**Tathagat Waghmare¹ and G. R. Naik*

Department of Biotechnology, Gulbarga University Gulbarga-585106, Karnataka, India.

Email ID – biotechnist@gmail.com

ABSTRACT: *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. Oil content of *Jatropha curcas L.* was subjected to various physicochemical parameters. The chemical parameters include determination of free fatty acids, peroxide value, Iodine value, saponification value, various physiological analysis of *Jatropha curcas L.* plant was also carried out in order to estimate the presence of total content of chlorophyll. By considering all the above parameters the present study reveals that the *Jatropha curcas L* plant is good for production of better quality of biodiesel.

Key words: Biodiesel, *Jatropha Curcus L*, Chemical properties, SDS-PAGE, Soxhlet extraction.

INTRODUCTION

J. curcas is native of tropical America, but is now found abundantly in many tropical and sub-tropical regions throughout Africa and Asia. *J. curcas* has spread beyond its original distribution because of its hardiness, easy propagation, drought endurance, high oil content, low seed cost, short gestation period, rapid growth, adoption to wide agro-climatic condition, bushy/shrubby nature and multiple uses of different plant parts.

Currently due to gradual depletion of world petroleum reserves and the impact of environmental pollution of increasing exhaust emissions, there is an urgent need to develop alternative energy resources, such as biodiesel. India consumes approximately 40 million tons of diesel and ranked 5th in the world after the US, China, Russia and Japan in terms of fossil fuel consumption. Recently, Government of India launched National Mission on Bio-diesel for a cheap and renewable liquid fuel based on vegetable oils. However, shortage of raw material to produce bio-diesel is a major constraint. The total number of oil-bearing species range from 100 to 300 and of them 63 belonging to 30 plant families hold promise for bio-diesel production. *Jatropha curcas* is one of the promising species for biodiesel production. It is necessary to investigate the production studies in terms of yield and quality of oil in different agroclimatic conditions. During present work, the results of seed studies in semiarid condition of Gulbarga are reported.

MATERIAL AND METHODS**Oil extraction.**

The seed kernels were ground, using a mechanical grinder, and defatted in a soxhlet apparatus, using hexane (boiling point of 40–60°C). Extracted seed oil was stored in freezer at –2°C for subsequent physicochemical analysis. The chemical analysis of seed oil such as oil content, Free fatty acid value, peroxide value, Iodin value, saponification value and the physical properties of *Jatropha curcas* seeds such as Moisture content, seed length, seed width and seed thickness measurement was carried out by vernier caliper.

Identification of Lipid profile in a given sample by thin layer chromatography

In biological material, lipids are found as lipoprotein complexes and these have to be extracted. Lipids, being soluble in non-polar organic solvents and proteins being soluble in polar aqueous solvents, the efficient lipid extraction can be achieved only when an aqueous solvents like ethanol or methanol is included in the non-polar organic solvents like chloroform and diethyl ether. This would help in breaking the lipoprotein complexes. Extracted lipid components can be separated on TLC based on their differential mobility along the porous stationary phase such as silica gel and these can be located by spraying the plates with either 2', 7'-dichlorofluorescein or 50% sulfuric acid. And further extraction of lipids from samples was carried out by using extraction solvent such as ethyl ether: ethanol (3:1) or chloroform: methanol 2: 1. The R_f value of the lipid components was calculated by using standard formula.

Electrophoresis (SDS- PAGE) Banding pattern of proteins in *J. curcas*

Proteins of leaves were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) on the basis of their molecular mass by following Laemli (1970) method. Fresh plant tissues (100 mg) was ground in 1 ml phosphate buffer (pH 7.2), using pestle and mortar and were centrifuged at 10000 rpm for 15 min at 4°C and extracts were immediately stored at -20 degree C.

Estimation of protein was done prior to loading by the following method of Lowry et al. (1951). And the samples of different concentration were loaded onto the wells of gel that consists of two equal amount of protein samples were mixed with sample buffer in eppendorf tubes and then layers, upper being the 5% stacking gel and lower 12% (w/v) resolving gel containing 1% SDS and 10% ammonium per sulphate in the vertical electrophoresis apparatus. 1x SDS running buffer (Tris -Glycine buffer) was used for running the gel and a voltage was applied to the gel. Proteins separated were stained with CBB stain and the gel was stored in 50% (v/v) methanol. The banding pattern of the gel was observed under UV Transilluminator and was photographed.

Physiological analysis of plant

Estimation of chlorophyll

The chlorophyll content of the plants was estimated by the method of Arnon (1949). The leaves were excised from the plant and washed with distilled water and blotted dry. One gram of leaf sample was homogenized with 80% acetone in pre chilled mortar. A pinch of CaCO_3 was added to facilitate easy grinding. The extract was then centrifuged at 5000 rpm for 15 min and supernatant was made up to 10 ml with 80% acetone. The supernatant was filtered through Whatman no 1 filter paper and the clear supernatant were transferred to one centimeter glass cuvette. The absorbance was measured using specific absorption coefficient for chlorophyll 'a' and 'b' at 645 nm and 663 nm using 80% acetone as a blank in Shimadzu (UV- 240) double beam spectrophotometer.

The following simultaneous equations were set up for measuring chlorophyll concentrations.

Chlorophyll a = (2.311 x OD at 663 nm) - (1.062 x OD at 645 nm)

Chlorophyll b = (2.757 x OD at 645 nm) - (2.241 x OD at 663 nm).

RESULTS

Chemical analysis

The aim of this study was to investigate the chemical properties of *Jatropha curcas* L seeds, as part of optimization of de-shelling and oil extraction of *J. curcas* L. The considered parameters oil content, iodine value, peroxide value, saponification value and acid value. These parameters were done in order to study the oil property of *J. curcas* L which makes the oil most suitable for biodiesel production.

Chemical properties

The chemical properties of oil are amongst the most important properties that determines the present condition of the oil. Free fatty acid and peroxide values are valuable measures of oil quality. The iodine value is the measure of the degree of unsaturation of the oil.

Table. 1 shows the physicochemical properties of the *J. curcas* seed oil compared to other biofuel oil like soya bean and castor seed oil. *J. curcas* seed oil in this study contained high oil content as per the data analyzed. The iodine value of the *J. curcas* seed oil was 348.3 ± 0.22 (mg/g) which is higher than the other biofuel oil. The oil analysis showed high iodine value due to its high content of unsaturated fatty acids. As a crude oil, the peroxide value of *J. curcas* seed oil showed a low value of 1.2 ± 0.44 miliequivalence/kg.

The high iodine value and oxidative stability showed that the seed oil upholds the good qualities of plant oil and semi-drying oil purposes (Eromosele et al. 1997). The acid value and free fatty acid content of the *Jatropha* oil were low in general. The saponification value of *J. curcas* seed oil (214.86 ± 0.25 mg/g) was higher. The slightly higher value of unsaponifiable matter in the Soxhlet method may be due to the ability of the solvent to extract other lipids associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments (Bastic et al., 1978; Salunke et al., 1992).

Table .1 Chemical Analysis of *Jatropha Curcus L* seed oil from Gulbarga Region.

S.No	Parameters	<i>Jatropha curcas</i>	Soya oil	Castor oil
1.	Oil Content	32.5 ± 0.5 %	25 ± 0.2 %	27.5 ± 0.5 %
2.	Acid value (mg KOH/g)	1.4 ± 0.25	1.12 ± 0.5	2.24 ± 0.1
3.	Peroxide value (mg KOH/g)	1.2 ± 0.44	4.1 ± 0.20	6.2 ± 0.41
4.	Iodine value (mg KOH/g)	348.3 ± 0.22	226.4 ± 0.12	104.5 ± 0.15
5	Saponification value (mg KOH/g)	214.86 ± 0.25	235.7 ± 0.17	246.0 ± 0.21
6.	Free fatty acid %	1.03 ± 0.10	2.01 ± 0.2	3.5 ± 0.10

Thin Layer Chromatography Plate showing Fatty acid profile

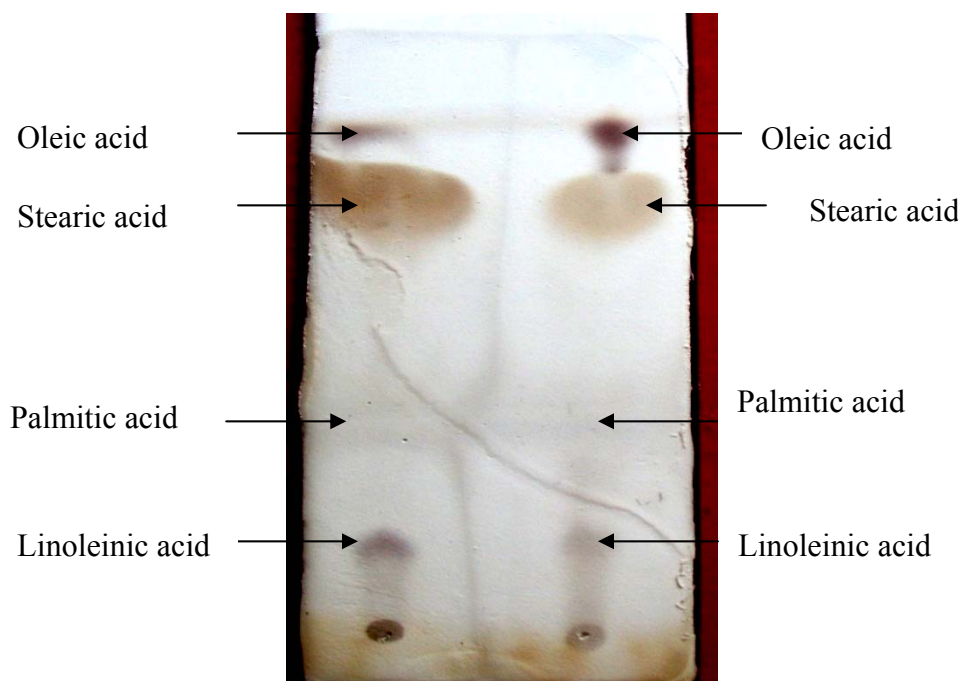


Fig. 1 Sample (Jatropha oil) Olive oil (Standard Oil)

The fatty acid profile present in the crude *J curcas* oil sample was identified by TLC with comparison with standard olive oil. With reference to the Rf value of the spots identified on the TLC plate saturated fatty acid like Palmitic acid (0.4), Stearic acid (0.8) and unsaturated fatty acid like linoleic acid (0.16) and oleic acid (0.93) were identified. The results obtained are similar to those of the Nzikou et al., (2009). (Fig-1).

Table. 2 TLC Plate showing lipid components

Spot No.	Rf of Jatropha oil Sample	Rf of Olive oil	Fatty acids
1	0.16	0.16	Linoleinic acid
2	0.25	0.23	Palmitic acid
3	0.44	0.44	Palmitic acid
4	0.8	0.79	Stearic acid
5	0.93	0.92	Stearic acid
6	-----	0.95	Stearic acid

Crude protein study on SDS-PAGE (Silver stain) of *J. curcas*

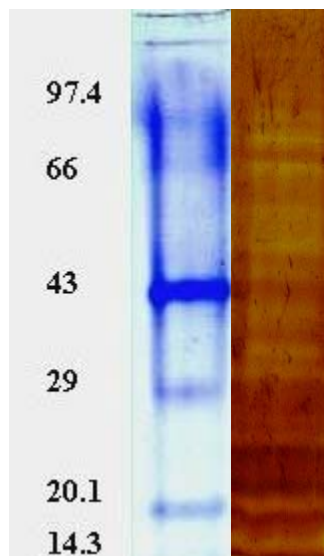


Fig .2. Protein profile

Crude protein extracted with phosphate buffer (pH 7.2) was run on SDS – PAGE and silver stained gel is shown in fig 8. The gel pattern shows that the protein specifically ranges from 14.3 KD to 97. 4 KD. (Fig-2).

DISCUSSION

Chemical Properties

The data collected from the study of chemical properties of the test samples (Table 5.1) shows the oil content of *Jatropha* seeds (32.5%), which was found higher than castor and soya seeds. High oil content of *J Curcas* indicated that *J Curcas* are suitable as non-edible vegetable oil feedstock in oleo chemical industries (biodiesel, fatty acids, soap, fatty nitrogenous derivatives, surfactants and detergents, etc).

The iodine value is a measured of the unstaruration of fats and oils. Higher iodine value indicated that higher unstaruration of fats and oils. The iodine value of *Jatropha* oil was determined as 348.3g. 12/100g standard iodine value for biodiesel was 120 for Europe's EN 14214 specification. The limitation of unsaturated fatty acids is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides. This can lead to the formation of deposits or to deterioration of the lubricating. Fuels with this Characteristic (e.g. Sunflower oil, soybean oil and safflower oil) also likely to produce thick sludge in the sump of the engine, when fuel seeps down the sides of the cylinder into crankcase. The iodine values of *Jatropha curcas* place them in the semi-drying oil group. High iodine values of *Jatropha* are caused by high content of unsaturation fatty acid such as oleic acid and linoleic acid. The iodine values of *jatropha* oil seed of suggest their use in production of alkyd resin, shoe polish, varnishes etc.

The usual method of assessment hydro peroxides (primary oxidation products) was determined by Peroxide value. The peroxide value of *Jatropha* oil seed showed a low value (as crude seed oil) of 1.2meq/kg, proving the oxidative stabilities of the seed oil relatively. The high iodine value and oxidative stability shows that the seed oil upholds the good qualities of semidrying oil purposes.

Saponification values of the studied oil were found to be 214.86. High saponification value indicated that oils are normal triglycerides and very useful in production of liquid soap and shampoo industries. Experimental result showed that a *Jatropha* oil seed has FFA content 14.025. The FFA and moisture contents have significant effects on the transesterification of glycerides with alcohol using catalyst.

Fatty acid profile

Fatty acid composition determination was another important characteristic carried out on this study (Table 2). Fatty acid composition of studied oil shown in table 2 compared with other vegetable oils such as soybean oil and castor oil. There are three main types of fatty acids that can be present in a triglyceride which is saturated (Cn:0), monounsaturated (Cn:1) and polyunsaturated with two or three double bonds (Cn:2,3). Various vegetable oil is a potential feedstock for the production of a fatty acid methyl ester or biodiesel but the quality of the fuel will be affected by the oil composition. Ideally the vegetable oil should have low saturation and low polyunsaturation i.e be high in monounsaturated fatty acid (Gunstone, 2004). Vegetable oils that rich in polyunsaturated such as linoleic and linolenic acids, such as soybean, sunflower (table 2), tend to give methyl ester fuels with poor oxidation stability. Vegetable with high degree unsaturation tend to have high freezing point. The predominant fatty acid in studied oil consists of monounsaturated (55.5%), followed by polyunsaturated fatty acid (35%) and saturated fatty acid (26.6%). Monounsaturated of jatropha seed oil higher than other seed oil such as soya oil and castor oil (table 1). The major fatty acids in *Jatropha* seed oil were the oleic, linoleic, palmitic and the stearic fatty acid. stearic acid showed the highest Rf value 0.93, followed by palmitic acid with Rf 0.44 and the Rf value of linoleic acid was 0.16. Thus, *Jatropha* seed oil can be classified as stearic-palmitic oil. Compared to other vegetable oil (table 2), jatropha oil seed has highest stearic contain than olive oil as olive oil was taken as a standard fatty acid.

CONCLUSION

In this present study the plant *J. curcas* has shown good results for chemical parameters. This indicates the plant has high free fatty acids, high Iodine number, high moisture content and saponification value and low peroxide value etc., when compared with the other oil yielding plants like Soya and Castor. The growth and development of our plant is considerably good. Plant seeds studies suggests that seeds have good quality and quantity which given plenty of oil when extracted. TLC for lipids and SDS-PAGE for proteins shown good results.

Hence we can be concluded that this plant is eligible for the production of better quality of Biodiesel.

Conflict of Interest

All the authors of this research papers have no conflict of interest.

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