Title

BIOPHYSIOCHEMICAL CHARACTERIZATION OF *JATROPHA CURCUS* FOR BIODIESEL PRODUCTION

Dissertation II

Submitted by

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ABSTRACT

Petrol, a synthetic based fluid is now used abundantly and commonly to serve different purposes. But the petroleum products are non-renewable and are also not environment friendly. Also their price is increasing day by day. So the biodiesel came up to the origin, which is envrironment friendly and is cheaper and is of biological origin. So is renewable and can be replenished. *Jatropha curcus* is a shrub belonging to Euphorbiaceae family. Jatropha's non edible seeds were used. The seeds were collected from different regions of India and these seed were dried, grinded and crushed and then it was put into thimble in soxhlet apparatus for the extraction of oil by n-hexane. After that distillation was done for separating oil and hexane and transesterified to produce biodiesel. Then, their biophysical determination was done such as oil yield, pH, iodine value, saponification value, free fatty acid, acid value, specific gravity, viscosity, flash point, fire point. The result shows that the best acceptable sample for the production of biodiesel was from Birsa Agriculture University. The factor which includes best for the production of biodiesel is fire point, flash point, oil content and free fatty acid.

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DATE: - **KUMARI NIKITA**

DECLARATION

With this I assert that the dissertation entitled "Bio-physical characterization of *Jatropha curcus* for biodiesel production" submitted for M.Sc (Hons) biotechnology degree is a result of my and my co-workers innovation and expertise. It does not involve any content which can be used for the completion of any other degree program.

DATE: - KUMARI NIKITA

CERTIFICATE

This to certify that Kumari Nikita student of M.Sc (Hons) Biotechnology, registration -11310760 has completed M.Sc Project entitled "Biophysiochemical characterization of *Jatropha curcus* seeds for biodiesel production" under my supervision. Up to my consideration, the performed dissertation is the outcome of her innovation and study. It is my assurance that the performed work does not involve any content from any other report of this university. This project report suits as well defined all demands required for evaluation leading to the award of Master of Science in Biotechnology.

DATE: -

Kuldip Chandra Verma

(Project Supervisor)

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CHAPTER:-1

INTRODUCTION

INTRODUCTION

Now-a-days the Production of Biodiesel is done by *Jatropha curcus* and it is having a significant attention due an alternative fuel as the predicting shortness of conventional fuel and concern about the environmental changes. It is also to overcome energy crisis problem but it is not used as nutritional sources. Biodiesel makes the environment eco-friendly. It is a nonpolluting, locally available, sustainable, and reliable fuel obtained from *Jatropha curcus*, vegetable oil and animal fats.

Fig 1:- *Jatropha* Leaf and seed (www.jatrophacurcusplantation.com)

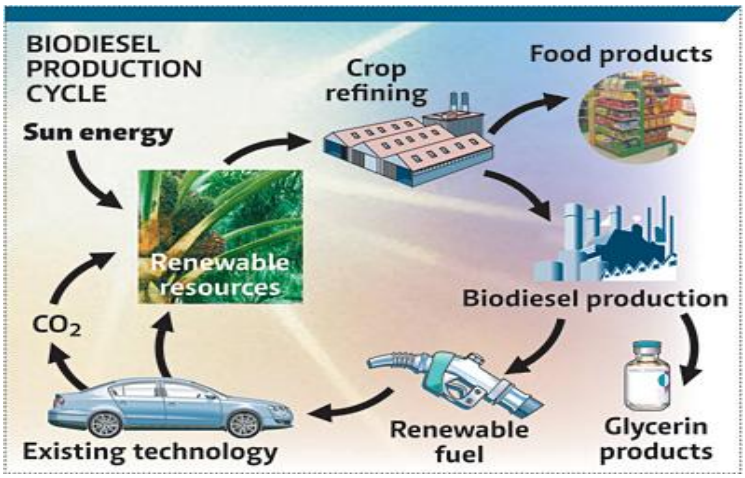

Fig 2:- Biodiesel production cycle (my palm oil.wordpress.com)

Jatropha curcus is a shrub belonging to Euphorbiaceae family known as widespread distributed species of India. It is a species having its origin in the Indies and present in the village of subtropical and tropical Africa, South America and South East Asia **(Adjanohoun *et al.*, 1989).** These plants are highly exploited for industrial purposes including textile, and pharmacopeia. *Jatropha curcus* is resistant to drought and having the qualities of various medicinal and non medicinal properties they are also famous for other useful aspects including production of soap and as an alternative fuel **(Gubitz *et al.*, 1990).**

Augustus *et al.*, 2000 has been shown that *Jatropha curcus* seed contain 20-40% oil content. *Jatropha curcus* is able to stay alive in unfavourable climatic situations due to its humidity or wetness, richness requirement, and high temperature.

All part of *Jatropha curcus* has their own uses. *Jatropha curcus* is a succulent tree. Being a succulent tree it can easily adopt the harsh condition and are efficient in preventing soil erosion. The leaves are used in ayurvedic medicine as a remedy for coughs or as antibacterial after birth **(Nayak and Patel 2010).**

The latex from the brushwood is used for wound healing. Every fruit contains four sided figure black seeds which produce oil. 40-60% (w/w) oil is contained by seed kernel. The extracts oil posse's insecticidal and remedial properties. They are useful for soap production and as alternative fuel 58% roughly. In Nigeria the composition of *J. curcus* oil consist of fatty acid such as stearic acid (2.53), palmitic acid (13%), oleic acid (48.8%) and linoleic acid (34.6%) **(Nayak and Patel 2010).**

The high unsaturated fatty acid 78-84% present in *J. curcus* shows effective production of biodiesel. However, the compositions of chemical in oil vary according to the environment and region. Biodiesel is substitute fuel prepared from natural sources such as vegetable oils and animal fats. Vegetable oils are more often glycolic ester with diverse chains length and saturation point. Vegetable contain a large amount of oxygen in their molecules. Vegetable oil need to be customized to bring the ignition related properties closer to that diesel oil. Modification of oil is mostly meant at dropping the viscosity and increasing volatility. In place of petroleum there are many substitute being measured are methanol, ethanol, and vegetable oils. *Jatropha*

curcus is a quick growing crop and has seed producing capacity for about 50 years. Depending on rainfall and soil quality oil can be extracted after 2 -5 yrs. The year give up of Jatropha seeds is in 0.5 to 12 tons in quantity. The continuous farming of Jatropha prevents soil erosion, and reclamation of wasteland. Oil from seed can be useful for manufacturer of candle and cosmetic industry (**Shivani *et al.*, 2011**). The amount of fatty acid in the oil should be less than 1% for the biodiesel production, if not then these FFAs react with the alkaline in the presence of catalyst during transesterification to produce soaps instead of esters. The latex of *Jatropha curcus* contains an alkaloid known as "jatrophine" which is supposed to have anti cancerous properties. The oil which is burned is not emitting smoke and it act as illuminants.

Jatropha curcus is used as a coloring dye as its bark produces a blue dye which is used as coloring agent. The derivatives of *Jatropha curcus* seeds consists of high nitrogen, phosphorous and potassium and it is used for domestic animals food, is also used as a fertilizers and fish food.

By the plant and animal oil, biodiesel can be prepared which can be used in straight diesel engines to serve as an alternative for petro diesel or it is mixed with petro diesel to decrease discharge of pollution. It is an oxygenated; free from sulphur, eco- friendly, non- hazardous, environmentally, pleasant substitute automotive fuel. The oil which is produced from the edible seed is becoming expensive in the market to use as a fuel and may increases the cost of food, so there is a substitute that is inedible seed to fulfill all the conditions for producing biodiesel (**Umaru *et al.*, 2012**).

Jatropha curcus is a toxic, semi-evergreen shrub or small tree, reaching a height of 6m (20 ft), allowed to grow in wasteland. The oil is present 27-40% in seed that can be processed to a high quality biodiesel which can be used as customary diesel engine. Toxalbumin curcin is a highly toxic is a source of the Jatropha seed **(Garba *et al.*, 2013).**

In the world about 175 species of Jatropha is present in which 200 name indicate its occurrence in various countries. The matured Jatropha seed color is brown and the leaves color is light green and large. The effect of temperature, light, and rainfall helps in inducing the dormancy by fluctuations (**Garba *et al.*, 2013**).

The alternative fuel which is biodiesel produced from natural resources like *Jatropha curcus* oil, vegetable oil and animal fats. Highly viscous oil produced from vegetable as compare to the biodiesel lead to the improper functioning of the pumping system and not mixing properly fuel with air lead to the partial ignition, higher the flash point lead to the higher deposition of carbon and internal choking of the system. So to reduce the problem, the viscosity is decreased and the volatility is increased. So, one of the most important factors which is used in converting the vegetable or Jatropha oil into methyl esters is tranesterification process which is the process of reacting alcohol in the presence of catalyst that is sodium hydroxides. So, Jatropha vegetable oil is one of the most important species for the production of biodiesel from the non edible seed **(Raja *et al.*, 2011)**.

Jatropha curcus is a plant which is attaining lots of attention due to the non edible and feasible seed which can produce biodiesel as compare to the remnant fuel such as petroleum. It is considered as a renewable source of energy and it is not emitting high amount of carbon dioxide in the atmosphere which can cause pollution and global warming. It was originated from Central and South America but now it is widely grown all over the world. It is a plant which is grown in shortage of water and stressful conditions and survives well with maintaining the osmotic level. These plants grow in different types of soil like clayey soil, sandy, loamy soil and with the soil which is having high salinity condition. The plant can be grown without any maintenance and it can be long live for 50 years with a good seed which is having good oil content. The oil content is from 25 to 37% in the viable seeds **(Nahar *et al.*, 2011).**

The uninterrupted investigation for the renewable source is because of the reduction of remnant oil. The oil or fuel which is obtained from plant and animal source is called as biofuels. As it is renewable source so it is having a lot of interest from all over the world for the production of biodiesel. Biofuels is the long fatty acid of monoalkyl esters which is found in plant and animals in the form of fats and oil. The changes made by chemical and natural process in the animal fats and vegetable oil are helpful in function of engines. The biodegradable waste products are also used in the production of biodiesel. This produced oil can be used with the little modification for running of engines. The biodegradable wastes are also useful in the

production of chemical and fertilizers which can be used in our day to day life **(Foloranmi 2013)**

Jatropha curcus has the prospective to become one of the key energy crops throughout the globe. The plant is found in tropical and sub tropical regions. In Nigeria Jatropha is even being cultivated by farmers majorly for border demarcations as well as for hedge. Half life of Jatropha is considered to be 30-40 years. It can be grown on wide range of land types such as non arable, marginal and waste land. Crude state of Jatropha is not edible. The oil content of the seed is 50-60%. One hectare of Jatropha can produce 2000 liters of fuel. Jatropha is not as efficient when compared with other oils such as, palm oil, castor oil and soybean oil. Jatropha is found to be as most economical viable livestock. (**Umaru *et al.*, 2012)**

Since the race for increasing the sources of the biofuels are increasing the Jatropha plant can be effective substitute. In this study we are trying to extrapolate the oil content of the plant and we are checking its efficiency of its oil as a biodiesel.

CHAPTER:-2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

20-40% percent of oil in Jatropha seed have concentration of both saturated and unsaturated fatty acids .These fatty acid 14.1% palmitic acid, 6.7%stearic acid, 47% oleic acid and 31.6 of linoleic acid **(Augustus *et al*., 2002**). However, only oleic acid constitutes the majority of concentration i.e. up to 41-48.8% in oil content. Linoleic acid and stearic acid (2.3- 2.8%), palmitic acid (34.6-44.6%). In contrast to Diesel, highly viscous vegetable oil (30- 200 centistokes) could be responsible for pump blockage; incomplete combustion is due to inefficient mixing of air with fuel, inferior coking and carbon deposit formation is due to increase flash point. These problems demands for the amendments necessary for making combustion suitable vegetable oil which can be regarded as good quality biodiesel.

Fig 3:-*Jatropha Curcus* Seed

(Wikipedia.org)

Further, tranesterification of the settled oil is considered using methanol (0.24% w/w) and NaOH (1.4% w/w) act as alkaline catalyst at 65^0C for biodiesel production. Finally, methyl ester of fatty acid was obtained in 2hr with ca. of 90% ***(*Berchmens and Hirata, 2007*).*** Acid catalyst (5% H_2So_4) and methanol (20% of oil) are used for biodiesel preparation from Jatropha oil through tranesterification. Taking

the 6:1 molar ratio of methanol and sodium hydroxide concentration 0.7 weight percentage of oil the reaction was done for 2 hrs. The total yield of methyl ester from Jatropha is 97 % **(Singh and Padhi, 2009).**

The seed of *Jatropha curcus* are enriched with high lipid content. It has approximately 20 to 39% of oil and it is energy source for fuel production. Different which are used for biodiesel production are tranesterification, soxhlet apparatus using petroleum ether and extraction of mixture of chloroform: methanol (1:1) ***(*Nzikou *et al.*, 2009).**

The oil concentration is 50% by soxhlet and 47% by chloroform: methanol. The mineral, viscosity, saponification value, free fatty acid, iodine value, unsaponifiable matter content, peroxide value, activation energy were determined. Petroleum ether was used to extract out Jatropha oil using light with temperature of 60-80^0 C **(Nayak and Patel, 2010).**

According to **Prathibhan *et al.*, 2010** oil was extracted by means of solvent extraction using hexane (40- 60^{0}C) with soxhlet apparatus the physical properties of seed is assessed like refractive index, specific gravity, acid number, iodine number, saponification number, cetane number. According to **Belewu *et al.*, 2010** the biodiesel is produced by tranesterification and the physiochemical character are optimized including specific gravity, oil yield, acid value, density, iodine value, free fatty acid, peroxide value, saponification value, flash point and viscosity. It was also estimated that the Nigerian Jatropha oil exhibits higher acid value, free fatty acid, iodine value and peroxide value as compared to Indian Jatropha oil. The calorific value of the Nigerian Jatropha oil was 48.31 MJ/Kg, while in case of Indian Jatropha oil it is 47.50MJ/k. Most of the properties of the Nigerian Jatropha diesel evaluated compared favorably with ASTM and EN (for biodiesel) standard value. Therefore it can be concluded from the study that Nigerian Jatropha oil has high potential and good economic condition of biodiesel production.

The iodine value of Jatropha seed oil is 106.0 in the area of Gujarat that is Bardoli and 105.20 is iodine value in Nigeria and 135.85 in Malaysia. Jatropha seed collected from Malaysia has saponification value 208.50mg/g and when it is compared to Nigeria then it is higher that is 198.85 mg/g. By the gas chromatography

analysis the fatty acid composition of *Jatropha curcus* is linolenic acid (0.28%) , stearic acid (7.67%) , oleic acid (40.39%) , palmitic acid (16.69%) , linoleic acid (33.09%) . 75.64% of unsaturated fatty acid is present in a Jatropha oil in a high amount **(Nayak and Patel, 2010).**

According to **Pedavoah (2010)** carbon atom between the range of 12 to 18 mixed with complex mixture is called as mineral diesel oil whereas organic compound mixture is a vegetable oil which is between range of a straight chain compound to multifaceted arrangement of proteins and fats is called as triglycerides .The oxygen molecules present in the mineral diesel oil is 10% higher than vegetable oil. Due to the molecular structure and chemical structure the vegetable oil is having higher viscosity than diesel. The most common solvent used for the extraction is methanol, ethanol and hexane. The methanol is having low cost and it is reacting solvent with triglycerides and NaOH is easily dissolved in methanol. The changing in vegetable oil is done only if oil is more viscous. So to reduce the viscosity and to increase the volatility there is a modification in the vegetable oil.

The most important method used for extraction of biodiesel is from soxhlet method and tranesterification method. Transesterification is a process of reaction of alcohol with fat or oil using sodium hydroxide and potassium as a catalyst and the alcohol mainly used is ethanol and methanol. It is process of converting alcohol into ester and water (**Raja *et al*., 2011).**

The optimized results revealed that the seed consist of protein (22.50%), 46.31% oil (dry w/w) and volatilities (5.8%), moisture content. Also, the analysis of physical and chemical properties of the seed extract shows the iodine value (106.00mg/g), saponification value (194.70) and acid value (34.46%). In presence of catalyst alcohol reacts with triglycerides of fatty acids during tranesterification reaction in vegetable oil. CI engine system is compatible with characteristics of vegetable oil. Vegetable oil is soluble with diesel in any proportions in large amounts. Nearly two third of diesel requirement is imported from other countries to India. The use of diesel is causing problems to the health and environment (**Raja *et al*., 2011**).

The physical properties such as density, flash point, kinematics viscosity is finded out. *Jatropha curcus* seed oil is extracted by soxhlet extraction method and gas

chromatography. The oil content on seed of Jatropha is 46.27% on dry basis. The physiochemical properties show acid value (36.46), iodine value (106.00mg/g) and saponification value (194.70 mg/g) **(Joshi *et al*., 2011).**

Jatropha *seed* was used for extraction oil by organic solvent. The acid value and antioxidant were also investigated. The maximum yield is obtained from soxhlet extraction method and hexane as a solvent ***(*Shivani *et al.*, 2011).**The acid value was 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. As a source of antioxidant oil can be used **(Shivani *et al.*, 2011).** Technique for effective biodiesel production from crude Jatropha seed with high free fatty acid concentration was developed. The two-step pretreatment process is used to reduce high FFA level of CJCO to less than 1%. The first step involves the presence of methanol in 0.60 w/w methanol-to-oil ratio in the in the presence of acid catalyst (1% H_2SO_4) for 1-h at $50^{\mathbf{0}}$ C. After that, the mixture is allowed to settled down for 2-h and the methanol-water layer is removed from the top.

In Jatropha seed it was found that poisonous and the unpleasant odor is because of protein. Oil content of Jatropha seed is 44- 46 %. The amount of saturation and unsaturation with the respect to hydrogen is depicting the fat and oils property. Thus it is necessary to the unsaturation level present in the sample. Thus different physico – chemical characterization is done like saponification value, acid number, flash point. The iodine value was 104.46 in Dehradun as compare to the Nigeria (105.20) and Malaysia (138.85). The saponification value is 175.12 mg/g **(Joshi *et al.*, 2011).**

According to **Raja *et al.* (2011)** transesterification process is method of production of biodiesel and biodiesel is separated from glycerin and both is purified by purification process. The different chemical properties were determined of Jatropha oil are acid value (38.2), viscosity at 31^0 C 40.4 cs. The Jatropha curcus seed which is waste after production of biodiesel is used as Jatropha seed oil cake which is rich in high content of nitrogen, potassium, phosphorous is used as fertilizers in the field crop as agricultural purpose for the fertility of the soil.

According to **Adebayo *et al.* (2011)** *Jatropha curcus* seed is extracted by chemical process. The oil yield was 39.7% and biodiesel production was 80.2which

the total yield. Different physical and chemical characterization was done from the produced biodiesel which includes flash point 170^0 C, specific gravity 0.875, viscosity at 40^0 C 4.8 cts and iodine value 7.64 which was compared with the petroleum. Biodiesel is the chemical reaction of alcohol and fats which gives alkyl esters. Currently the Government of Nigeria has revealed huge attention in Jatropha plant to reduce the dependency on other nation for petrol and to reduce pollution and make environment free from global warming and to increase the industrial scale.

According to **Wei He (2011),** from past centuries, plants, animals and microorganisms, biological sources, are providing food, energy and other products which are fulfilling the need of human beings. Living organisms naturally produce organic matters such as wood, cultivated crop and their co-products, waste produced from food material and waste from metropolitans are all termed as biomass. In the past, incineration was the process for producing energy from the biomass which was used efficiently. However, there was a major demand by the automobile industries for the petroleum. So to satisfy this demand, biodiesel was produced by the fermentation of sugars and ethanol (bioethanol and biobutanol) and via transesterification of triacylglycerol resulting in production of biodiesel. On the other hand, for conversion of plant biomass into fuel, thermochemical processes has been developed such as gasification, pyrolysis and liquefaction. The use of petroleum is problem because it releases a greenhouse carbon dioxide. From 1970 to 2009, the release of carbon dioxide is twice. The burning of petroleum is the major contributor to the increase of carbon dioxide concentration in atmosphere from 390 ppm in 2010. So, this increasing concentration carbon dioxide is the major problem of global warming. Combustion of petroleum is also releasing number of pollutants, with particles like NO^X and SO^X which react with hydrogen molecule in the environment to causes an acid rain.

According to **Nahar *et al.* (2011)** different parts of plants of *Jatropha curcus* were explained such as leaves, flowers and fruits. Leaves color is green to pale green, leaves are arranged in opposite to each other and 3 to 5 lobed. In the same inflorescence male and female flowers are produced, for every one female flower there are 20 male flowers. The ranges of petiole length are 6.1- 23.1 mm and plants are monoceious. Mostly the fruits are produced in winter season. The seed capsule

color changes, from green to yellow when seed is matured. The oil yield by weight is 20-40%. 20% saturated fatty acid and 80% unsaturated fatty acid is present in seeds.

According to **Wei He (2011)** *Jatropha curcus* seed shows the toxicity. Non edible if consumed accidently by human then it causes vomiting, diarrhoea and giddiness. In laboratory animals, experiment conducted, and then it shows the mortality of animals. Animals used for the experiment was chickens, goats and mice. Different toxins and suppressant of nutrients are present such as phorbols esters, curcins, lectins, trypsin inhibitors, phytates, saponins and tannins. Different varieties of Jatropha seeds are present in Mexico. The most toxic compound present in non edible seed in *Jatropha curcus* is PEs and curcins. PEs is the diterpenoids, which is not present in edible seeds but present in non edible seed. Curcin is found in non edible variety and it belongs to the ribosome inactivating protein family. And it is not known till now that curcin is present in edible seed or not. Trypsin inhibitors are the protease inhibitors which are not easily digested by monogastric animals. Phytates are the phytic acid which is not easily digested by non ruminants and reduces the mineral intake, phosphorous and dietary proteins. To decrease the PEs content by 97.9% from Jatropha curcus and help in reducing toxicity by extracting with 90% ethanol and treating with 0.07% Na HCO_3 and heating it considerably.

Iodine value is to determine the unsaturation point of oil. Greater is the unsaturation of fats and oil and heating unsaturated fatty acid lead to the glycosidic polymerization which lead to the configurationally change of lubricating properties. The determined iodine value is 105 g l_2/100g. Iodine value of vegetable is in the range 100 to 130 which fall in the group of semi drying oil (**Umaru and Aberugba *et al.*, 2012)**

The determined saponification value is 190. In *Jatropha curcus* oil is having a very high saponification value so due to this Jatropha oil is having normal triglycerides. The acid value is 36.2mg KOH/g which shows high fatty acid content. Due to the higher percentage free fatty acid by weight it causes soap formation during degradation of alcohol process and having difficulties in separation of biodiesel from its side product as it decrease the production of biodiesel **(Umaru *et al.*, 2012)**

According to **Owolabi *et al.* (2012)** Diesel engine was invented by Dr. Rudolf diesel to run on a multitude of fuels which includes coal dust floating in water, heavy mineral oil and vegetable oil. Dr. Diesel first failed in his experiment. But by the time he showed that the engine was running on 100% of peanut oil, which was held on the World Exhibition, Paris. In 1911 he said that the engine can be run in the vegetable oil. In 1912, Diesel said that "The use of vegetable oil for engine fuels may seems insignificant today" but after sometime it will become important as petroleum causing pollution and harm to the world. As now a days this statement becomes true and everyone is choosing biodiesel as an engine fuel.

The Biophysio-chemical classification of refined oil from *Jatropha curcus* seed are as follows: the content of acid value was 0.1428mg KOH g/oil, production of soap value is 155 mg KOH g /oil, the saturated and unsaturated fatty acid value was 0.0718 mg KOH g / oil, iodine value was 51.27 g/100g oil, flash point was 150^0c and the pH was 5.15. The value which was found is equivalent to ASTM standard so jatropha oil can be used as biodiesel for making the environment free from pollution because it has low acid content **(Garba *et al.*, 2013).**

According to **Chandra .P *et al.* (2013)** the collection of Jatropha seeds was done from Mohali, Punjab and the seed extraction for biodiesel production is done by soxhlet method using methanol as a solvent at 70^0 C. Different Biophysiochemical characterization was done and the result was dry oil content 21.15% v/w, acid value is 30.67 mg/g and saponification value was 195mg/g. Different concentration of KOH catalyst is used for clear and purified biodiesel with 0.5, 1, 2 and 3 KOH to have different physiochemical properties. Jatropha can be grown with other crops such as coffee, sugars, vegetables and fruits.

The high content of free fatty acid present in the Jatropha oil is converted into Jatropha methyl ester and ethyl ester which is also called as Biodiesel. The different Biophysico – chemical characteristics of Jatropha curcus seed oil was used to determine the fire point, flash point, acid value with comparison with JEE 20, JEE 100, JME 20, JME 100 to determine it compatibility with the CI engine. The table is showing the different values are as follows: **(Shambhu *et al.*, 2013).**

TABLE 1:- Acid value, flash point and fire point of different biodiesel.

S. No	Oil type	Fire point	Flash point	Acid Value
1	Diesel	70.0	63.2	0.38
2	Jatropha oil	235.2	229.3	32.8
3	JEE 20	97.2	91.2	0.44
4	JEE 100	201.0	195.0	0.55
5	JME 20	101.0	95.5	0.40
6	JME 100	204.7	198.9	0.46

TABLE 2:- The standard value of biodiesel is shown according to **Patel *et.al* (2013) and Chandra .p *et.al* (2013)**

S.NO	BIOPHYSICO-CHEMICAL CHARACTERIZATION ACCORDING TO ASTM	UNIT	ASTM VALUE
1	Oil content	% (v/w)	22.15
2	pH	-	6.5- 6.8
3	Fire point	^{0}C	220- 240
4	Flash point	^{0}C	186
5	Saponification value	mg KOH/g	194
6	Iodine value	mg KOH/g	101.0
7	Acid value	mg KOH/g	11.0

8	Free fatty acid value	mg KOH/g	5.5
9	Specific gravity	(40^0c) gm	0.95
10	Viscosity	mm^2/ s	20.49

According to **Patel *et al.* (2013)** 97% oil yield and methyl esters was found from Jatropha seeds which is non edible. It is growing all over the planet or earth under different climatic conditions but mainly it is growing in tropical and sub-tropical regions of the planet. Jatropha curcus is a small shrubs or tree. Esterification process is done with acid catalyst (5% H^2SO^4) and 20% of oil is mixed with methanol. Simultaneously transesterification process is also done for 2 hours with 6:1 ratio of methanol and oil and 0.7 gm of sodium hydroxide is used.

According to **Shambhu *et al.* (2013)** to some extent the renewable source of energy has made the country self dependent and decreases the import of oil from other countries. In India the crude oil production from 2008-2009 was 33.51 million tones and the 128.16 million tones is imported from other countries. Biodiesel is produced from the process termed as tranesterification. The source which is used to produce biodiesel is vegetable oil and animal fats.

According to **Patel *et al.* (2013)** fuels produced naturally by vegetable oil is an alternative to petroleum. In past firstly biodiesel was produced by soybean, sunflower, palm, canola, rapeseed, cottonseed and Jatropha. By using all edible sources leads to the shortage and increasing the cost of for production of biodiesel. So, it was unfeasible in India. So, In India production of biodiesel is done from non edible seed which show the feasibility as comparing from others. An additional quality of non edible seed is that it can be cultivated in wasteland and cash crops and on a large scale production.

According to **Garba *et al.* (2013)** Jatropha oil can be used as jet fuels, power house and high amount of hydrocarbon is present. The seed oil can be used in treatment of rheumatic disease, fever, jaundice, gonorrhea, itching and parasitic skin

disease. It can be used as stimulants for hair growth, making candle and soaps. It can be used as insecticides and pesticides which include as a pest of cotton, pulses, potato and corns.

According to **Okullo *et al.* (2014)** Higher the concentration of free fatty acid, it leads to the corrosion of engines. According to the ASTM D 6751, standard free fatty acid should not be more than 0.5% in Biodiesel because it may lead to the corrosion in rubber parts and deposit in the engines. Higher the saponification value, lower the oil content and form an emulsion and produce glycerin which is difficult to separate.

CHAPTER:-3

SCOPE OF THE STUDY

SCOPE OF THE STUDY

As per geologists, petroleum engineers and economists worldwide production of oil has conceded its highest point which means in world oil production has increased and will decline slowly. As the population is increasing the demand for energy continues to growing more rapidly. In India and China which is developing nation more and more energy is consuming item is used. Now the forth coming generations may witness little or petroleum available for energy.

So we have a number of options in case of non edible plant to be used for biodiesel production extraction, so *Jatropha curcus* is termed as the best source of fuel.

Advantages of biodiesel

1) Biodiesel can be named as dirt free fuel as it does not contain sulphur content and cancer causing properties.
2) It consists of high biodegradability and lubricating property.
3) Improve engine efficiency and operating life cycle.
4) Domestic source.
5) Readily mixes with petroleum diesel fuel in any ratio.
6) Its property of higher flash point makes it safe in transporting and its storage.
7) Particulate matter and carbon monoxide emissions are greatly reduced.
8) It is useful controlling the pollution which is causing harm to the atmosphere.
9) By decreasing the viscosity of oil and producing the biodiesel by the process of tranesterification help in reducing the choking of engines, deposition of carbon, and problem in functioning of automobiles.

CHAPTER:-4

OBJECTIVE OF THE STUDY

OBJECTIVE OF THE STUDY

1) Seed collections and production of biodiesel from different biotypes of *Jatropha curcus*.

2) Biophysiochemical characterization of *Jatropha curcus* oil for biodiesel production

CHAPTER:-5

RESEARCH METHODOLOGY

RESEARCH METHODOLOGY

Sample was collected from interstate and intrastate regions of India.

1) Holy cross Krishi Vigyan kendriya (Hazaribag),
2) Near S.P Kothi (Hazaribag),
3) Forestry College (Ranchi),
4) Birsa Agriculture University (Ranchi).
5) Udaipur (Rajasthan)
6) Punjab university (Punjab)
7) Gurgaon
8) Hyderabad (Comboitre)
9) Gwalior

PREPARATION OF SEEDS SAMPLE

Jatropha curcus matured seed were collected from different region of India. The seed were cleaned and de- shelled and air dried after that it is dried in hot oven at 60- $65^{0^{\circ}}$C for 6 hour. Seeds were powdered using mortar and pestle and electric grinder prior to oil extraction.

Fig 4:- Homogenized seed of *Jatropha curcus*

EXTRACTION OF OIL USING SOXHLET APPARATUS

The crushed and grinded seed were weighed that is 50 gm and then the sample was put into filter paper pouch. And filter paper pouch with sample were put into a thimble of soxhlet apparatus. 300 ml n- hexane (solvent) were taken in round bottom flask to extract a sample which is attached to soxhlet extractor .The extraction was carried out for approximately 8 hours at temperature of 65- 70^{0}c and then sample were collected as crude oil. Again different sample were extracted using this process.

Fig 5:- Extraction of oil using soxhlet aaparatus

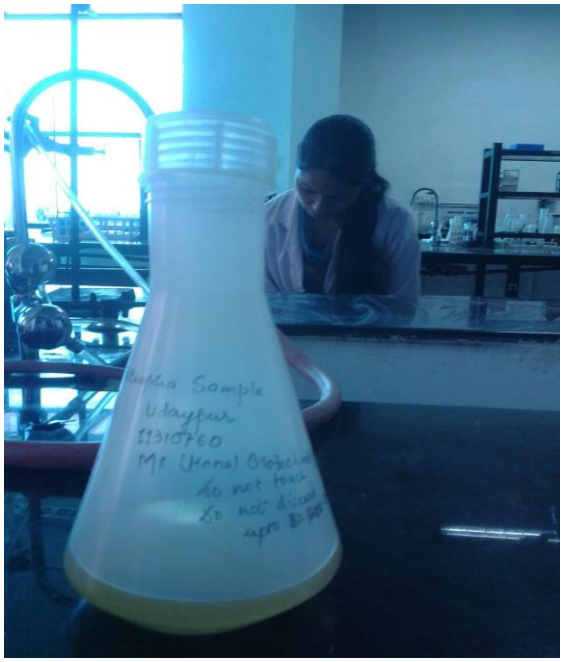

Fig 6:- Extraction of oil using hexane from Jatropha

DISTILLATION OF OIL

After a completion of extraction process of different *Jatropha curcus* sample, the oil and the solvent (hexane) mixture was added to the distillation unit so that the solvent and oil could be separated from each other.

Fig7:- Distillation for separation of hexane and oil

TRANSESTERIFICATION

It is the process of chemically reacting at and oil with an alcohol in a presence of a catalyst. Alcohol used is methanol and ethanol. Catalyst is usually sodium hydroxide or potassium hydroxide. The main product of transesterification is biodiesel and the co –product is glycerin.

TRIGLYCERIDES + FREE FATTY ACID + ALCOHOL ⟶ Alkyl esters + Glycerin

PROCESS OF TRANESTERIFICATION

1)1 ml of oil sample was weighed and put into a round bottom flask.

2) 25 ml of methanol was added into it

3) 0.4 gm of sodium metal was weighed quickly and added into to the round bottom flask containing the sample.

4) The content was refluxed at 60^0C for 2 hours for the separation of salts and impurities.

Fig 8:- Tranesterification – Oil sample kept for reflux

5) After 2 hours, the refluxed sample is allowed to cool and then the sample is ready to use for the next step of biodiesel production.

BIODIESEL PRODUCTION

Biodiesel is produced from the oil extracted from different seed sources. For producing biodiesel, tranesterification is being performed.

1) Took separatory funnel and the funnel stand for holding the funnel.
2) Transesterified oil and the 25 ml of distill water was added into the funnel.
3) After that mixed it gently.
4) Added 0.2 ml of methyl red indicator to it. It gave a yellow color on its addition.
5) Added 50% H_2SO_4 until it develop a pink color and mixed it properly.
6) 100 ml of petroleum ether was added to the sample and again mixed it.
7) Petroleum ether form two layers – the upper layer contain ether mixed with oil and lower layer having glycerin and impurities.
8) Allowed the two phases to stand for 5 minutes.
9) Removed the lower layer and washed the upper layer 2-3 times with distill water and allowed to stand for overnight which contain biodiesel.
10) After washing with distill water, the upper phase is added to the beaker with 5 gm of anhydrous sodium sulphate and kept it until only the biodiesel is left.
11) This step is repeated for the other sample of different regions.

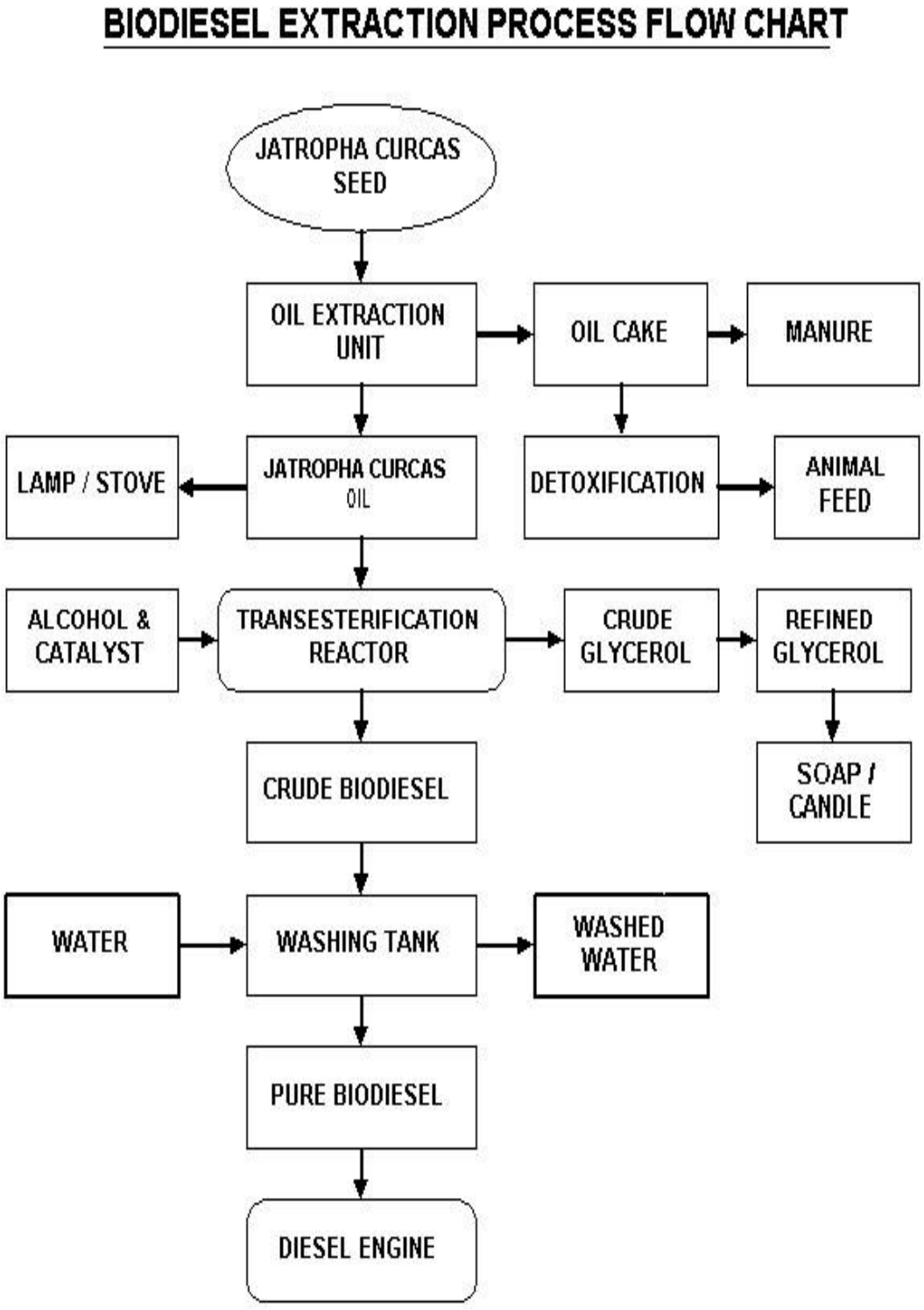

Fig 9:- Flow chart of extraction of biodiesel (Scialert.net)

Biophysio-chemical characterization of biodiesel produced from Jatropha curcus

.1) Oil content (Joshi *et al.*, 2011)

By the distillation unit oil was separated using hexane. The percentage of oil content was calculated as given below

$$\% \text{ of oil} = \frac{\text{Wt of oil obtained in gm} \times 100}{\text{Wt of seed taken in gm}}$$

2) Viscosity (Chandra *et al.*, 2013)

A viscometer with flow time above 200 sec was taken .to eliminate dust and salt material to the liquid sample, the sample was filtered through sintered glass. The liquid suction was drawn up to the upper mark of viscometer which was filled with sample. The temperature of water bath was at 29^0C and was allowed to for 10 min to match the bath temperature. The suction was applied to thinner arm and drawn to the upper arm till that time marks the timing and the viscosity is recorded.

$$\eta 1 = \rho 1 * t1 * \eta 2 / t2$$

η1= viscosity of biodiesel

ρ1= density of b/d

t1= flow time of b/d

η2= viscosity of water

t2= flow of water

3) **Determination of specific gravity**

Firstly empty container was weighed and then the container was weighed with water which was fully filled and then the container was filled with same volume of oil and it was weighed. It is calculated as follows

$$W3 - W2 / W1$$

Where, w3 = weight of container and oil

W2= weight of empty container, W1 = weight of water

4) Determination of Acid value (Garba *et al.*, 2013)

1 gm of oil was weighed in a 250 ml conical flask and then 10 neutral ethanol was added and then flask was heated on the water bath for 3 min and after that it is left to cool and the content was titrated with 0.1 N alcoholic KOH solution using phenolphthalein as an indicator till it develop pink color and by also side by side titration was also conducted.

Acid value (mg KOH/g) = Titer value X56.1X N / Weight of sample

Titre value = sample –blank

N- Normality of KOH

5) Free Fatty Acid Determination :(**Umaru *et al.*, 2012)**

The amount of free fatty acid value is just the half of acid value that is calculated as follows:

$$\text{FFA (mg KOH/g)} = \frac{\text{Acid value}}{2}$$

6) **Determination of Iodine value (Joshi *et al.*, 2011)**

In 250 ml iodine flask 0.1gm oil was poured and 10ml of chloroform was added. By means of pipette Wij's reagent (10ml) was added. The flask was kept in dark for one hour with intermittent shaking then to the flask 15%potassium iodide solution (5ml) and 25ml distilled water was added and shaken well. Titration of liberated iodine was done with 0.1N sodium thiosulphate using fresh starch solution as an indicator. Simultaneously blank titration was carried out.

$$\text{I.V} = 12.69\ C\ (V1-V2)/M$$

Where C = Concentration of sodium thiosulphate used;

V1 =Volume of sodium thiosulphate used for blank;

V2 = Volume of sodium thiosulphate used for determination,

M = Mass of the sample.

7) Saponification Value Determination: (Umaru *et al.*, 2012)

In 25ml round bottom flask 1gm oil was weighed then 0.5N alcoholic potassium hydroxide solution(12.5ml) was added by pipette and then using air condenser for 1 hour reflux was carried out on heating mantle and then flask was cooled .The contents were titrated with 0.5N HCL solution using phenolphathein as an indicator Simultaneously blank titration was carried out

$$\textbf{Saponification value} = \frac{56.1 \times T (v_0 - v_1)}{M}$$

Where, T= molarity of the standard KOH solution used,

V0= volume of acid used for the first titration with oil sample

V1=volume of acid used for the second titration of the blank solution,

M= mass of the oil sample used

8) Determination of pH (Akpan *et al.*, 2014)

With the help of digital pH meter the Jatropha oil was measured by maintaining the pH buffer solution that is 7. Firstly pH electrode was cleaned and then it maintained at 7 by dipping it into pH buffer and then it was swabbed and dipped into oil sample and measured the pH.

9) Determination of flash point (Garba *et al.*, 2013)

The flash point is measured by the help of pensky martin apparatus. In this 1 gm oil is weighed by the means of pipette and then in poured in the cup of pensky martin and thermometer was fitted and as temperature rises flame is induced which

causes popping sound which gives the reading. And then the flash point was obtained when application of attest flame causes the vapour above the oil to ignite.

10) Determination of Fire point (Shambhu *et al.*, 2013)

The fire point is that point at which fuel vapour catches fire and stays for minimum five seconds. And at this point it was recorded in the pensky martin apparatus.

Fig 10:- Pensky Martin apparatus used for the determination of fire point and flash point

CHAPTER:-6

RESULTS AND DISCUSSION

RESULT AND DISCUSSION

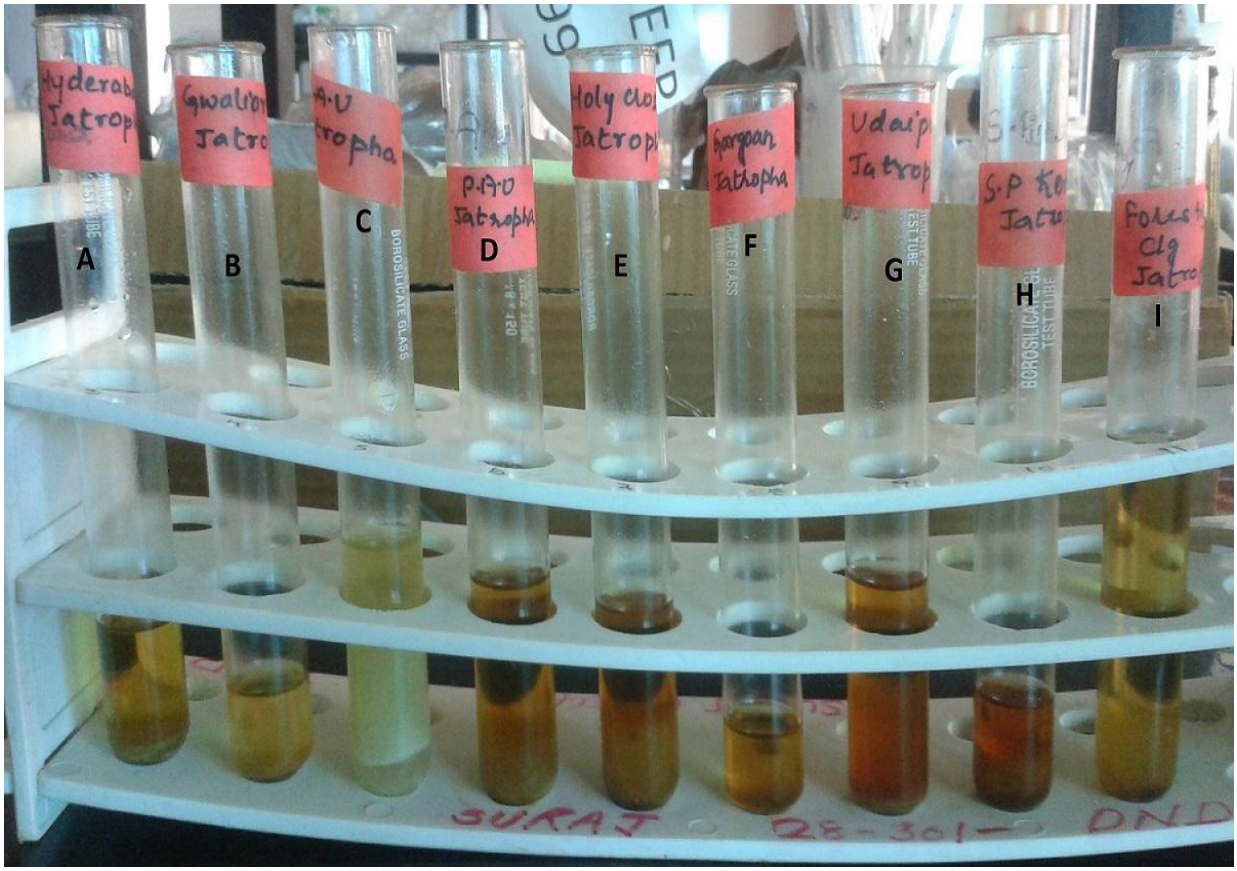

Fig 11:- Oil separated from hexane after distillation

Sample A- Hyderabad, Sample B- Gwalior, Sample C-B.A.U, Sample D- P.A.U, Sample E- Holy Cross, Sample F- Gurgaon, Sample G- Udaipur, Sample H- S.P. Kothi, Sample I- Forestry College

Fig a:- 1 ml oil is mixed with 25 ml of distill water which result in yellow color

Fig b:- Methyl red indicator is added and 50% H_2SO_4 is added which gives pink color

Fig c:- 100 ml petroleum ether is added and shaked which form two layers. Lower layer contain glycerin and upper layer contain biodiesel.

Fig d:- Washed it with distill water 2 to 3 times and left it for overnight and repeated for other sample.

Fig 12:- Biodiesel production by tranesterification process

Fig 13:- Produced biodiesel from Jatropha seeds

In Fig 13 it was showing biodiesel produced after transesterification process.

Sample A- Forestry College

Sample B- Punjab Agriculture University

Sample C- Udaipur

Sample D- S.P.Kothi

Sample E- Holly cross

Sample F- Birsa Agriculture University

Physical properties were evaluated from *Jatropha curcus* seed. Physical properties of seeds are given in Table 3:-

Table 3:- Biophysical Characterization of *Jatropha curcus* Seed

S.NO	SAMPLE	SOIL	CLIMATE	SEED COLOR	ODOUR
1	FORESTRY COLLEGE	Laterite	Hot and cold	Dull brownish black	Disagreeable
2	HOLLY CROSS	Sandy	Hot and cold	Dull brownish black	Disagreeable
3	B.A.U	Laterite	Hot and cold	Dull brownish black	Disagreeable
4	S.P KOTHI	Sandy	Hot and cold	Dull black and smooth	Disagreeable
5	UDAIPUR	Sandy soil	Hot	Dull brownish black	Disagreeable
6	P.A.U	Sandy soil	Extreme hot and cold	Dull black	Disagreeable
7	GURGAON	Fertile soil	Hot and humid	Dull black	Disagreeable
8	HYDERABAD	Red soil	Wet and dry	Dull brownish black	Disagreeable
9	GWALIOR	Alluvial soil	Hot and humid	Dull brownish black	Disagreeable

Table 4: Biophysical characterization of Jatropha oil from different regions of India

S.no	Sample	Oil content (%)	Biodiesel (gm)	pH
1	Forestry college	31.6	25.23	6.5
2	Holy cross	26	22.67	6.9
3	Birsa Agriculture University	29.36	17.05	7
4	S.P Kothi (Hazaribag)	16.48	24.29	7.2
5	Udaipur	33.4	24.3	8
6	Punjab Agriculture University	25.6	29.6	7.5
7	Gurgaon	24.48	15.5	7.2
8	Hyderabad	24.16	17.9	8
9	Gwalior	23.04	22.5	6.9

- The above value is mean of triplicates.

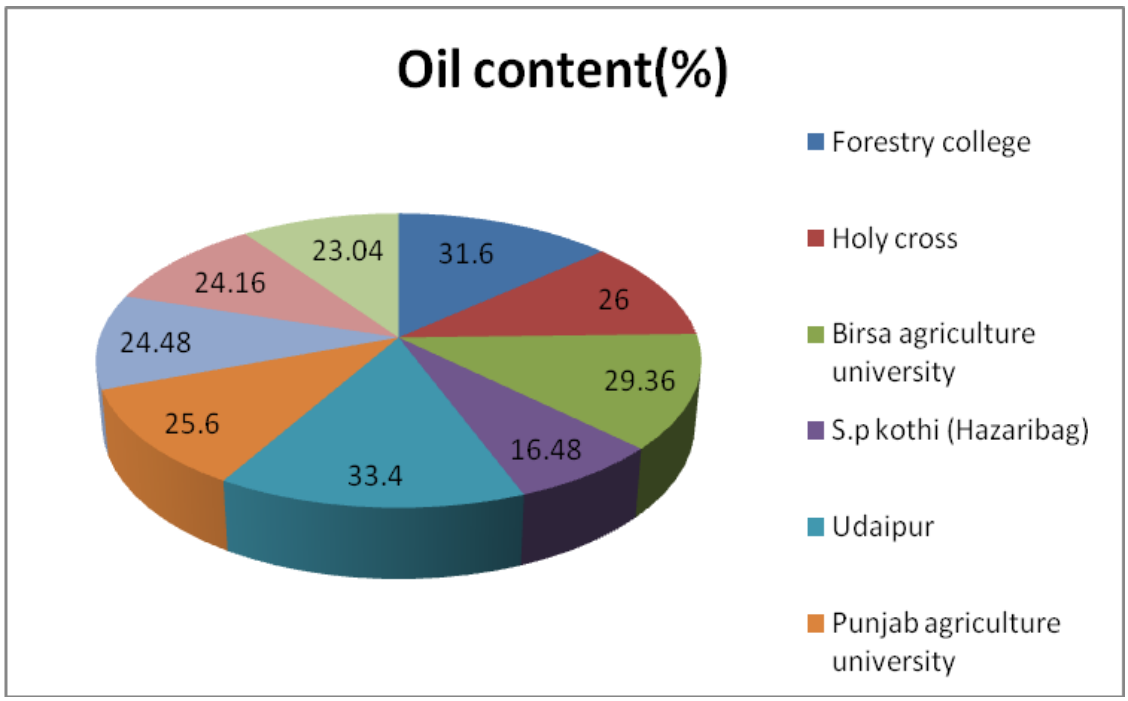

Fig 14:- Graph Showing the oil content in % in different regions of India.

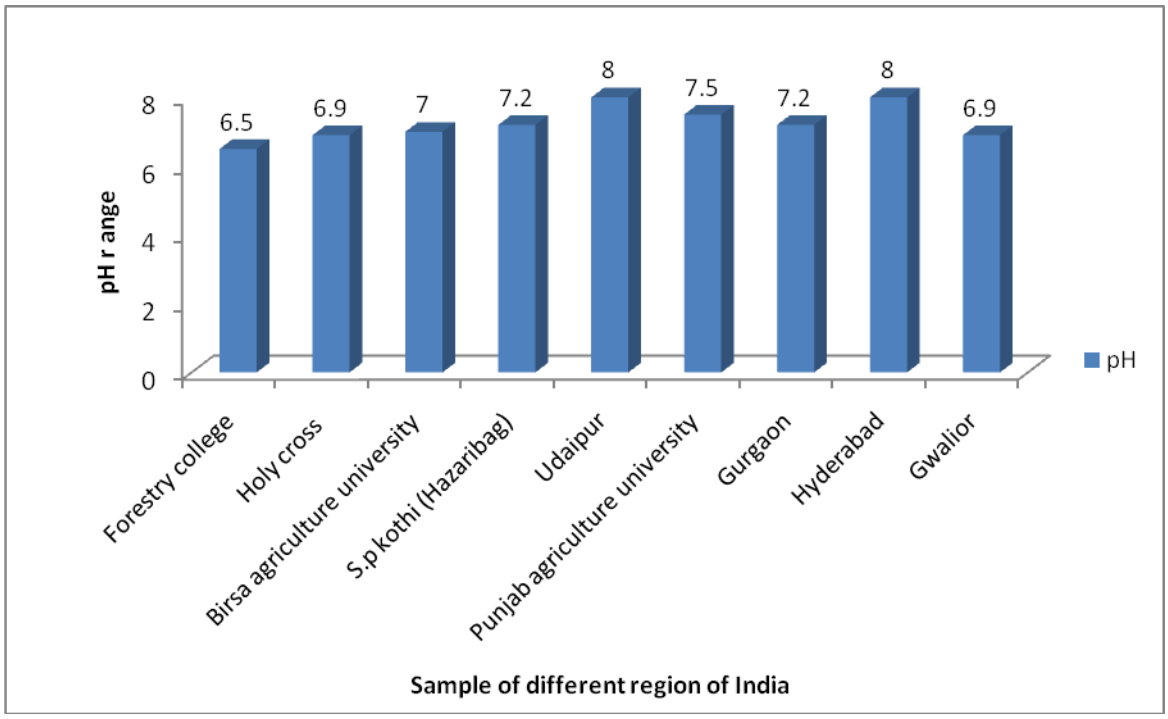

Fig 15:- Graph showing the pH value of different samples from different regions of India

The oil yield percentage of *Jatropha* seed collected from different regions of India showed the highest amount of oil is 33.4% in the seeds collected from Udaipur and lowest in S.P Kothi (Hazaribag) 16.48 % similar result were recorded by **Joshi *et al.* (2011).** The variation in oil yield is due to the gene variation, typical weather, different species of plant, soil condition and not good handling techniques such as delaying and long exposure in harvesting seeds to sunlight can affect the oil yield of *Jatropha curcus* **(Umaru *et al.*, 2012).** And Biodiesel produced after tranesterification collected from nine different regions of India showed the highest amount of biodiesel is 29.6 gm in the seed collected from Punjab Agriculture University and the lowest in Gurgaon is 15.5. The highest pH value is 8 in Udaipur and the lowest pH value is 6.5 in Forestry College.

Biophysiochemical characterization was done in which fire point, flash point, iodine value and saponification value is determined which is mentioned in table 5. In the Table 5 the highest fire point is 280^0c in Birsa Agriculture University and the lowest fire point is 190^0C in P.A.U and similar result were recorded by **Shambhu *et al.* (2013)**. The flash point is 260^0C in Birsa Agriculture University and the lowest flash point is 170^0c in P.A.U and similar results were recorded by **Shambhu *et al.* (2013).** In higher temperature, Flash point and Fire point of *Jatropha curcus* oil prevent from explosion and danger of fire. Flash point is important condition for the safety during use or management, storage space, and transportation and carrying (**Garba *et al.*, 2013).**

The highest iodine value is 90.17 in S.P Kothi and the lowest iodine value is 76.2 in Gurgaon and similar results were recorded by **Folaranmi (2012).** Greater the degree of saturation point higher is the iodine value. The oil concentration below 100 is non dried oil. If there is decrease in unsaturation of oil and fats then it means that oxidation stability is greater (**Garba *et al.*, 2013)**. And the highest saponification value is 297.33 in S.P.Kothi and the lowest saponification value is 201.96 in Gwalior similar result was recorded by **Belewu *et al.* (2010).** Saponification value is the rate of reacting alkali group in oil and fats. High saponification value was found better because it causes fatigue release during the fire of the engine. *Jatropha curcus* oil was found to be better with high saponification value (**Garba *et al.*, 2013).**

TABLE 5:- Biophysical characterization of Jatropha oil from different regions of India (the below Table is mean of triplicates)

Sn .no	Sample	Fire point (^{0}c)	Flash point (^{0}c)	Iodine value (mg KOH/g oil)	Saponification value (mg KOH/g oil)
1	Forestry college	220	200	88.9	280.5
2	Holly cross	240	220	82.55	258.06
3	Birsa Agriculture University	280	260	86.36	252.45
4	S.P Kothi	250	210	90.17	297.33
5	Udaipur	210	200	82.55	260.86
6	Punjab Agriculture University	190	170	88.9	210.37
7	Gurgaon	220	200	76.2	266.47
8	Hyderabad	260	220	77.47	204.76
9	Gwalior	260	230	86.36	201.96

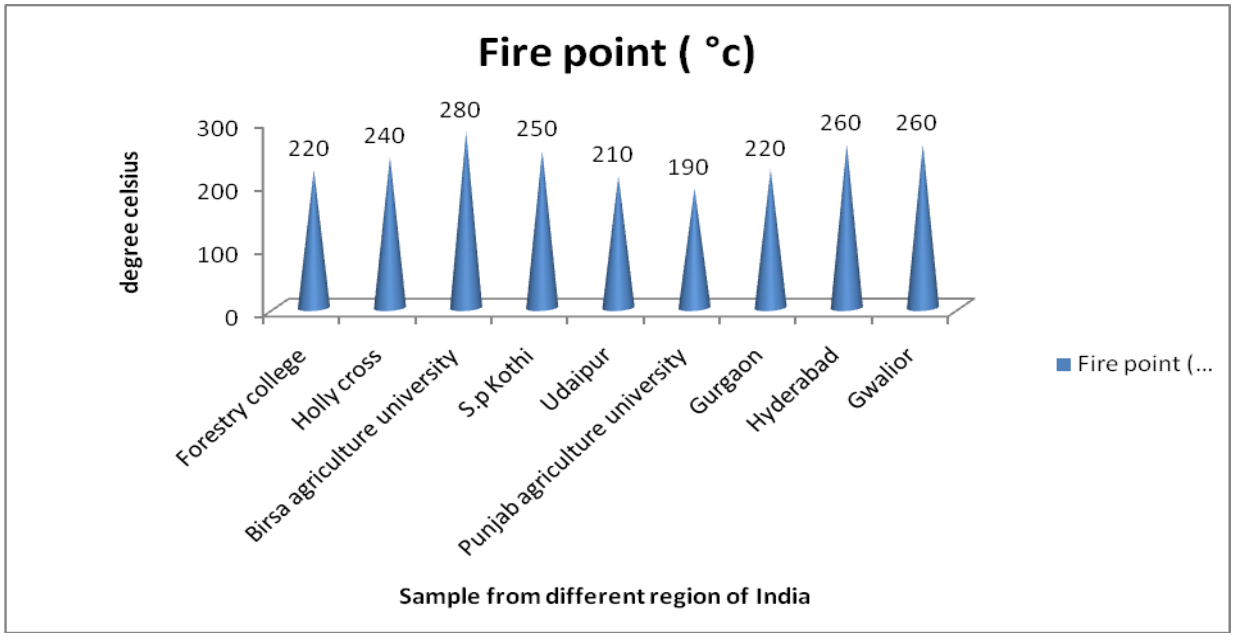

Fig 16:- Graph showing the fire point of different sample from different regions of India

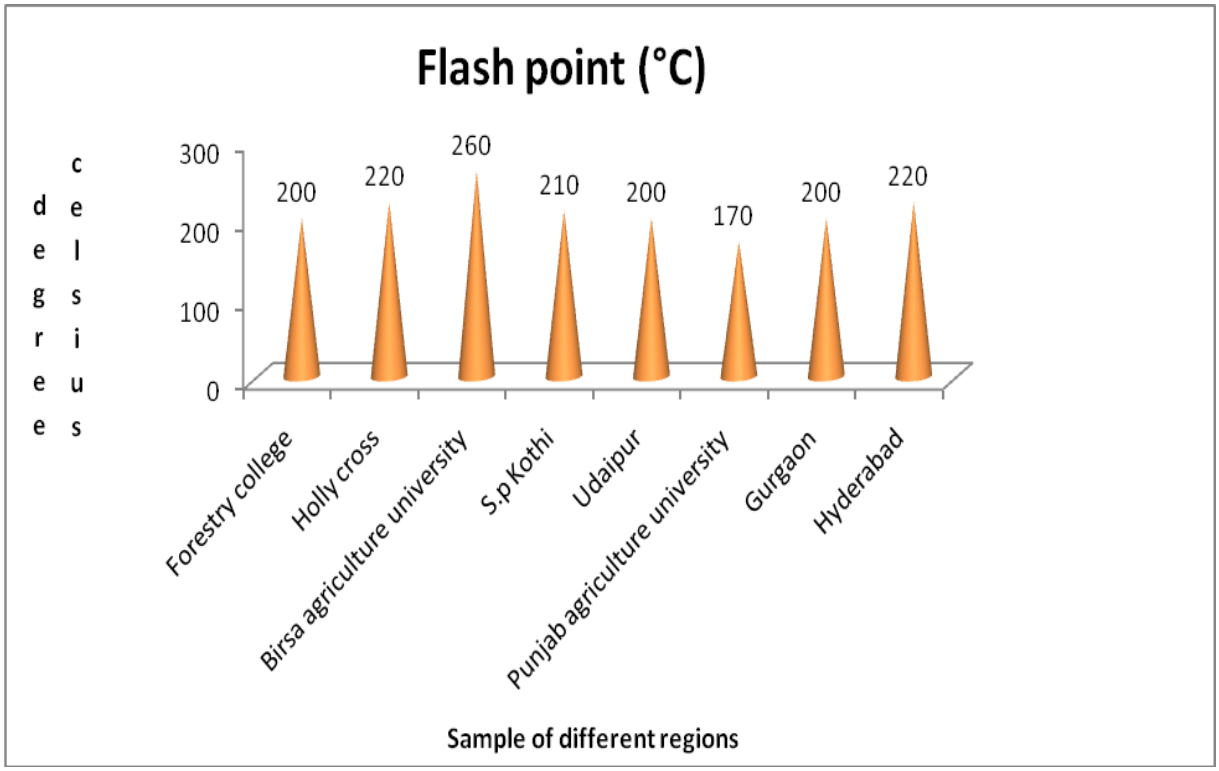

Fig 17:- Graph showing the result of flash point of different sample from different regions of India

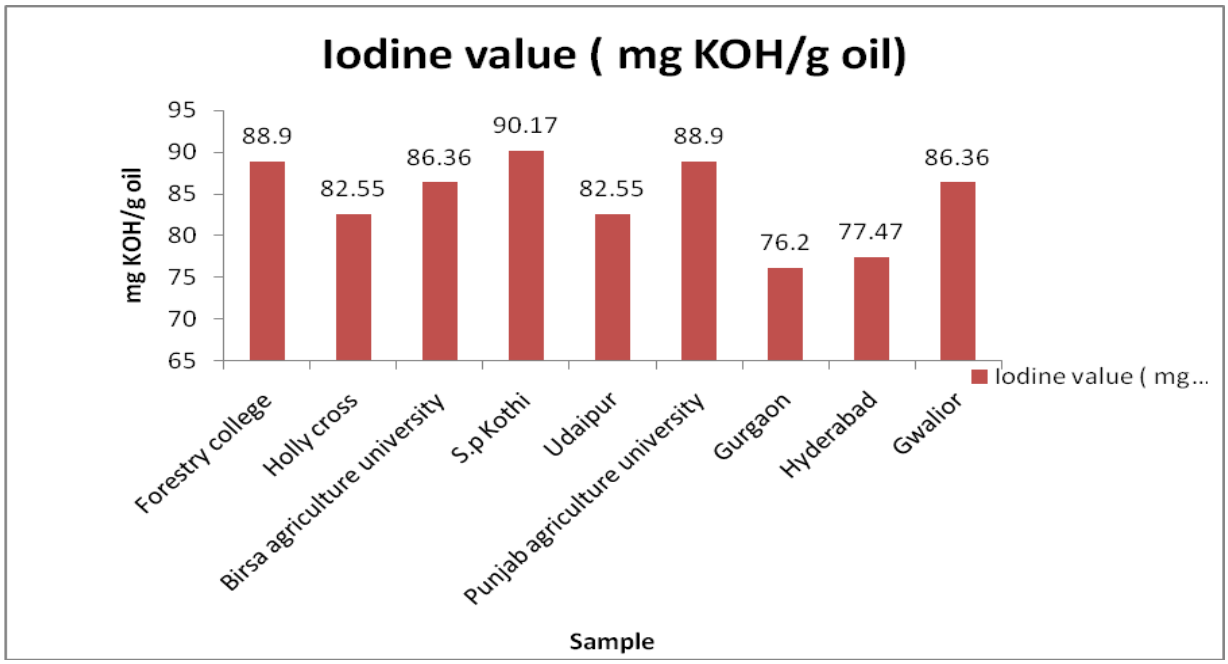

Fig 18:- Graph showing the result of Iodine value of different sample from different regions of India

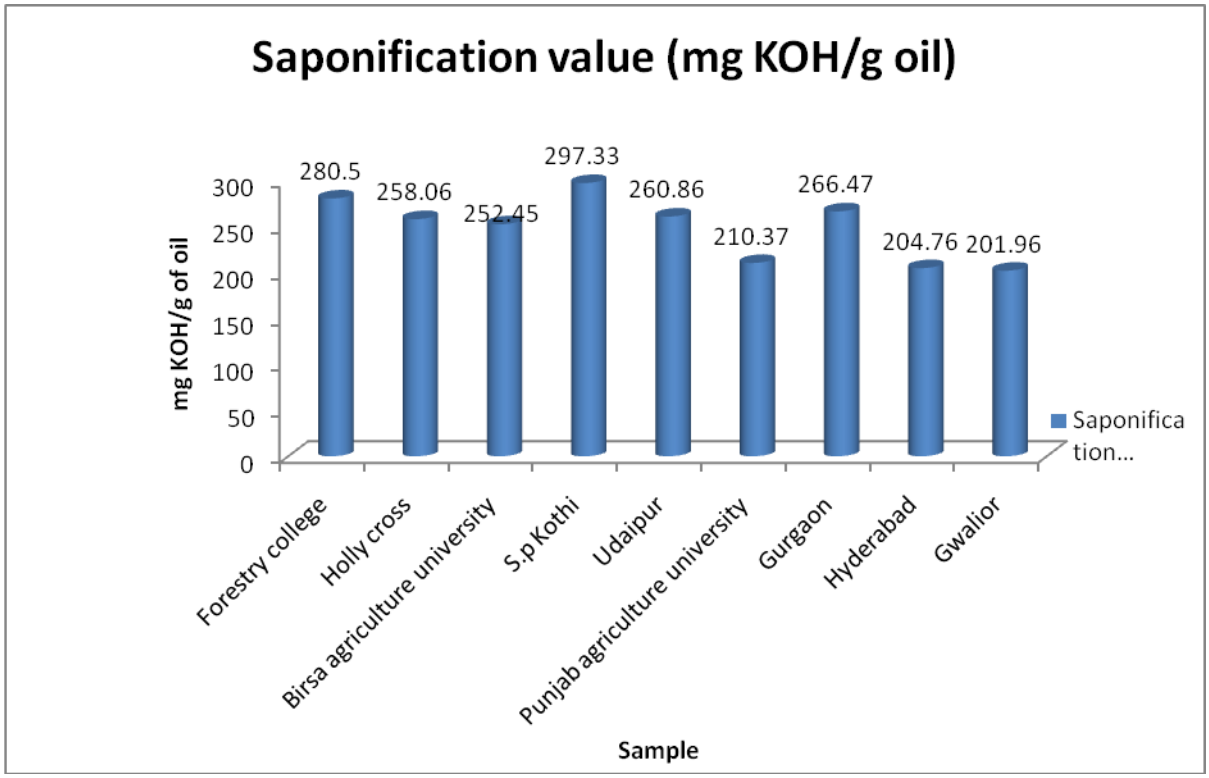

Fig 19:- Graph showing the result of saponification value of different sample from different regions of India

TABLE 6:- Biophysical characterization of Jatropha oil from different regions of India (The below value is mean of triplicates)

S.NO	SAMPLE	Acid Value (mg KOH/ gm oil)	Fatty Acid (mg KOH/ gm oil)	Specific gravity (40^0c) gm	Viscosity (40^0c) mm^2/s
1	FORESTRY COLLEGE	0.68	0.34	0.895	34.6
2	HOLY CROSS	2.40	1.20	0.861	30.4
3	BIRSA AGRICULTURE UNIVERSITY	1.37	0.68	0.811	32.9
4	S.P KOTHI	3.09	1.50	0.872	30.9
5	UDAIPUR	1.89	0.94	0.855	33.1
6	PUNJAB AGRICULTURE UNIVERSITY	3.78	1.89	0.922	35.6
7	GURGAON	0.60	0.30	0.876	32.1
8	HYDERABAD	1.46	0.73	0.894	35.9
9	GWALIOR	0.86	0.43	0.917	36.4

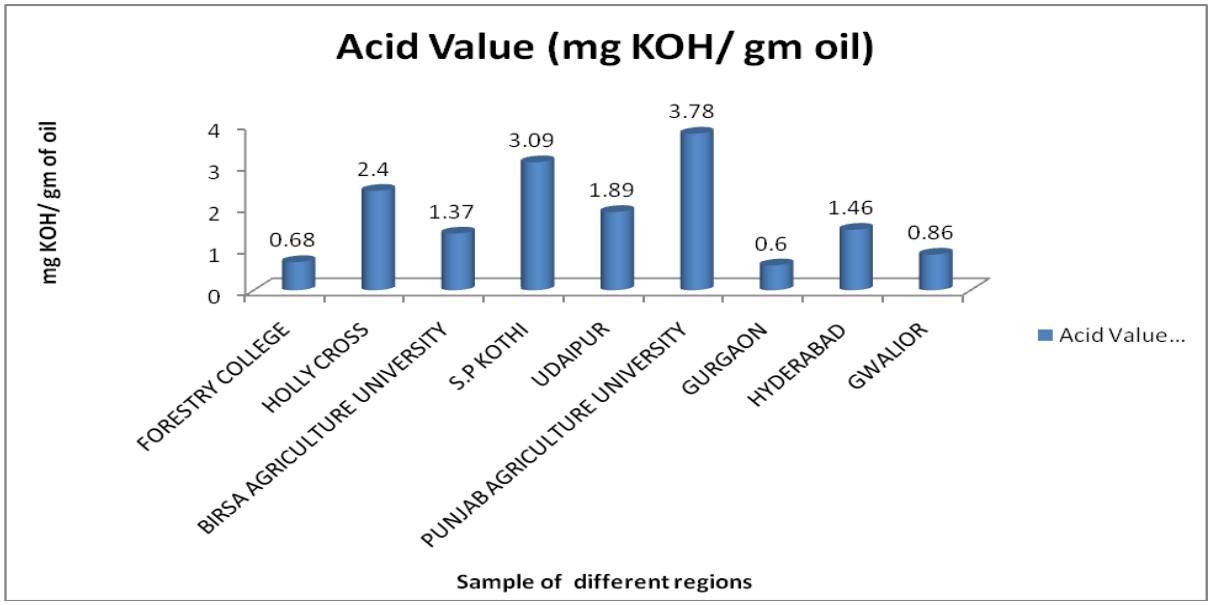

Fig 20:- Graph showing the result of acid value of different sample from different regions of India

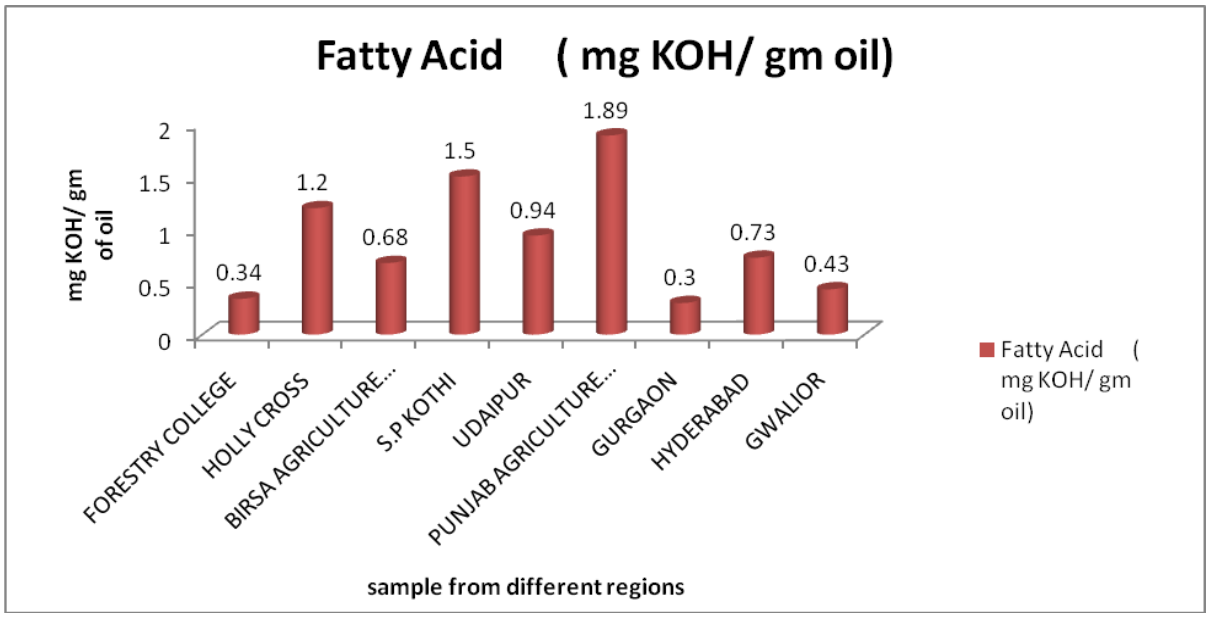

Fig 21:- Graph showing the result of fatty acid of different sample of different regions of India

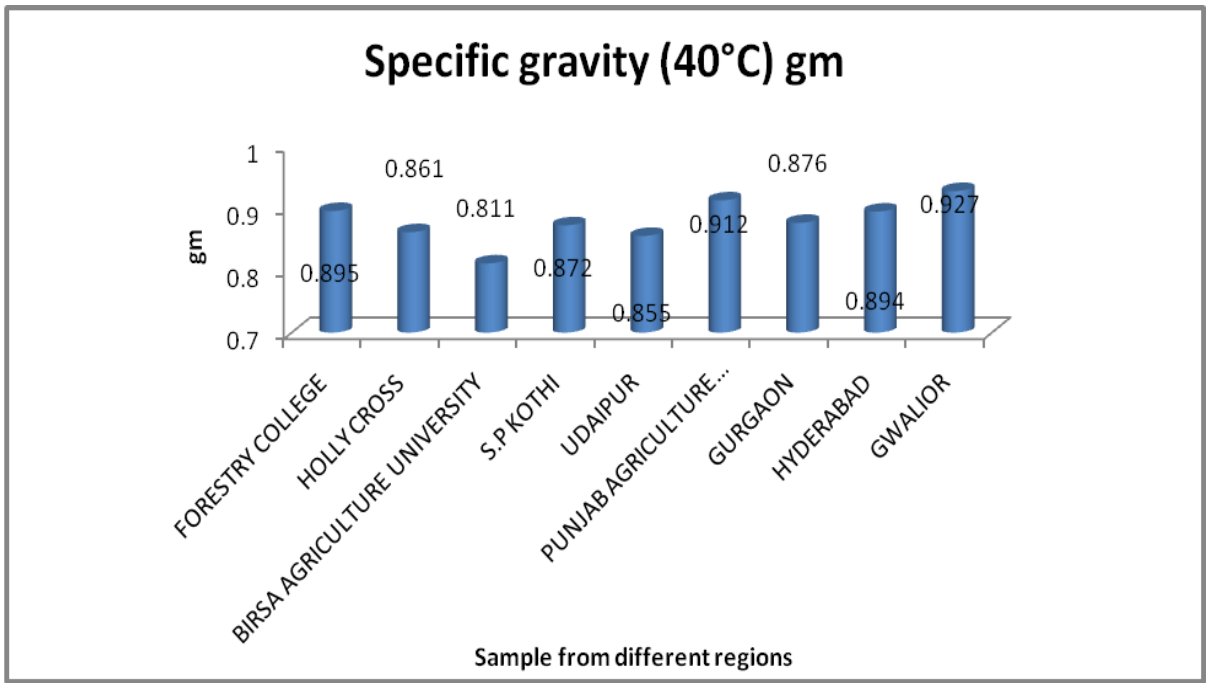

Fig 22:- Graph showing the result of specific gravity of different sample from different region of India

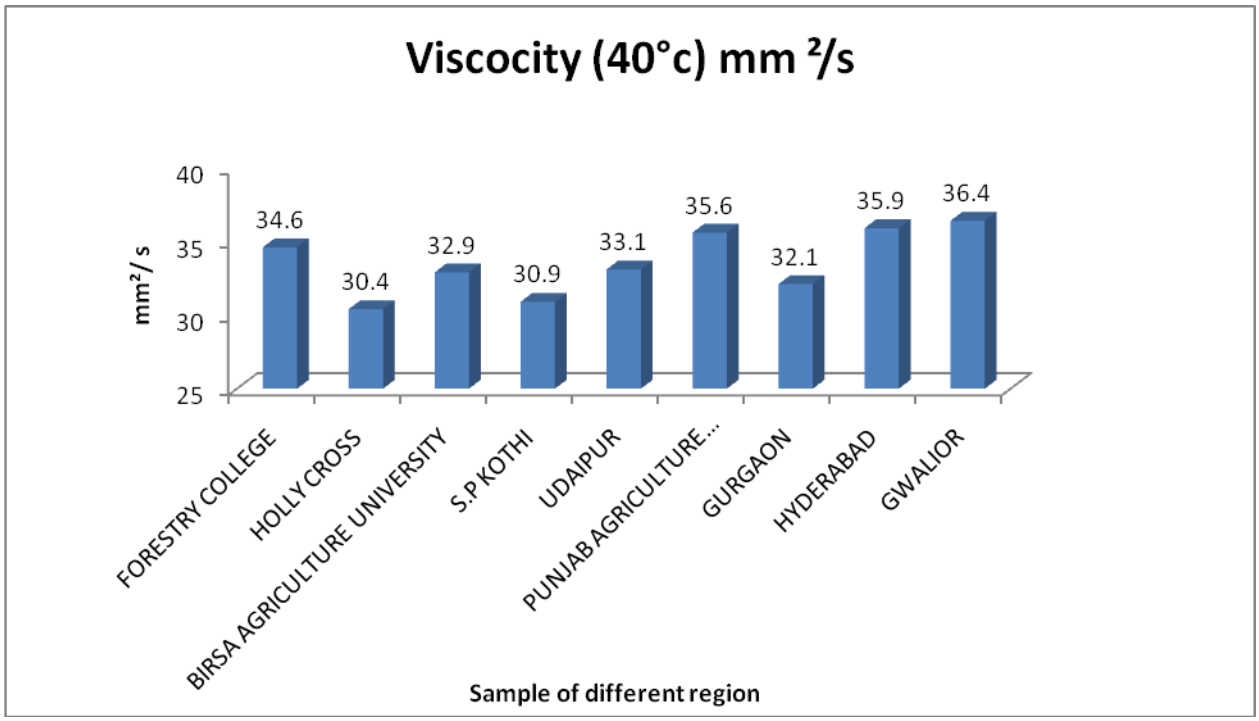

Fig 23:- Graph showing the result of viscosity different sample from different regions of India

The highest acid value is 3.78 mg KOH/ gm in Punjab agriculture university and the lowest acid value is 0.60 mg KOH/gm in Gurgaon similar result were recorded by **Okullo *et al.* (2012)**. Higher the acid value content then its shows the presence of oxidation which can cause deterioration and sludge in system. An acid value is an indication of grow old or mature and value of the oil (**Umaru *et al.*, 2013)**. The highest free fatty acid value is 1.89 in Punjab Agriculture University and the lowest free fatty acid value is 0.3 in Gurgaon similar results were recorded by **Okullo *et al.* (2012)**. Higher the concentration of free fatty acid value then it leads to formation of soap during the process of alcholysis which lead in complex separation of biodiesel from its soap and glycerin present in the oil which results in the less production of biodiesel (**Umaru *et al.*, 2013).** The highest value of specific gravity 0.922 in P.A.U and the lowest value 0.81 in Birsa Agriculture University and similar result were recorded by **Garba *et al.* (2013)**. The specific gravity depends as the oil density decreases with molecular weight with increasing unsaturation level (**Umaru *et.al* 2013).** The highest value of viscosity is 36.4 in Gwalior and the lowest value is 30.4 in Holly cross and similar result were recorded by **Patel *et al.* (2013)**. Lubricant is good factor for viscous oil. Hence, *Jatropha curcus* oil is having good lubricating properties. Higher the viscosity means there is impurities in oil. For the biodiesel production the viscosity is reduced from Jatropha oil because highly viscous is not suitable for the production of biodiesel. If highly viscous is used in the engine as fuel then it may causes several problem and bad operational functioning of engine. The problem is like deposition of carbon, sticking of oil as a ring formation, formation of thick layer and gelling substance of viscous oil. Transesterification is the process for the reduction of viscosity and making oil suitable for using in engine as a good fuel which reduces the problem of choking of engines and heating (**Umaru *et al.*, 2013).**

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CHAPTER:-7

CONCLUSION

CONCLUSION

TheJatropha oil from Birsa Agriculture University was best for the production of biodiesel which was having flash point 260^0 C and fire point 280^0C. The factor which was involved best for the biodiesel production was flash point, fire point, oil content and free fatty acid in which two factors was showing best properties for the production of biodiesel. The flash point and fire point is higher so, it is reducing from auto ignition, for safety during use or management, storage space and transportation. The highest oil content was found in 33.4 % in Udaipur and lowest oil content was found in 16.48% S.P.Kothi. Highest Iodine value was found in S.P.Kothi 90.17 mg KOH/ gm of oil is best in Industrial scale in the production of shoe polishes, varnishes and alkyl resins. Highest saponification value was found in S.P Kothi 297.3 mg KOH/ gm of oil is best in Industrial scale in the formation of soap. Highest viscosity was found in Gwalior 36.4 mm^2/s is best for the production of candles, waxes, soap formation, cooking, lighting, for the production of paraffin's, in the cosmetic industry and medicines.

On confirmation it is observed that Jatropha oil is an alternative to biodiesel. Results depicts that it is a promising area of research on large scale basis for the production of biodiesel. It has found its application in surface coating. So, it is amenable to continue more research over it to explore its potentials for future industrial oilseeds crop.

It is also concluded from this study that the biodiesel produced from *Jatropha curcus* has potential to reduce the petro-diesel which is very harmful for environment and population and protecting from the hazards by using a fossil fuel and hence will boost the economy of the country.

CHAPTER:-8

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CHAPTER:-9

APPENDIX

APPENDIX

Abbreviations

1) CJCO – Crude Jatropha curcus oil

2) FFA - Free fatty acid

3) ASTM – American society for testing and material - provisional specification for Biodiesel

4) F.A- Fatty acid

5) I.V- Iodine value

6) S.V – Saponification value

7) FAME- Fatty acid of methyl esters

8) H_2SO_4- Sulphuric acid

9) NaOH- Sodium hydroxide

10) KOH- Potassium hydroxide

11) A.V- Acid value

Chemical preparation

1) Starch preparation:-

1 gm of starch was added in 10 ml of distill water and mixed well and then it was poured into 100 ml of boiling distill water and stirred thoroughly and boil for 1 min. and leave to cool down.

2) 0.5 N Alcoholic KOH:-

Prepared 95% ethanol: - 95 ml ethanol and 5 ml distill water. Then for 100 ml of alcoholic KOH added 2.8055 gm of KOH and mixed in 95% ethanol.

3) 0.5 N HCL:-1.824 ml in 100 ml distilled water.

4) 15% potassium iodide:-1.5 gm in 100 ml distilled water.