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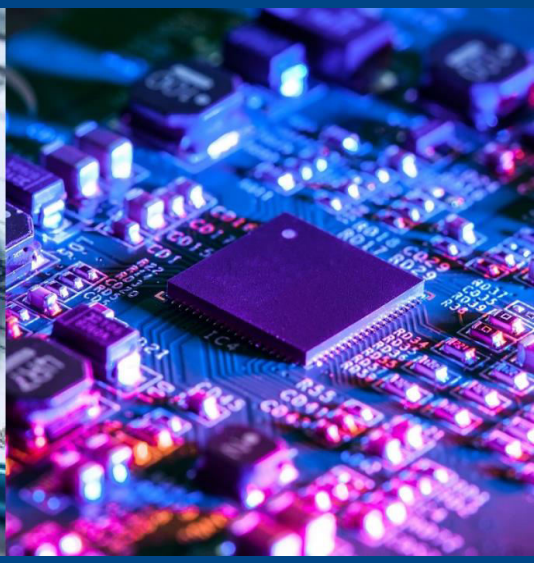

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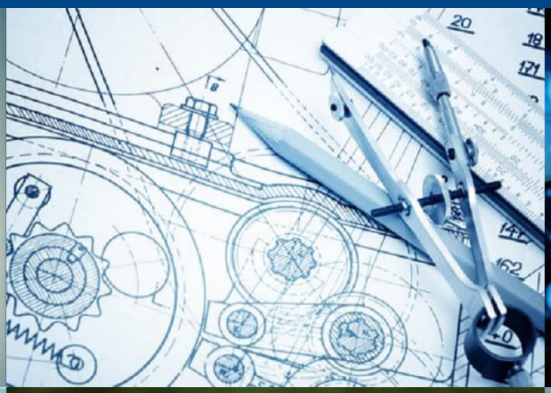

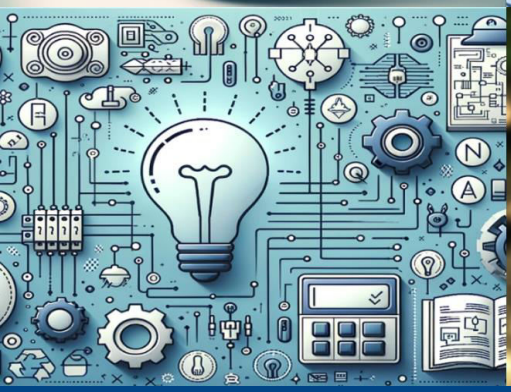

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Transcriptome Analysis of *Jatropha Curcas* L. Seed Oil: A Review

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ABSTRACT: *Jatropha curcas* L. is a multipurpose shrub with a variety of applications, including the production of biodiesel. The present study deals with the optimization of oil extraction from *Jatropha* seeds using various organic solvents and techniques. The effects of type of organic solvent and extraction technique on oil yield were investigated. The acid value and antioxidant property of the oil were also determined.

The maximum oil yield was obtained using the Soxhlet extraction method and hexane as a solvent. Petroleum ether was found to be the best solvent for producing pure and colorless oil, though its yield was lower than that of hexane and isopropanol. The acid value of the oil was found to be 2.24±0.01 mg KOH/g. The scavenging activity of the leaf and oil extract was found to be 29.92±4.72 and 19.94±1.39%, respectively. The results suggest that *Jatropha* oil can be used as a source of biodiesel and antioxidants. (Shivani P., Khushbu P., Faldu N. et al., 2011)

The effects of natural aging on *Jatropha curcas* L. oil, a popular biodiesel source. Results show that prolonged aging decreases oil yield and deteriorates the oil's quality. However, natural aging seeds, even up to a year, can still be used due to their resistance to deteriorating effects. (Suresh A. et al, 2019)

A modified SDS-Trizol method was used to isolate total RNA from maize seeds stored at regular intervals for four months. The method reduced co-precipitation problems due to high carbohydrate content in the seeds. The RNA yield from seeds was 978.6 ± 65.46 ng/µl, while fresh leaves had 1008.2 ± 77.088 ng/µl. DNase treatment improved the A260/280 ratio in both seeds and leaves. The extraction of RNA from stored seeds and fresh leaves opened new possibilities for understanding developmental steps. (Dutta S., Muthusamy V., Chhabra R .et al.)

I. INTRODUCTION

In recent years, the global focus on renewable energy sources, particularly biomass, has become fundamental for the sustainable development of civilizations. Liquid bio-energy production from vegetable oils has emerged as a viable option to reduce greenhouse gas (GHG) emissions. The global bio-diesel production during 2004–2005 was estimated to be 2.36 million tonnes, with the European Union (EU) countries and the USA contributing significantly, accounting for 88% of the total production. Bio-diesel production is expected to grow, with projections indicating it will reach 24 billion litres and become the largest share of biofuel production by 2017. (Divakara B. , Upadhyaya H. , Wani S et al., 2010)

India, as the fifth-largest consumer of fossil fuels globally, has launched the "National Mission on Bio-diesel" to explore renewable liquid fuel options based on vegetable oils. However, the shortage of raw materials poses a significant challenge. While many developed countries utilize edible oil-seed crops for bio-diesel production, developing countries like India explore non-edible oil seeds such as Pongamia (Pongamia pinnata) and Jatropha (*Jatropha curcas*) to meet the demand without compromising food and fodder security.

Jatropha curcas, a hardy plant native to tropical America, has gained importance in tropical and sub-tropical countries due to its numerous advantages. It can thrive in diverse agro-climatic conditions, has a high oil content, low seed cost, and is adaptable to wastelands. Additionally, sustainability assessments indicate favourable environmental impacts when cultivated on degraded lands. Despite its potential, *Jatropha curcas* faces challenges in realizing its full potential. Limited research, especially in genetics and agronomy, has hindered its commercial viability. Large-scale plantations in countries like Brazil, Nicaragua, and India have shown low productivity, leading to commercialization difficulties.

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A comprehensive review of existing literature is necessary to address these challenges. This review aims to consolidate information from various sources to provide a holistic view of the genetic improvement of *Jatropha curcas*. The lack of comprehensive studies, bench-mark descriptors, and information on genetic variability and genotype × environment interaction necessitates a concerted effort to bridge these gaps in knowledge. By integrating available information and experiences, a more informed approach can be developed for the successful genetic improvement of *Jatropha curcas*, thus unlocking its full potential as a renewable energy source.

The plant has spread beyond its original distribution due to its advantages, such as its hardiness, easy propagation, drought endurance, high oil content, low seed cost, short gestation period, rapid growth, adoption to wide agro-climatic conditions, bushy/shrubby nature, and multiple uses of different plant parts. Biodiesel fuel is rapidly expanding due to demand, policy support, and technological availability. India, ranked 5th in the world in terms of fossil fuel consumption, launched the National Mission on Bio-diesel to develop a cheap and renewable liquid fuel based on vegetable oils. The National Biodiesel Accreditation Program, BQ-9000, is a cooperative and voluntary program for the accreditation of producers and marketers of biodiesel fuel. The program helps companies improve their fuel testing and reduces the chance of producing or distributing inadequate fuel. In the interim period since the original Washington State Ferries biofuel test of 2004-05, there have been continued improvements in manufacturing and handling processes and monitoring of these systems. Biodiesel byproducts, such as crude glycerol, have been used as a carbon source for the production of industrially important chemical components (Waghmare T. et al, 2017).

II. JATROPHA CURCAS L. : AN OVERVIEW

Jatropha curcas L. , a versatile, drought-resistant perennial plant from the **Euphorbiaceae** family, holds significant promise for biodiesel production. This tropical plant thrives in a wide range of rainfall areas, making it suitable for commercial cultivation and field boundaries, serving as both a protective hedge against grazing animals and erosion prevention. To harness its industrial potential effectively, comprehensive knowledge of the plant's biology, chemistry, and diverse applications is imperative. This paper explores the taxonomy, botanical description, distribution, and ecological requirements of Jatropha. It critically evaluates various propagation techniques, including tissue culture, enabling the production of large, disease-resistant plantlets. The study meticulously examines the presence of chemicals, including toxins, in different plant parts. It delves into the possibilities of harnessing the plant's potential across multiple applications. Additionally, the paper investigates toxins and detoxification methods, offering a holistic overview encompassing the plant's biology, chemistry, seed toxicity, detoxification, and industrial uses. Emphasizing the socio-economic benefits for both rural and urban economies, this review provides a comprehensive understanding of Jatropha curcas. (Kumar & Sharma , 2008)

The study explores the multifaceted environmental impacts of *Jatropha curcas* biodiesel cultivation in India, a strategic move by the country to achieve energy security and reduce its reliance on crude oil imports. India set ambitious biofuel blending targets to foster energy self-reliance and employment opportunities, primarily focusing on utilizing marginal lands for Jatropha cultivation.

Jatropha curcas, identified as a suitable biodiesel feedstock due to its ability to grow on marginal land and produce high-quality oil, was promoted extensively. However, the cultivation of Jatropha curcas on these lands led to the displacement of existing land use patterns, affecting local communities' activities like livestock grazing and wild product gathering.

While *Jatropha curcas* cultivation has the potential to prevent desertification and enhance ecosystem functions on marginal lands, the environmental impacts vary significantly based on cultivation intensity and former land use. The study highlights that using Jatropha curcas biodiesel can reduce greenhouse gas emissions compared to fossil diesel, but its environmental sustainability is complex and context-dependent. Factors such as irrigation, processing efficiency, and usage of Jatropha products and byproducts play crucial roles in determining its overall environmental impact.

The study specifically assessed nine agronomic trials in Andhra Pradesh, considering inputs like water, fertilizer, pesticides, and arbuscular mycorrhizal fungi. Additionally, it compared *Jatropha curcas* methyl ester (JME) with fossil diesel in terms of non-renewable energy consumption, global warming potential, eutrophication, acidification, ecotoxicity, water demand, and land use.

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The research aims to determine the optimization potential of the *Jatropha curcas* value chain, emphasizing the need for comprehensive, context-specific assessments to ensure the environmental sustainability of biodiesel production from *Jatropha curcas* in India. (Gmünder S., Singh R., Pfister S. et al., 2012)

III. METHOD & METHODOLOGY

In this study, *Jatropha curcas* seeds were collected from a nursery in Rajkot, India. Healthy and clean seeds were selected, and damaged ones were discarded after which they were dried before use. Various extraction methods were employed to obtain oil from these seeds. (Shivani P., Khushbu P., Faldu N. et al., 2011)

1. Solvent Extraction Techniques:

The seeds were cracked, and the shells were removed, leaving the kernels. These kernels were ground and subjected to solvent extraction using organic solvents like petroleum ether, hexane, and isopropanol. The extracted lipids were separated from the solid residues using techniques like filtration, centrifugation, and separating funnel. The extracted seed oil was stored at -20°C for further analysis.

2. Aqueous Enzymatic Oil Extraction (AEOE) from Seed Kernels:

Seeds were soaked in water and ground into a paste without adding extra water. The paste was mixed with distilled water, and cellulase enzyme was added. After incubation, the upper oil phase was collected through centrifugation. A control experiment without enzyme addition was also conducted.

3. Soxhlet Extraction:

Seed kernels were ground and defatted using a soxhlet apparatus with solvents like hexane, isopropanol, and petroleum ether. The extraction continued for 6 hours. The extracted oil was then recovered by removing the solvent through vacuum evaporation and heat exposure.

4. Acid Value (%FFA) Determination:

The acid value, indicating free fatty acids (FFA) content, was determined by titrating the oil sample with 0.1 N KOH in a solvent mixture. The acid value was calculated using a standard formula.

5. Antioxidant Assay:

Extracts from fresh leaves and oil were prepared using methanol. After filtration and solvent evaporation, the dried extract was used for antioxidant assays. The DPPH (1,1-diphenyl picryl hydrazyl) free radical scavenging activity was measured by mixing the extract solution with a DPPH methanol solution. The radical scavenging capacity was calculated as a percentage of DPPH radical scavenging effect.

Physical properties of J. curcas seeds

Moisture content: This study used dry seeds of J. curcas for experiments, which were cleaned manually and oven dried to determine their initial moisture content. Samples were prepared by adding distilled water and stored in a refrigerator for a week. The seeds were then equilibrated at room temperature for 2 hours before testing. The physical and mechanical properties of the seeds were determined at five moisture contents in the 7.78–21.67% d.b. range with four replications at each moisture content.

Plant seed studies: Analysis of 100 irrigated seeds of J. curcas using a Vernier Caliper to measure axial dimensions. The geometric mean diameter (Dg) was determined using the formula DP = (abc) 1/3, where a, b, and c are the dimensions along the longest axis in mm. Sphericity was also determined using Mohsenin's formula (Ψ = (abc) 1/3).

To identify Lipids in a given sample by thin layer chromatography: Lipids, found in biological materials, are extracted as lipoprotein complexes. Efficient extraction requires aqueous solvents like ethanol or methanol in non-polar organic solvents like chloroform and diethyl ether. Extracted lipid components can be separated using TLC, spraying plates with different chemicals.

Extraction of lipids from sample: The tissue was ground in extraction solvents, separated, and evaporated. The lipid extract was prepared using TLC plates and analyzed using petroleum ether or hexane: ethyl ether: glacial acetic acid.

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Lipid spots were identified using UV light and charred areas. The Rf value of lipid components in the sample was calculated and compared with lipid standards. The lipids were identified by comparing their Rf values with lipid standards.

Electrophoresis (SDS- PAGE) Banding pattern of proteins in J. curcas: Proteins from leaves were separated using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using the Laemli (1970) method. Fresh plant tissues were ground, centrifuged, and stored. Protein estimation was done using Lowry et al.'s (1951) method. Samples were loaded onto a gel, mixed with sample buffer, and electrophoresed. The proteins were stained with CBB and stored in methanol. The banding pattern was observed under UV Tran's illuminator.

RNA extraction & isolation:
The seeds of *Jatropha curcas* L. were collected from Dahod, Gujarat, India and were initially sun dried for 48 hours to remove moisture. Seeds were grouped into three categories: fresh seeds, accelerated aging treatment, and seeds of natural aging. Aging treatments were given for various durations, including 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 10 days, 12 days, and 15 days. The oil content of the seeds was determined using the Soxhlet extraction method, milling them to 1-mm particle size. The oil was extracted using a solvent-oil mixture and collected and weighed. The percentage oil yield was calculated using the following expression. Qualitative analysis of the oil involved various biochemical tests such as iodine value (IV), saponification value (SV), peroxide value (PV), acid value (AV), and percentage of free fatty acids (%FFA). Acid value was determined by titration with 0.1 N KOH solution, while free fatty acids were determined by titration with 0.5 N HCl and 1% phenolphthalein indicator. Fatty acid analysis of oil by gas chromatography involved adding 0.4 N sodium methylate to oil and heating it in a water bath at 65°C. The resulting homogenous solution indicated the composition of esterification. The GC was equipped with a SS packed column and flame ionization detector, and individual peaks of the samples were identified by comparing their relative position or retention time with the standards. Statistical significance of the results was determined using the one-way ANOVA method, with values considered statistically significant at p-values of 0.05, 0.01, and 0.001, respectively.

RNA Isolation: The study involved the isolation of RNA from seeds and leaves using the Trizol method. Young leaf samples were collected at 30 days after sowing and stored at -80°C until RNA extraction. Mature seeds were collected after self-pollination and stored in a muslin cloth bag under ambient room temperature. Seeds from the muslin cloth bag were stored for 4 months at ambient temperature. At monthly intervals, seeds were kept under deep freeze condition at -80°C until RNA extraction. The Trizol method was optimized in Maize Genetics Unit, IARI, New Delhi. Three to four seeds stored in muslin cloth at each month interval were ground using liquid nitrogen to make a fine powder. The samples were then transferred to a pre-chilled 1.5 ml DEPC treated micro-centrifuge tube and 500 µl of sodium dodecyl sulphate (SDS) extraction buffer. The samples were centrifuged at 12,000 rpm for 12 minutes at 4°C, and the supernatant was transferred to a fresh 1.5 ml centrifuge tube. Trizol was added to the reaction mixture, and the samples were kept in ice for another 10 minutes. The samples were then centrifuged at 12,000 rpm for 12 minutes at 4°C, and the aqueous phase was carefully transferred without disturbing the interface. An equal volume of pre-chilled isopropanol was added and kept in -20°C for 45 minutes to 1 hour to allow the sample for precipitation. The samples were then dried in ice and dissolved in DEPC treated water. The Trizol method was also used for the isolation of RNA from tender leaves. Fresh leaf samples were collected and fixed with liquid nitrogen, and the samples were thawed before being dispensed into DEPC-treated eppendorf tubes. (Dutta S.et al.,2020)

The study used a Verso cDNA Kit for first-strand cDNA synthesis for PCR amplification of the Alcohol dehydrogenase1 (Adh1) gene. The primers were designed by Delobel et al. (2008) and used for specific amplification. The PCR reactions were performed using a Veriti96-Thermal Cycler, with initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation, annealing, and primer extension at 72°C for 45 s. The PCR product of Adh1 gene was separated through 4% agarose gel electrophoresis and visualized under a gel documentation system.

IV. TRANSCRIPTOME ANALYSIS TECHNIQUES

1. Total RNA extraction and purification
This method involves total RNA extraction and purification, which involves grinding samples into a fine powder using sterilized mortar and pestle. The samples are ground into a fine powder and stored in ultralow temperature freezers until

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the day allocated for batch RNA extraction. A crude RNA extract is obtained by adding 200 mg of the original powdered sample to a nuclease-free microfuge tube containing 750 µL of RNA Extraction Buffer and 500 µL of phenol: chloroform: isoamyl. The crude RNA extract is separated from debris by centrifugation at 14,000 x g for 10 minutes. The supernatant is transferred to a new 2.0 mL microfuge tube containing 400 µL of 1.2 M NaCl and 700 µL of isopropanol. The RNA is precipitated in a regular or ultralow temperature freezer for at least 1 hour or overnight. A crude RNA pellet is obtained by centrifugation at 18,800 x g for 15 minutes and purified using a Mini Kit. The dissolved RNA pellet is passed through a purple mini spin column and purified by passing through a pink mini spin column. The purified RNA is collected and dried by adding 50 µL of nuclease-free water and incubating for 3 minutes at 50 °C heat block. The total RNA extract is then eluted by centrifuging at 9,000 x g for 1 minute and stored on ice.

2. cDNA synthesis followed by cRNA transcription and labelling

This document describes a process for processing 24 samples simultaneously, including cDNA synthesis, cRNA transcription and labeling. The process involves pre-warming three microfuge tube heat blocks at 80°C, 65°C, and 37°C before beginning the steps. The preparation of one-color Spike Mix, T7 Promoter Mix, and cDNA master mix is based on the manufacturer's instructions. The RNA extract is dilute 100-fold to fit within the 50-100 ng range, and a 1:100 dilution is made by adding 1 µL of purified RNA extract to 99 µL of nuclease-free water in a 1.5 mL microfuge tube. A second dilution of each total RNA sample is made to make 50 ng of total RNA in a final volume of 1.5 µL. The Spike Mix is prepared using the Low Input Quick Amp Gene Expression Labeling Kit and stored on ice. The T7 Promoter Mix is prepared and stored on ice for 24 samples. The cDNA synthesis Master Mix is prepared from the Low Input Quick Amp Labelling Kit and mixed by pipetting.

The cDNA is synthesized for 2 hours in a 40°C heat block, and the temperature is adjusted to 70°C for heat inactivation. The tubes are then incubated at -80°C overnight or stored at -80°C after transcription.
The Transcription Master Mix is prepared while the samples are on ice for 5 minutes, and the tubes are briefly spin-downed to collect liquid at the bottom of each microfuge tube. The tubes are then incubated at 40°C for 2 hours to generate Cy3-labeled cRNA.
After cRNA transcription and labeling, the labelled cRNA is purified using a RNeasy Mini Kit.

3. cRNA Purification:

This process involves purifying labelled cRNA using a RNeasy Mini Kit, preparing buffers, and mixing the mixture. The cRNA is collected by centrifugation at 4°C for 30 seconds, washed in 500 µL of Buffer RPE, dried, and transferred to a nuclease-free 1.5 mL microfuge tube. The samples are then eluted with nuclease-free water and incubated in a 55°C heat block for 60 seconds. The cRNA is collected by centrifugation at room temperature for 30 seconds and stored at -80°C. The cRNA is quantified using Nanodrop using the microarray feature and recorded in a spreadsheet. The cRNA yield and specific activity are computed as detailed in Püffeld et al. 2019.

4. Microarray hybridization and scanning

Microarray hybridization and scanning are crucial steps in the process of analyzing samples. This step takes 3-4 hours, allowing one operator to run up to 4 slides (32 samples). The next day is dedicated to washing and scanning the slides, with additional runs performed in the afternoon. It is recommended to use color-free, powder-free latex gloves for handling and processing the slides to prevent contamination.

The Gene Expression Hybridization Kit is used for microarray hybridization, and 10x Blocking Agent is prepared according to manufacturer's specifications. The Fragmentation Mix for each sample is prepared as described by the manufacturer, and samples are mixed gently using a vortex mixer. Incubation is conducted in a 60°C heat block for 30 minutes, followed by adding 2x GEx Hybridization Buffer HI-RPM to each tube. Centrifuge all tubes for 1 minute at 15,750 x g and load each sample as quickly as possible.

Hybridization assembly is prepared before leaving the lab for 17 hours of incubation. Dispensing the hybridization mix slowly in the center of each gasket well is crucial, and the microarray slide is placed on top of the gasket slide. The hybridization chamber assembly is then placed in the hybridization oven rotator and hybridized at 65°C for 17 hours.

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Wash hybridized microarrays using the Gene Expression Wash Buffer Kit is prepared, and wash buffers are prepared the next day. After 17 hours of hybridization, one hybridization chamber is disassembled on a lab bench lined with lint-free paper. The microarray sandwich is transferred to Dish 1, and the slides are distributed evenly along the rack. The slides are then dried and placed in a slide box, and the process continues until all samples are hybridized and scanned.(Butardo V. et al., 2020)

V. SEED OIL PROFILING

Fatty acid profile: Jatropha curcas oil is predominantly unsaturated, with high iodine index values. It contains oleic, linoleic, and palmitic acids. The oil of Congo has a high C18:2 acid rate and a fatty acid profile similar to Mexico. Other countries have similar profiles, indicating minimal variation in seed chemical composition (Waghmare T. et al, 2017).

Chemical Analysis:
This study aimed to analyze the chemical properties of Jatropha curcas L seeds to optimize deshelling and oil extraction. The parameters considered included oil content, iodine value, peroxide value, saponification value, and acid value. The results showed that J. curcas seed oil had high oil content, with a higher iodine value due to its high unsaturated fatty acid content. The peroxide value was low, indicating its potential for plant oil and semidrying oil purposes. The saponification value was higher, and the higher unsaponifiable matter value may be due to the solvent's ability to extract other lipid-associated substances (Naik G., Waghmare T. et al, 2015).

TLC:
The crude J curcas oil sample's fatty acid profile was identified using TLC compared to standard olive oil, revealing saturated, stearic, and unsaturated fatty acids. The crude protein was also extracted and analyzed using SDS-PAGE, showing a silver-stained gel ranging from 14.3 KD to 97.4 KD (Naik G., Waghmare T. et al, 2015).

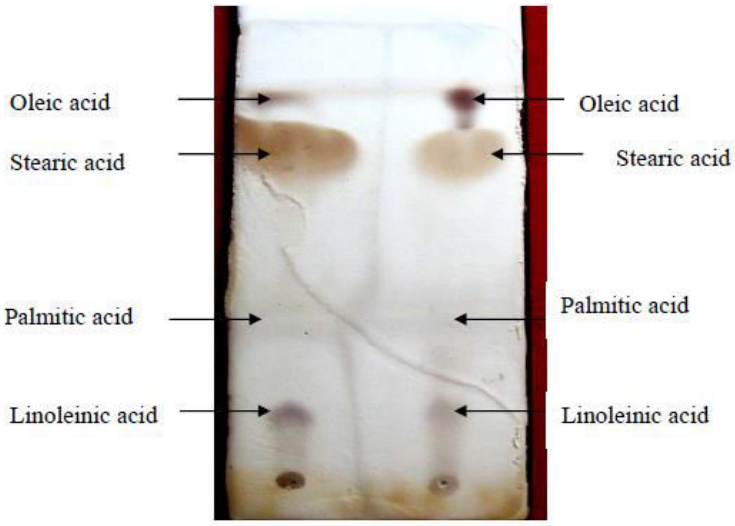

Fig. 1 Sample (Jatropha oil) Olive oil (Standard Oil)

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).

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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

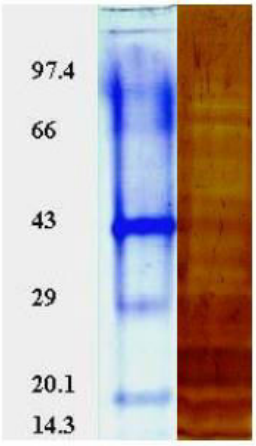

Fig .2. Protein profile

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).

Source : chemical analysis of seed and oil of jatropha curcas l' a biofuel plant Cultivated in hyderabad karnataka region, north karnataka. (Waghmare, T., & Naik, G. R. , 2015)

VI. DISCUSSION & RESULTS

Physico-chemical property of oil: The physical property that is, colour of the oil extracted by different organic solvents was also studied. All solvents except hexane yields colorless oil and without impurities. Hexane yield faint yellow colour oil. The chemical property that is %FFA content of the oil was found to be 2.24% (mg KOH/g). The free radical scavenging activity, expressed in percentage inhibition of the leaf and oil extract of *J. curcas.* (Shivani P., Khushbu P., Faldu N. et al., 2011)

The Soxhlet method yields oil in the 53-78% range, with hexane being the best solvent due to its maximum yield. However, the oil recovered by hexane and isopropanol is slightly yellow, causing issues for biodiesel production. Petroleum ether is recommended for efficient biodiesel production. Enzymes are used in AEOE to facilitate oil release from oil bodies. Jatropha oil seeds have a high FFA content, affecting transesterification of glycerides with alcohol. High FFA content leads to soap formation and low biodiesel product yield. The leaf of J. curcas contains more indigenous antioxidants, demonstrating the highest antioxidant property.

The study aimed to extract high-quality RNA from seeds using a modified SDS-based Trizol method. The RNA yield was recorded across four months of storage, with a mean of 978.6 ± 65.46 ng/µl. The RNA yield from freshly harvested seeds (0 DAS) was 922.4 ± 85.958 ng/µl, while the average RNA yield at different storage intervals was not affected by their duration. The quality of the isolated RNA was assessed through spectrophotometric absorbance at 260 nm and 280 nm. The mean A260/280 ratio was improved across all samples, indicating the removal of contaminated genomic DNA. The use of DNase treatment further intensified the sharpness of 28S and 18S rRNA bands and lack of smear in the 1%

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agarose gel. The next step was cDNA preparation through reverse transcription using isolated RNA as a template. A sharp and intact band of 135 bp of fragment of Adh1 gene was separated on 2% gel electrophoresis, confirming the accurate synthesis of cDNA from all RNA preparations isolated from seed samples under storage using the modified SDS-based Trizol method. This result suggested the equal effectiveness of both methods for RNA isolation from seeds and leaves. (Dutta S.et al.,2020)

The commercial success of Jatropha curcas L. cultivation relies on oil yield. Oil content decreases significantly with storage period, accelerated aging, and saturated salt accelerated aging. This is consistent with Sisman and Delibas' 2004 study, which found a three-month storage of sunflower seeds resulted in a significant decrease in oil content. The oxidation process, a reaction between unsaturated fatty acids and oxygen, is responsible for seed deterioration. During storage and germination, lipids are utilized as energy by the seed, leading to seed oil reduction. Jatropha curcas L. is a potential biodiesel feedstock as it retains its maximum oil quantity even up to 12 months of natural aging.

The study found that free fatty acid content in Jatropha curcas L. seeds significantly increased from 15 months of natural aging to 24 months of natural aging. This increase was similar to the results from the 4th day of accelerated aging and the 5th day of saturated salt accelerated aging. The study also found that seeds exposed to extreme temperatures and moisture had similar deteriorative effects as those from 6 months, 12 months, and 24 months of natural aging. The acid value of a natural oil is determined by its degree of unsaturation and free fatty acids. Oils with free fatty acid levels below 1% are unfit for biodiesel conversion due to low quality and soap formation. The study found that seeds with free fatty acids levels below 1% can be easily chosen as feedstock for biodiesel production. However, seeds with high acid values can cause severe lipid peroxidation, damaging engine parts. The study also found that Jatropha curcas L. seeds are the best feedstock for withstanding extreme temperature and moisture conditions in tropical regions like India.

The saponification value of Jatropha curcas L. seed oil showed a gradual increase during storage, with a significant increase from NA15m to NA24m. This increase was also observed in AA5d to AA15d and SSAA5d to SSAA15d. However, no significant increase was observed from 6 months to 24 months of natural aging. The increase in saponification value may be due to aging treatment, leading to bond breakage of long chain fatty acids. Jatropha curcas L. oil with high saponification value is not suitable for biodiesel production.

Peroxide values are a crucial indicator of oxidation and rancidity in oil storage. A significant increase in peroxide values was observed from NA15m to NA24m, AA4d to AA15d, and SSAA7d to SSAA15d. This increase is evident from 12 months to 24 months of natural aging, indicating the rapid onset of peroxide formation. The peroxide value in AA1d and SSAA2d was the same as NA6m, indicating that accelerated aging or saturated salt accelerated aging can cause the same negative effect. Increased peroxide value can also cause denaturation of nucleic acids and proteins, inactivation of enzymes, and other negative effects. To protect biodiesel from oxidation and increase stability, its peroxide value must always be kept low. Oil extracted up to 12 months of natural aging, up to 4 days of accelerated aging, and 5 days of saturated salt accelerated aging serves as the best feedstock for biodiesel conversion.

Iodine value is a parameter that indicates the degree of unsaturation of fatty acids in triacylglycerol. A significant decrease in iodine values indicates higher unsaturation in certain groups. However, no significant change in iodine values was observed in half-yearly and yearly analyses, indicating gradual unsaturation. Exposure to AA, SSAA, and AA and SSAA can decrease iodine values, affecting oil stability. The decrease in iodine value cannot be attributed to aging or accelerated aging, as oxidation stability is determined by the position and number of bis-allylic methylene moieties.

The study reveals that oil extracted from seeds of Jatropha curcas L. still maintains stability up to 15 months of natural aging, 5 days of accelerated aging, and 7 days of saturated salt accelerated aging, based on fatty acid methyl esters analysis. The study found significant changes in oleic acid and palmitic acid methyl esters, while stearic acid and palmitoleic acid methyl esters remained unchanged. The study suggests that prolonged storage and extreme conditions of heat and moisture can lead to the oxidation of double bonds in oleic acids. The findings suggest that oil extracted from Jatropha curcas L. seeds may have greater stability in storage conditions.(Suresh A. et al.,2019)

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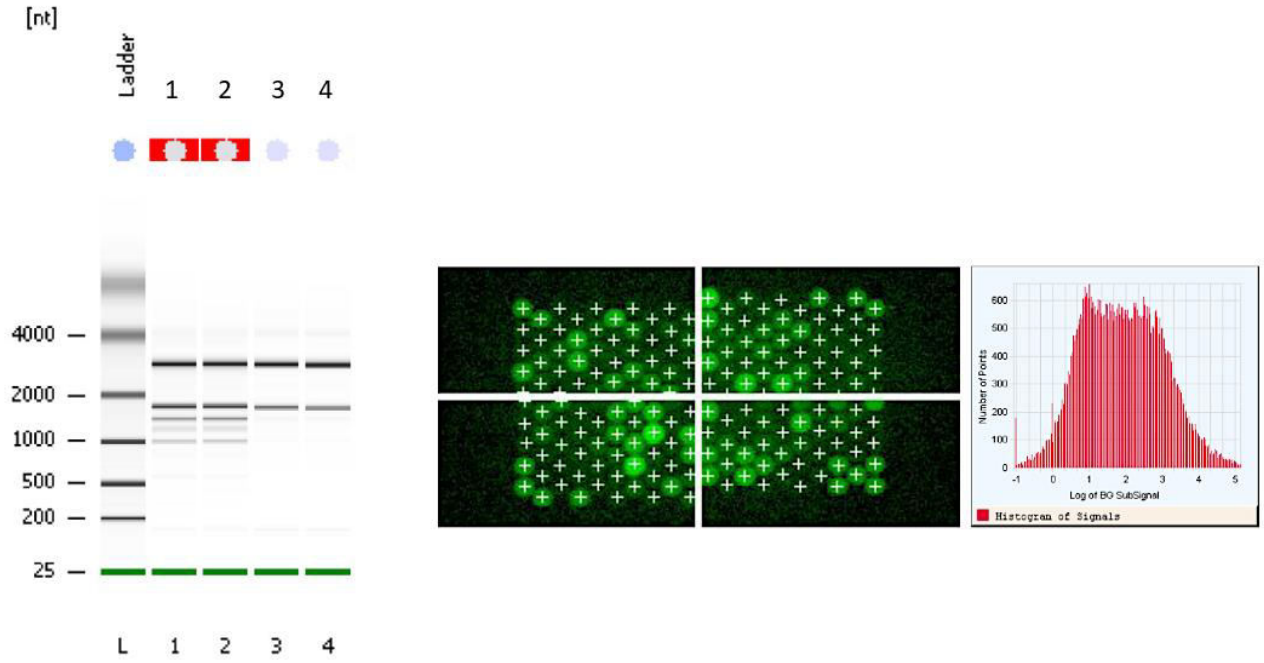
The quality of RNA in barley leaf and seed tissue samples was confirmed using Bioanalyzer. The RNA quality was very good, with a RIN factor of 10. The barley microarray hybridization was successful, with detected signals on the grid and a histogram. (Butardo V. et al., 2020)



This method is designed to extract total RNA from 24 cereal seed samples per day, ensuring high quality and yield. Commercially available plant RNA extraction kits are not suitable for seeds due to significant amounts of starch, proteins, sugar, and/or lipid contamination. The method involves chemical extraction of crude RNA followed by column purification using a commercial RNA extraction kit. The RNA integrity (RIN) value for all samples is typically 10 using the protocol described in this paper. A time course experiment of two elite barley inbred lines (Sofiara and Victoriana) was used for analysis of malting quality. RNAs were extracted from germinating seeds at different times after imbibition in biological triplicates. The results were further evaluated using the GeneSpring software, and the collected data were presented as principal component analysis (PCA). The closer the samples are, the more similar they are. Technical replicates should be closer together than biological ones, and biological replicates should cluster closer together than samples from different time points, tissues, or conditions.

VII. CONCLUSION

The soxhlet extraction method yielded higher oil yields due to continuous extraction for 6 hours, while the titration method for FFA determination was faster, efficient, and sensitive. (Shivani P. et al., 2011)

In this study, an effective, robust, and efficient method for RNA extraction from seeds was developed. The optimized method was effective for leaf sample to isolate high quality RNA. This method can be used for studying regulatory genes under dormant condition of seed during storage and also deciphering the role of the key genes in adult stage. (Dutta S.et al.,2020)

The study investigates seed storage of Jatropha curcas L. seeds, finding a reduction in oil content and oleic acid methyl esters after one year. However, seeds maintain high levels of free fatty acids, saponification value, and peroxide value. Controlling moisture can delay seed deterioration (Suresh A. et al,2019).

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