

Extraction of underexploited *Vernonia cinerea* oil with comparative pretreatment techniques for conversion into biodiesel

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An examination by a conventional Soxhlet method and pretreatment by ultrasonication was performed for the oil extraction from non-edible *Vernonia cinerea* seeds. Pretreatment was conducted by ultrasonication, microwave and autoclave methods. After optimizing the process parameters, ultrasonication provided the best results with a yield of 40.9%. A study on the kinetics of oil extraction was performed. The first-order kinetics was found to fit for the oil extraction with activation energy of 82.2 kJ/mol. The physical and chemical properties of the extracted oil were determined by ASTM method and all properties fit within the requisite limits for the production of biodiesel. The oil was subjected to two-step transesterification process and biodiesel produced was within standard limits.

Keywords: Pretreatment, ultrasonication, extraction, *Vernonia cinerea*, kinetic studies, biodiesel

INTRODUCTION

Fossil fuels are in the depletion phase and the pollution created by ignition of such non-renewable fuels is highly hazardous to the environment. Biodiesel is fuel which would be a solution to overcome the above mentioned constraints. Due to the heavy demand prevailing all over the world, biomass provides a wide choice as the feedstock for production of alternate renewable fuels [1]. Biodiesel production from different biomass sources has grabbed the limelight in the recent years. In particular, non-edible seeds have been proved undeniably as a second-generation resource for the production of biodiesel [2]. The prime reason for its significance is that no major cost of input is utilized for the raw materials, as these seeds are plentifully available in tropical regions.

The oil extracted from the seeds of plants consists of a combination of saturated and unsaturated fatty acids, the significant biomolecules of which have to be brought under the focus of research. These biomolecules may serve as good raw materials in pharmaceutical, food and cosmetic industries [3-5]. If the lipid content of the extracted oil meets the fuel standards, it is a welcome source for the production of biodiesel. For the current economic situation pertaining in the world, voluminous scale of biodiesel production would help saving in foreign exchange [6, 7].

Proper selection of feedstock is the major parameter in production of biodiesel as the production cost could be excised to a great extent,

which leads to economical and feasible production. Seeds have to be chosen as the feedstock for transesterification process in such a way that the oil yield is above 20 % [8]. The degree of unsaturation of fatty acid in the raw oil extracted determines the cetane number, iodine value and oxidation stability. These are the key factors that ascertain the fuel standards [9].

In the present investigation *Vernonia cinerea* is the chosen seed for the extraction of oil. This is a novel seed as no earlier works have been documented for extraction of oil from *Vernonia cinerea*. It belongs to Asteraceae family. The objective pertains to the optimum yield of oil using Soxhlet extraction method which is the conventional and the best method for oil extraction. Pretreatments are performed using autoclave [10], ultrasonication [11-13] and microwave assistance methods [12] for enhancement of lipid extraction. Comparative studies on the highest oil yield using the mentioned pretreatment methods are deliberated.

The extracted oil is the lipid source which can be converted into FAME. No research work has been carried out earlier in conversion of *Vernonia cinerea* oil into biodiesel. In the present work all the key parameters, namely particle size of the chosen seeds, their moisture content, the best solvent utilized for extraction, the solvent-to-solid ratio and the extraction temperature required for conformity to be used as lipid source for biodiesel production, were studied. The biodiesel obtained from the transesterification reaction of the extracted *Vernonia cinerea* oil can be utilized as a fuel for automobiles.

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MATERIALS AND METHODS

Extraction of oil

Vernonia cinerea plants are copiously available even in the barren lands all throughout the year. The seeds collected were sun-dried. The solvents n-hexane, chloroform, petroleum ether, acetone, isopropanol and dichloromethane of analytical grade with purity of 99.9% required for oil extraction were purchased from SRL Chemicals private ltd. Mumbai, India.

For the maximum extraction yield of oil, the moisture content in the seeds has to be negligible. To remove the excess moisture, the seeds were kept in a hot-air oven at 60°C for 20 min. The moisture content of the seeds was determined using the formula:

$$\text{Moisture content} = \frac{\Delta W}{W_i} \times 100 \% \quad (1)$$

where ΔW = difference between initial and final weight of the seed, W_i = initial weight of seed.

The seeds were then crushed to fine powder using a crusher. Mechanical sieving was later performed for uniform segregation of the powders.

Pretreatment methods

Pretreatment was performed to improve the extraction yield of oil. The outer wall breaks thereby providing a better interaction with the solvent for extraction. Three options of pretreatment were chosen.

Microwave assistance pretreatment

Microwave assistance pretreatment was performed with a 1200 W microwave oven (Model No. IFB 23SC3IFB). Whole seeds (10 g) were arranged randomly on a petri dish placed in the middle of the turn table of the microwave oven. The pretreatment by microwave irradiation was carried out for 30 sec [14].

Pretreatment using autoclave

The seeds were autoclaved at 121°C and at a pressure of 15 lbs. The presence of excess thermal stress prompts the rupture of cell walls inducing the release of intracellular lipids. After the pretreatment the seeds were brought back to room temperature [15].

Ultrasonication pretreatment

Ultrasonication was carried out using ultrasonic probe at 24 KHz (Model No: Sonics vibra cell 500 watts) with 10 sec of on and off cycle at constant room temperature. As solvents n-hexane, petroleum ether and chloroform were tested. The best solvent was used for pretreatment. The change in solid-to-solvent ratio (1:2-1:4 g/ml) and the ultrasonication time (2.5 - 15 min) were further studied [12].

Soxhlet apparatus was employed for extraction of oil from pretreated biomass. The organic solvent chosen for extraction was taken in a double-neck round-bottomed (RB) flask. The Soxhlet extractor was fixed to the center neck of the RB flask and the other end of the extractor was connected to a bulb condenser. Water inlet and outlet was provided on either ends of the condenser. The biomass of known weight was packed in the thimble. The entire set-up was placed in a heating mantle. Temperature was maintained little above the boiling point of the organic solvent chosen. Thermometer was inserted in the side neck of the RB flask to check the temperature of the system. The vaporized solvent moved up and on condensation dripped back to the thimble and got in contact with the biomass. Extraction of oil occurred here and the volume of solvent rose inside the thimble. Once the chamber was filled with the solvent, it flowed back to the RB flask through the side siphon tube. The extraction process was continued for 'n' cycles and the important parameters were optimized for highest yield of oil [16]. The solvent was removed by using a rotary evaporator. The extracted oil was stored in tightly packed vials. The yield of oil was calculated using equation 2:

$$\text{Yield of oil (\%)} = \frac{\text{weight of oil (g)}}{\text{weight of seed powder (g)}} \times 100\% \quad (2)$$

Biodiesel synthesis

To check the conversion of the synthesized biodiesel a single run of two-stage transesterification was performed. For the esterification process, oil was treated with 1% sulfuric acid. 10:1 Methanol to oil and 1 wt.% acid mixture were taken in a flat-bottomed flask and kept in a water bath. The neck of the flask was connected to a condenser. The whole set-up was placed on a hot plate with magnetic stirrer. After thirty minutes of stirring, 0.5 wt % of KOH (homogeneous base catalyst) was added. The operating conditions were 65°C, 400 rpm stirring speed and 5 hours of process time [17]. After the reaction, the mixture was brought to room temperature and was allowed to settle for few hours. Two distinct layers were observed. The biodiesel formed at the top layer was separated and washed with distilled water.

RESULTS AND DISCUSSION

Comparison of pretreatment methods on oil yield

Pretreatment of *Vernonia cinerea* biomass was done using ultrasonication, microwave assistance and autoclave methods. The highest oil yield was

observed in ultrasonication-pretreated biomass. Without pretreatment the oil extraction yield was observed as 34.55 wt %. The yield significantly increased in all three pretreatment processes as observed in Figure 1.

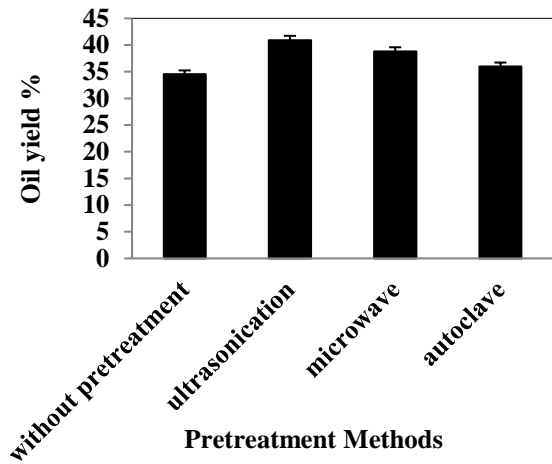


Fig. 1. Oil yield obtained using the different pretreatment methodologies.

Ultrasonication pretreatment process has given a maximum increase of 6.35% oil yield. Earlier, the increase in oil yield was reported as 4.6% with the same pretreatment process [12]. The result obtained in the current work is appreciably high.

Hence, the oil extraction was performed with optimization of other important parameters to obtain high yield.

Optimization of process parameters for oil extraction using ultrasonication

The yield of oil with ultrasonication pretreatment gave the highest yield, thereby optimization of solvent, solid-to-solvent ratio and time was performed.

Influence of solvents used for ultrasonication

n-Hexane, petroleum ether and chloroform were the solvents chosen for ultrasonication. Solid-to-solvent ratio was taken as 1:3 and ultrasonication time was 10 minutes.

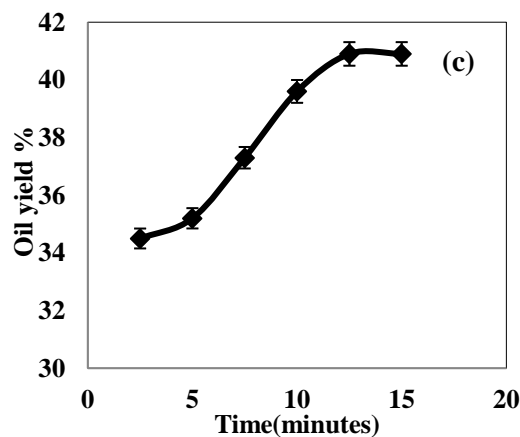
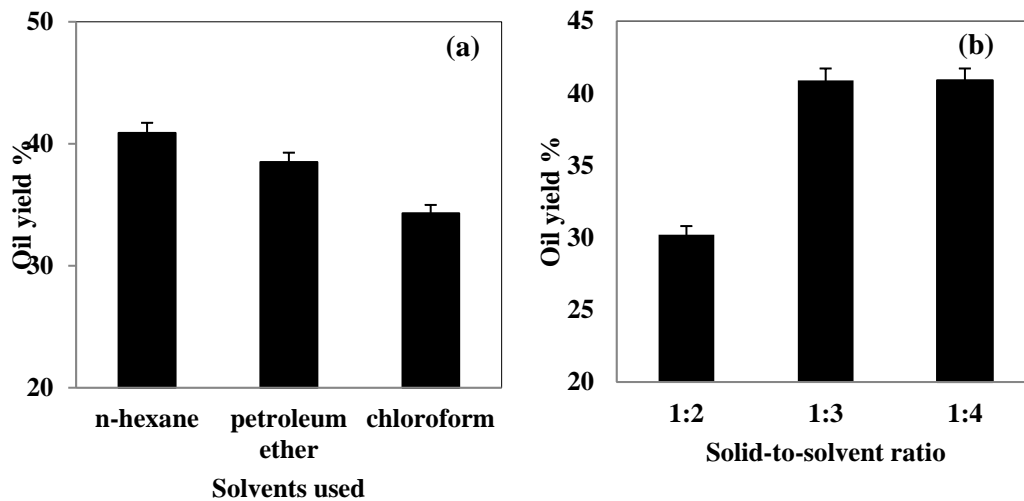


Fig. 2. Optimization of ultrasonication pretreatment process: (a) influence of solid-to-solvent (n-hexane) ratio; (b) effect of different solvents used; (c) effect of ultrasonication time.

The best yield was obtained using n-hexane solvent (Figure 2a). Many earlier works on extraction of oil have proved n-hexane as the best solvent for the maximum yield of oil from non-edible seeds [17].

Solvent-to-solid ratio in ultrasonication

Different ratios of 1:2, 1:3, 1:4 n-hexane-to-seed were taken and subjected to ultrasonication pretreatment. The process was carried out at room temperature for 12.5 minutes (Figure 2b).

Ultrasonication pretreatment time

Time of ultrasonication pretreatment was optimized with n-hexane as the solvent. The solvent-to-solid ratio was 1:3. Time was varied from 2.5 minutes till 15 minutes. The maximum yield of oil was observed at 12.5 minutes (Figure 2c).

Extraction of oil following ultrasonication pretreatment

Influence of solvents used for extraction. After ultrasonication pretreatment, few solvents, polar and non-polar in nature, namely acetone, chloroform, dichloromethane, petroleum ether, methanol and n-hexane were chosen for extraction of oil (Table 1). The yield of oil with methanol as solvent was the lowest with 20.3%. Non-polar solvents as listed in the table below yielded high oil extraction. The maximum yield was observed using n-hexane as the solvent. For extraction of oil, non-polar solvents are better suited than polar solvents as more of free fatty acids would be extracted using polar solvents [18].

The graph evidently shows that as the polarity of the solvent increases the extraction yield decreases. The maximum yield was observed to be 40.9% with the non-polar solvent n hexane (Figure 3a).

Influence of moisture content in seeds. Moisture content in seeds is a very important parameter for oil extraction. Presence of water would be an impeding factor for oil extraction with hydrocarbons. The moisture content on dry basis was read by the AOAC method [19]. Four samples beginning with the moisture content of 7% reduced till 2% were taken. Maximum oil extraction of 40.9% was observed with 2% moisture content. The extraction of oil got improved by 9.85% when the moisture content in the seeds was brought to the minimal one (Figure 3b). The seeds were dried for the removal of moisture content. By the process of drying the seed membrane wall the hydrolyzing enzymes get stimulated which leads to denaturalization of the protein structure inside the seed membrane. This helps in extraction of more oil from the dried powdered seeds [20]. Oil

extraction was highest at the minimum moisture content in earlier works too [21-23].

Influence of particle size of the powdered seeds. The powdered seeds with moisture content of 2% were subjected to mechanical screening where the powder was discriminated into different fractions as per their cumulative size. Particles retained above the mesh 20, 40, 80, 100, 120, 200 were taken for analysis (Table 2). The oil extraction of 40.9% was observed to be the highest at the particle size range of 200 mesh. Lesser the particle size better is the observed oil yield. The wall of the seeds gets broken with the process of milling. While milling the seeds into finer particles, heat gets produced which is thereby making the cell walls of the seeds easily broken. Due to this phenomenon extraction of oil gets influenced by the reduction in particle size [24].

Influence of temperature. Temperature is one important parameter to be optimized. Extraction was performed in the temperature range of 45-70 °C using n-hexane as solvent for 5 hours. The optimized parameters of powder taken as the feed were moisture content of 2% and particle size of 200 mesh. Oil yield improved and was noted to be high at 65°C and maintained to be the same at next increment of 70°C too (Figure 3c). Thus, the maximum yield is obtained at boiling range of n-hexane solvent. Many previous works have observed the highest oil yield only around the boiling range of the solvent. When the temperature reaches the boiling point of the solvent, an unparalleled improvement of diffusion coefficient between the solvent and the oil can be observed thereby improving the extraction of oil [25, 26]

Influence of extraction time. The main parameters to evaluate the economy of the extraction process are extraction time and amount of solvent utilized for oil extraction. Extraction of oil was performed from 3 hours till 5 hours 30 min (6-9 cycles per hour). The oil yield was calculated for every 30 min. It was found to be maximum at 5 hours of extraction time. After five hours there was no huge incremental yield of oil observed (Figure 3d). So, the optimized extraction could be concluded as 5 hours since extending the extraction beyond that time frame would mean spending high energy thereby increasing the cost of extraction. The inclusion of ultrasonication pretreatment process made the entire process time-effective as the Soxhlet extraction without pretreatment was extended up to 7 hours whereas the pretreatment made the entire extraction process in 5 hours. The reduction of time by two hours may be due to expeditious mass transfer of oil from the seeds to the solvent [27].

Table 1. List of solvents used for oil extraction with their properties

Name of the solvent	Chemical formula	Molecular weight (g/mol)	Density (kg/m ³)	Boiling point (°C)	Polarity index	Polarity
n-hexane	C ₆ H ₁₄	86.17	655	69	0.1	Non polar
Petroleum ether	C ₆ H ₁₄	86.18	640	60-80	0.1	Non polar
Chloroform	CHCl ₃	119.38	1490	61.2	4.1	Non polar
Acetone	C ₃ H ₆ O	58.08	784	56	5.1	Polar
Methanol	CH ₃ OH	32.04	792	65	5.1	Polar

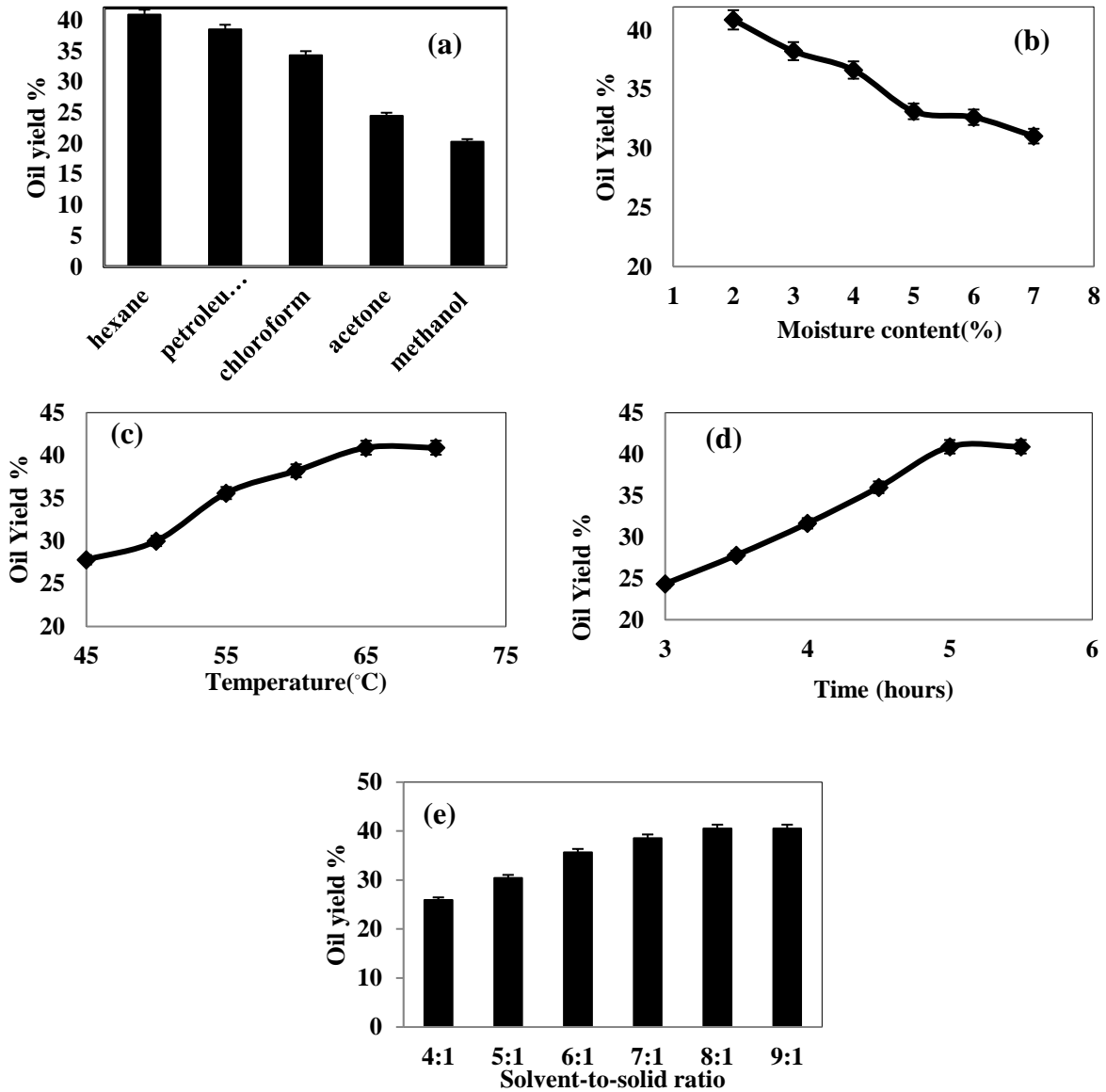


Fig. 3. (a) Influence of different organic solvents used for oil extraction (process conditions: solvent-to-solid 9:1, 65°C, 5.5 h, 2% moisture); (b) Influence of moisture content in powdered seeds (process conditions: n-hexane solvent 9:1, 65°C, 5.5 h); (c) Influence of extraction temperature (process conditions: n-hexane solvent 9:1, 2% moisture, 5.5 h); (d) Influence of extraction time (process conditions: n-hexane solvent 9:1, 2% moisture, 65°C); (e) Influence of solvent-to-solid ratio (process conditions: n-hexane solvent, 2% moisture, 65°C, 5 h), 200 mesh particle size of powdered seeds in all cases.

Influence of solvent-to-solid ratio. All the above-mentioned parameters were taken at their optimized values to study the effect of solvent-to-solid ratio. Powdered seeds of particle size of 200 mesh with 2 % moisture content were subjected to extraction at the temperature of 65°C. The extraction process continued for 5 hours. Different solvent-to-solid ratios of n-hexane solvent, i.e. 2:1 incremented till 9:1 were taken and the significance of this parameter was studied. The observed highest yield at 8:1 ratio was 40.9 % (Figure 3e). At higher solvent-to-solid ratio extraction yield was observed to be high. This could be due to the immense driving force resulting in high oozing out of oil in more diluted solvent system [28]

Kinetic studies on oil extraction

The rate equation for extraction of oil can be expressed as equation (3):

$$\frac{dY}{dt} = kY^n \tag{3}$$

From the yield (Y) of oil and the extraction time (t), the rate constant (k) and the order of the reaction (n) can be found.

At different temperatures, namely 50, 55, 60 and 65°C the yield of oil was experimentally determined for the kinetic studies. The values of the respective yield are tabulated in Table 3.

A plot of ln(dY/dt) versus lnY is drawn and the values of the rate constant are obtained from the slopes of the plot (Fig 4 a). Regression coefficient (R²) average was obtained as 0.956. From the ‘n’ values, we could conclude it to be first-order kinetics.

Parthiban and Perumalswamy [1] have also reported first-order kinetics for oil extraction from *Annona squamosa* seeds.

Using Arrhenius equation, the rate constants can be found from equation (4) and are listed in Table 4 obtained at the different temperatures:

$$k = Ae^{-\frac{Ea}{RT}} \tag{4}$$

Table 3. Oil yield in the temperature range of 50-65°C with respect to extraction time

Time, hours	Temperature, °C			
	50	55	60	65
3.0	20.2	22	23.3	24.35
3.5	22.2	24.8	26.3	27.8
4.0	24.5	27.9	29.8	31.65
4.5	27.1	31.5	33.8	36
5.0	30	35.6	38.2	40.9

Table 4. Values of reaction rate constants with respect to temperature

Temperature (°C)	Rate constant k (min ⁻¹)
50	1.521 × 10 ⁻³
55	3.251 × 10 ⁻³
60	3.934 × 10 ⁻³
65	6.44 × 10 ⁻³

From the slope of the line in Fig. 4b, the activation energy was found to be 82.2 kJ/mol.

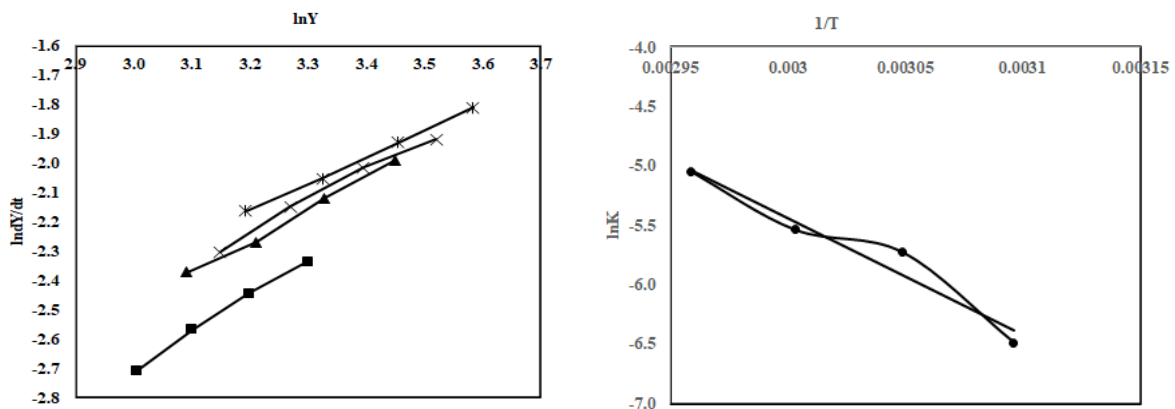


Fig. 4. (a) Plot of ln dY/dt vs lnY at 50, 55, 60, 65°C for *Vernonia cinerea* oil; (b) Graph of lnk vs 1/T for the determination of activation energy.

Physical and chemical properties of extracted oil

The basic physical properties of extracted *Vernonia cinerea* oil were determined using a standard method and are listed in Table 5.

Three important properties of the oil which determine whether the oil can be further subjected to transesterification process for the production of biodiesel are iodine value (IV), saponification value (SV) and cetane number (CN). These properties were determined for *Vernonia cinerea* oil.

Iodine value defines the degree of unsaturation. When the presence of unsaturated molecules is high, the FAME produced will not be suitable to be used as fuel. These double-bond molecules will react with the atmospheric oxygen leading to the formation of peroxide. Crosslinking may occur which further may lead to polymerization into a gummy material. On ignition at high temperature, this gummy polymer can clog the engine. The iodine value must be less than 133.5 [30].

Table 5. Properties of *Vernonia cinerea* oil

Property	Obtained value
Color	Dark brown
Odor	Repulsive
Specific gravity at 25°C (ASTM D1840 method)	0.927
Kinematic viscosity (cst) (ASTM D445 method)	41.1
Acid value (ASTM D664 method))	10.2
Iodine value	68.5
Saponification value	205.2
Cetane number	57.51
Long chain saturation factor (LCSF)	15.3
Higher heating value (HHV)	39.997

Cetane number tells about how rapid the fuel ignition occurs. The cetane number as per ASTM should be above 47 and should not exceed 65. The upper limit is fixed, as the increase in cetane number would decrease the iodine value which again would lead to gumming of fuel [30].

Transesterification of Vernonia cinerea oil

The acid value of extracted oil was high thereby before transesterification process, esterification process was carried out. The yield of biodiesel with the process conditions of 10:1 methanol-to-oil ratio and 0.5 wt % KOH was 89%. With proper

optimization of process parameters, the conversion of oil could be improved.

CONCLUSION

Vernonia cinerea seeds were subjected to pretreatment using ultrasound, microwave and autoclave techniques. Ultrasonication pretreatment was highly effective resulting in comparatively higher yield of oil. Oil extraction was performed by the Soxhlet method for oil extraction and the parameters for high yield were optimized. n-Hexane, a non-polar solvent, was best suited for extraction of oil. The parameters like moisture content, particle size, time, temperature and solid-to-solvent ratio were optimized. The highest yield of oil with all the optimized parameters is 40.9 %. The chemical parameters are well within the ASTM standard limits. A trial run was performed to check the conversion of oil and the yield of biodiesel was 89%. Further optimization of process conditions would enhance the yield of biodiesel. Thereby, *Vernonia cinerea* could be the best second-generation (non-edible oil) feedstock to produce biodiesel.

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