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BIOFUEL PRODUCTION, SILVER NANOPARTICLE EXTRACTION AND THE THERAPEUTICANALYSIS FROM *JATROPHA CURCUS* SEEDS USING MICROBES BY SOLID STATE FERMENTATION

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Abstract

Biodiesel is the most common biofuel produced in Europe by transesterification of vegetable, animal, and algal lipids. It is utilized as a fuel in its pure form (B100) and includes methyl/alkyl esters of long-chain fatty acids. About 100 g of Jatropha curcas seeds were fed into the oil extraction machine, and the oil was extracted according to the machine's operating process at 210 °C and the Jatropha curcas seeds produced around 42% seed oil. The pink color cocci was observed in the gram staining for microorganism isolated from water bodies of Arpa river, Bilaspur (CG) shows the presence of gram negative bacteria (probably *E.coli*). The antibacterial activity of microbial oil extracts, and *Jatropha curcas* seed oil was tested. The *Candida, E. coli, Neurospora,* and *Staphylococcus*, the seed oil hasn't demonstrated any effectiveness. Petroleum ether or ethanol that was dissolved in microbial oil (50:50) showed less antibacterial action**.**

Keywords: *Jatropha curcas* seeds, Biofuel, antimicrobial activity, oil extraction, gram staining

INTRODUCTION

Biofuel is any fuel obtained from biomass, such as plant or algal waste or animal waste (Voloshin et al.,2016). Biofuel is considered arenewable energy source, as opposed to fossilfuels such as petroleum, coal, and natural gas,because such feedstock material may be easilysupplied. Plant-based biomass is usually usedprimarily for heating, although waste-based biomass is often liquid and can be utilized in vehicles. There are sporadic references to the two main biofuels: bioethanol

and biodiesel (Brunschwig et al., 2012). Ethanol, the alcohol present in drinks, can be combined with petrol but not used by itself. On the other hand, diesel engines can usually run on biodiesel without any modifications. Additionally, conventional diesel and biodiesel can be combined.
Jatropha curcas is a Brazilian shrub that has flourished in a number of African nations(Kashe et al., 2018). The plant may grow up to 10 metres in height and has a 50-year lifespan. It will be able to start producing afterfive years. All plants yield fruits that contain between 30% and 40% oil (Achten et al.,2013). Jatropha is one of the plants used todayto produce biodiesel because of its ability to thrive in areas that are unfavorable forconventional agriculture. By doing this, the problem of food rivalry is avoided.

MATERIALS AND METHODS

All of the substances used in the study were bought from HiMedia in Mumbai, Maharashtra. The experiment was carried outaccording to a self-created procedure.

Collection of Seeds

Fresh *Jatropha curcas* seeds (Figure 1 and 2)were collected locally throughout the summerfrom the Bilaspur (C.G.)

Figure 1: *Jatropha curcas* plant

Figure 2: *Jatropha curcas* seeds Extraction process

The following extraction materials and methods are used to prepare $AgNO_3$ biodiesel:A pipette, a measuring cylinder, a water bath,a hydrometer, a conical flask, Test tube, beaker, a digital weighing balance, and substrate, methanol, NaOH, Hexane, ethanol, sucrose, glucose monohydrate, silver nitrate($AgNO_3$), ethanol, petroleum ether and Jatropha oil. Oil extraction is a crucial phase in the manufacturing of biodiesel, and there are various methods and techniques used for this process.

Feedstock Preparation

Preparing the seeds is a need for oil extraction.In order to expose the kernels or seeds, the fruit's outer layers must be removed, and the fruit must then be dried to remove moisture. Fruits are divided into their seeds and thefruits that do not dehisce are manually crackedopen. The kernels or seeds that have been separated are cleaned, sieved, and kept at room temperature.

Biofuel Production

Oil was extracted from the air-dried seeds. For the production of biofuel, an oil extraction equipment was utilized.

Microbial biofuel production

Jatropha curcas seed pulp was mixed with theyeast and allowed to settle for a week following the extraction of the oil. A seed pulpand yeast were kept to extract oil from the ethanol. Another burning test was performed on the acquired product.

Antimicrobial activityMicroorganisms

The investigation employed microorganisms from the MTCC (Microbial Type Culture Collection). Gram positive bacteria *Staphylococcus aureus* (MTCC 740) and *Enterococcus faecalis* (MTCC 439) were used in the current investigation, while gram negative bacteria *Pseudomonas aeruginosa*(MTCC 424) were used. The fungus *Candida albicans* MTCC 227 was employed in theinvestigation.

The bacteria and fungi were kept on nutrient agar slants at -20°C and cultivated in Muller-Hinton medium (HiMedia Pvt. Ltd., Mumbai,India) at 37°C for 24 hours and Sabourand Dextrose Media (HiMedia Pvt. Ltd., Mumbai,India) at 25°C for 72 hours. The pure isolate used for the test organisms' inoculum wasgrown in nutrient broth for an overnight subcultured period. To obtain a log phaseculture, the overnight broth cultures were in fresh nutrient broth and grown for three hours.Muller-Hinton agar (MHA) medium andSabourand Dextrose agar (SDA) media were used to make the agar plates using the pour plate technique. One millilitre of the test organism's growing culture ($1x10^8$ cells) was completely combined with the sterile MHA/SDA medium, which had been chilled to 45°C, before being put into sterile petri dishes and allowed to set. Using a sterile borer, 8mm-diameter wells were created, to which test extracts were introduced. The MHA plates were given a 24-hour bacterial incubation period at 37°C.The SDA plateswere cultured for fungus for 72 hours at 25°C.Using a HiMedia zone reader, the diameter ofthe inhibitory zones was measured in millimetres (Kaladhar *et al*., 2014)

.

Gram's staining

Gram staining technique was performed to identify the type of bacteria present in the culture isolated from Arpa river, Bilaspur (CG).

Evaluation of Silver nanoparticles

The bacterial strain *Escherichia coli* was usedin this study to evaluate the microbial synthesis of silver nanoparticles.

RESULTS

About 100 g of *Jatropha curcas* seeds (Figure3) were fed into the oil extraction machine, and the oil was extracted according to themachine's operating process at 210 °C and the*Jatropha curcas* seeds produced around 42% seed oil (Figure 4 and 5).

Figure 3: plant and seeds of *Jatrophacurcas* % oil extracted= amount of oil in ml/ 100 gmof seed X100= 42/100 X100= 42%

Figure 4: *Jatropha curcas* Seed oil

Figure 5: *Jatropha curcas* seed, substrateand oil

Figure 6 shows the extracted seed oil of *Jatropha curcas* was burned in the sand pot and the oil was burned for about 10 minutes per 10ml of oil (yellow colour).

Figure 6: Sand pot used for flame testing

For oil isolation, extracted microbial oil using*Jatropha curcas* seed waste material was employed. The seed debris containing*Saccharomyces cerevisiae* was stored for oneweek for reaction, as indicated in (Figure 7).

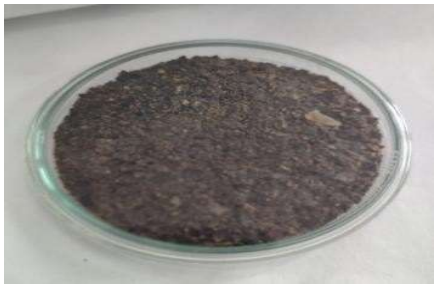

Figure 7: *Jatropha curcas* Seed waste as asubstrate mixed with *Saccharomyces cerevisiae*

Characterization of AgNP

The silver nanoparticles confirmed based on color change after incubation and absorption peak at 410 nm in UV–vis absorption spectra.

Gram Staining

The pink colored cocci was observed in the gram staining for microorganism isolated from water bodies of Arpa river, Bilaspur(CG) shows the presence of gram negative bacteria (probably *E.coli*).

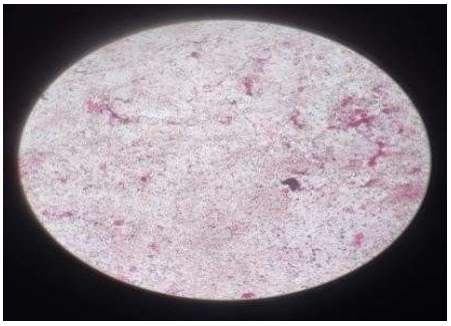

Figure 8: Gram Negative Bacteria

After one week, ethanol with substrate and *Saccharomyces cerevisiae* was utilized for oil extraction and its ability to burn was examinedin a sand pot. The extract had a good orange hue and

a yellow flame, as was observed(Figure 9)

A)Seed and microbial AgNPoils B) Burning of Seedand microbial oils

C) Antimicrobial activity of M-AgNP microbial (25mm) oil O-oil(10mm) and W-sterile water (0mm)

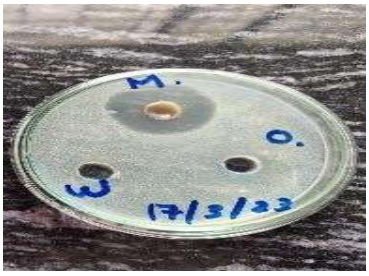

Figure 9: Microbial AgNP oil Vs Seed oilfrom *Jatropha curcas*

Antimicrobial activity

The antibacterial activity of microbial oil extracts, and *Jatropha curcas* seed oil was tested (Table 1 and Figure 10). The *Candida,*

E. coli, Neurospora, and *Staphylococcus*, the seed oil hasn't demonstrated any effectiveness. Petroleum ether or ethanol thatwas dissolved in microbial oil (50:50) showedless antibacterial action**.**

Table 1: Antimicrobial activity *Jatrophacurcas* seed oil [Zone of inhibition (in mm) along with zone size of 8mm]

Sample	***Candida***	***E.coli***	***Neurosp ora***	***Staph ylococ cus***
Seed oil	10	11	11	**12**
Petroleum ether mix with microbial oil (50:50)	10	10	10	11
Ethanol dissolved with microbial oil (50:50)	8	8	8	10

Figure 10: Antimicrobial Activity

DISCUSSION

The oil recovery estimation was used as the benchmark (Sun and Pollitt, 2022). Soxhlet extraction is superior to other techniques because it is continuous and fully recovers allof the oil.
Hexane is considered the best solvent since ithas the highest yield when compared to othersolvents (Johnson and Lusas, 1983). However, the oil recovered with hexane and isopropanol has a somewhat yellow tint. Thismight make it more difficult to use oil toproduce additional biodiesel. As a result, petroleum ether is preferable for producing biodiesel effectively. InThe aqueous enzymatic oil extraction (AEOE), enzymes are employed to speed up the release of oil from oil bodies that aretangled up in protein and cellulosic/hemicellulosic networks (Shah etal., 2005). The use of just cellulase and pectinase had no effect on oil yield. Additional enzyme preparations, such asProtizyme, Pectinex Ultra SP-L, Promozyme,and others, are required for increased oil output. According study results, the free fattyacid content of Jatropha oil seeds is 2.24%. When using a catalyst, the amounts of free fatty acid (FFA) and moisture have a significant influence on how successfully glycerides are transesterified with alcohol. Due to the high FFA content (> 1% w/w), soapwill develop and the products will be exceedingly difficult to separate, resulting ina low biodiesel production yield.

In the present experiment, the oil was extracted using the heat press process from the dried seeds of the *Jatropha curcas* L plant. From 100 g of seeds, 42 ml of oil were obtained. *Jatropha curcas* L. fresh seeds yielded about 42% seed oil. In the sand pot, the extracted oil was burned for approximately 10 minutes per 10 millilitres ofoil (yellow colour). After one week, the oil from *Jatropha curcas* L was extracted using ethanol with substrate and *Saccharomyces cerevisiae,* and its ability to burn in a sand potwas assessed. The extract had a nice orange colour and a yellow flame, as was seen.

The study has demonstrated that the majority of the biodiesel's tested qualities meet American society for testing and materials(ASTM) and Europian standards (EN)standard values. From this study, it can be inferred that biodiesel made from Jatrophacurcas oil is a potential substitute for fossil diesel. Additionally, the production and efficient use of biodiesel will help to lower thecost of protecting the environment from the risks associated with using fossil diesel, which will

strengthen the nation's economy (Dey et al., 2021).

The numerous advantages of Jatropha plants and the oil released from its seeds are not onlyhelpful in reducing environmental pollution but also encourage the creation of jobs and thegrowth of businesses (Pandey et al., 2012). The plant's potential for usage as an energy crop, agro-forestry crops, soil conservationtechniques, and industrial applications make itappealing to grow it on under unutilized and waste land. The expenditure needed to produce a unit quantity of biofuel is decreasedsince the Jatropha plant requires extremely little input to harvest oil (Openshaw, 2000).

As an energy crop, it has already been proventhat it can serve as an alternative fuel forstationary and motive diesel engines by adequate processing and blending with petro- diesel. The thermodynamic characteristic of biodiesel also indicates that it may be used indiesel engines (Shahmirzae et al., 2014). The manufacture of biodiesel byproducts such glycerine and oil cake can yield advantages financial returns, making the cultivation of Jatropha and its oil commercially viable. In addition to Jatropha, other plant species such as karenji, rubber seeds, neem seeds, pongmiarapeseeds, etc. are possible sources of biodiesel that can be used in diesel engines affordably when blended in the right amounts.

CONCLUSION

The research findings have conclusivelyshown that *Jatropha curcas* seed oil may be used as a feed to make high quality biodiesel.The findings were discovered that the createdbiodiesel made from Jatropha seed oil had qualities that met the specifications set forth in the literature.

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