

# STATISTICAL OPTIMIZATION AND SYNTHESIS OF BIODIESEL FROM ACACIA NILOTICA SEEDS BY SOLVENT-ASSISTED DIRECT TRANSESTERIFICATION

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## Abstract

Biofuels have emerged as a prominent and environmentally-friendly alternative to traditional fossil fuels. Biodiesel, in particular, is recognized as a top contender for replacing petro-diesel, associated with pollution and environmental concerns. In the present research work, *Acacia nilotica* seeds were used as an indigenous non-edible feedstock for biodiesel production. The objective of current study was to employ the solvent-assisted direct transesterification approach for biodiesel production based on the simultaneous oil extraction and transesterification in one step. Ethyl acetate was used as a co-solvent, and due to the high free fatty acid content in *Acacia Nilotica* seed oil, the concentrated  $H_2SO_4$  was used as a catalyst. The purpose of co-solvent is to improve the miscibility of *Acacia* oil into methanol which leading to attain maximum yield (98%) in shorter time (300 min) therefore this could be a promising approach for biodiesel synthesis economical and viable. To achieve maximum product yield, the study utilized a computational statistical optimization method rooted in the Box-Behnken response surface methodology (RSM) design. This method optimised key process parameters, including reaction time, temperature, molar ratio (methanol to oil), and catalyst quantity. The results of RSM (optimization of reaction parameters) shows that, 300min reaction time, a temperature of 55°C, a molar ratio of 21:1, and a catalyst quantity of 6% by weight of  $H_2SO_4$ , these were identified as the optimized process parameters that achieved a biodiesel yield of 98%. FT-IR and Gas chromatography (GC-FID) analyses indicated that the predominant fatty acids in *Acacia nilotica* oil were Linoleic acid (31%) and oleic acid (30%). Our investigation unveiled the effective utilization of co-solvent, enabling mild conditions for acid-catalyzed direct transesterification. This approach leads to the production of high-quality biodiesel from acid oil.

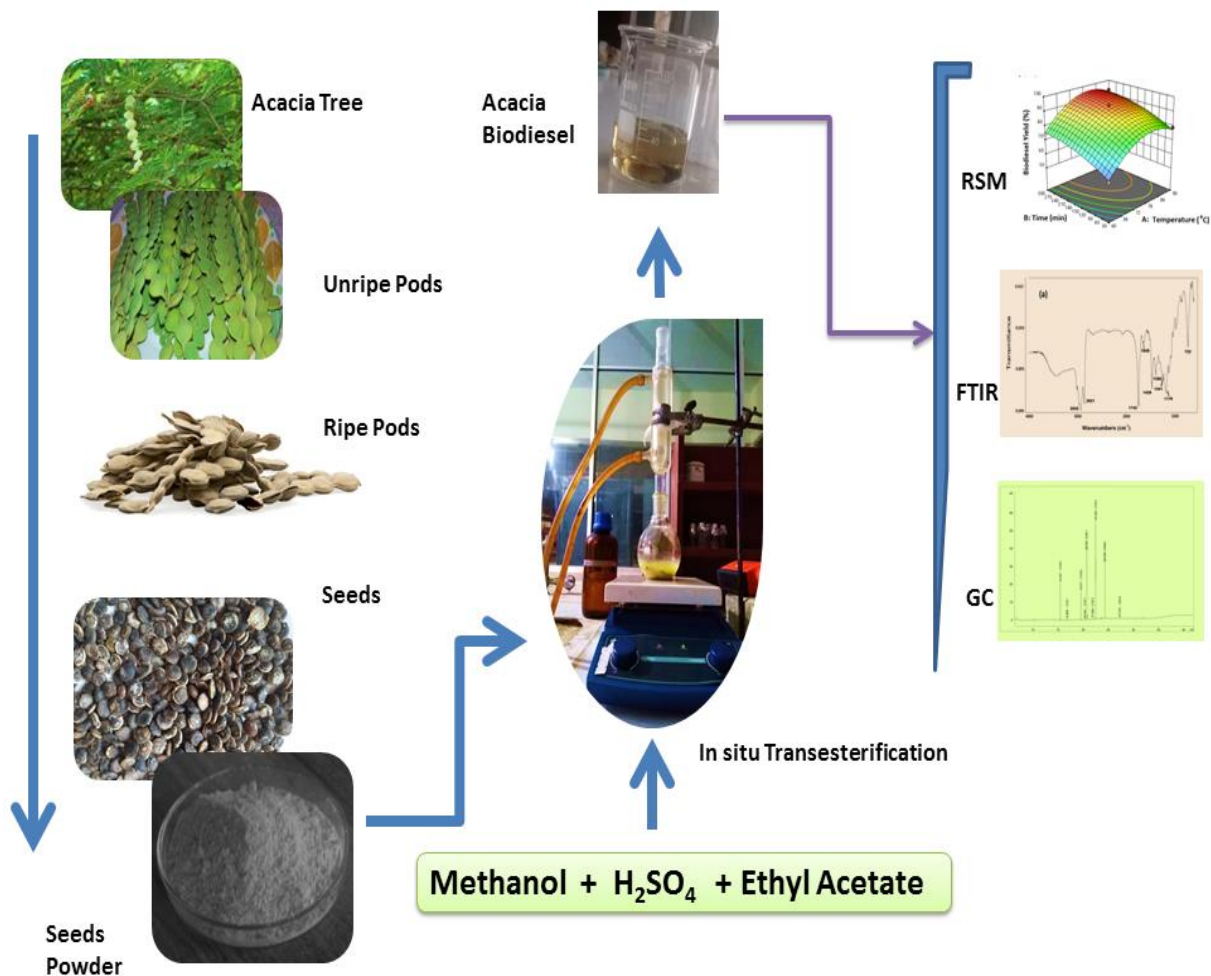
**Keywords:** *Acacia Nilotica*, Non-Edible, Acid-Catalyzed Transesterification, Box-Behnken Design, Direct Transesterification.

## 1. INTRODUCTION

Energy is a fundamental requirement for sustaining life and supporting civilization as it plays a pivotal role in industrial operations and transportation. There is a strong focus on harnessing renewable energy sources due to their increased feasibility and sustainability compared to conventional fossil fuels. [1, 2]. As the global population grows, the demand for traditional fossil fuels is on a sharp incline, leading to the rapid depletion of finite reserves. Moreover, the combustion of fossil fuels has severe ecological consequences. Consequently, scientists must explore cost-effective, environmentally friendly alternatives that can serve as clean replacements for these polluting fossil fuels [3-8]. Biodiesel is a liquid biofuel derived from vegetable oils or animal fats primarily composed of triglycerides. Chemically, it combines fatty acid methyl esters (FAMES), featuring both saturated and unsaturated fatty acid chains. [9-12]. The production of biodiesel primarily involves a catalytic transesterification reaction of plant feedstock, which can be either edible or non-edible. While both plant oils and biodiesel are chemically classified as triglycerides, plant oils cannot be directly utilized as a fuel source due to their high viscosity and unfavorable fuel characteristics. Therefore, transesterification serves as a crucial process to lower oil viscosity and enhance the fuel properties of biodiesel. [10, 13-15]. In recent times, the production of biodiesel has predominantly relied on edible feedstock. However, the elevated cost of edible oils and concerns surrounding the "food versus fuel" dilemma have prompted researchers to focus on investigating innovative and non-edible feedstock options [4, 16, 17]. Numerous studies have documented the successful production of biodiesel using readily available non-edible feedstock sources, including *Jatropha curcas*, *Azadirachta indica*, *Nicotiana tabacum*, and *Pongamia pinnata*. [3, 18]. The oil extracted from *Acacia nilotica* seeds, known locally in Pakistan as Kikar or Babol, represents a promising non-edible feedstock for biodiesel production. Biodiesel exhibits several noteworthy characteristics, including: 1) biomass-based fuel instead of fossil fuel, 2) biodegradable fuel, 3) neat and clean fuel due to its emission profile has fewer particulates such as soot, oxides of sulfur, oxides of nitrogen, carbon monoxide, and hydrocarbons [19]. In addition, it is considered a carbon-neutral fuel because its combustion does not increase carbon dioxide in air. Moreover, biodiesel has a higher flash point, cetane number, greater lubricity, and minimum sulfur content [20, 21].

Non-edible oils often contain elevated levels of free fatty acids (FFAs), making them suitable for base-catalyzed transesterification. However, this method has its drawbacks, notably the occurrence of side reactions like saponification and emulsification, which can decrease the biodiesel yield. To overcome these issues, acid-catalyzed transesterification can be employed as an alternative. However, it's worth noting that acid-catalyzed transesterification is often considered less economical due to its requirements for higher temperatures and longer reaction times [22]. The use of solvent-assisted direct transesterification represents a promising approach to address the drawbacks associated with conventional acid-catalyzed transesterification. This method offers a milder, faster, single-step, and more convenient process for biodiesel synthesis from non-edible feedstock, as shown in Fig 1. Efficiency is crucial, and conventional experimental

biodiesel production can be time-consuming, often requiring more than 24 of hours. However, researchers can significantly expedite the process by leveraging computational-based statistical optimisation techniques. These techniques can swiftly and accurately optimize experimental parameters, potentially leading to higher biodiesel yields and reduced research duration. This innovative approach holds the potential to revolutionize biodiesel production. Furthermore, physicochemical properties were determined and compared with international standards. Thus, non-edible *Acacia nilotica* seed oil and solvent-assisted direct acid-catalyzed transesterification technique were both extremely effective and affordable candidates to boost the developing biodiesel industry.

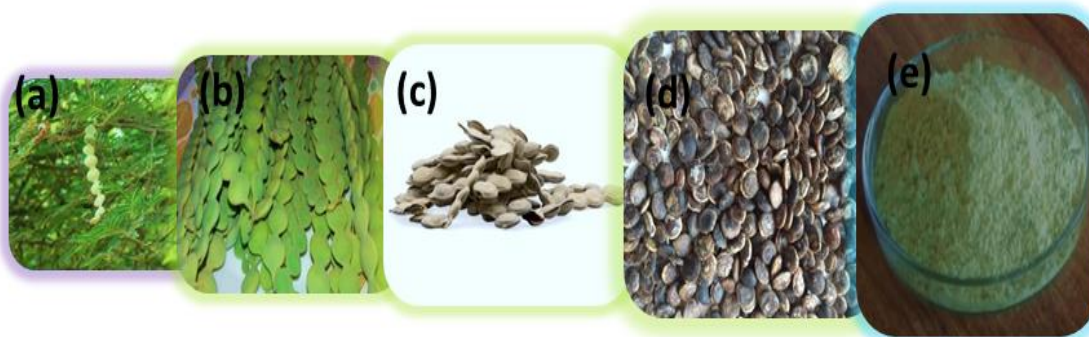


**Figure 1: A schematic diagram of biodiesel production from non-edible *Acacia nilotica* seed using Solvent-assisted acid-catalyzed Direct Transesterification.**

## 2. EXPERIMENTAL PROCEDURES

### 2.1. Materials

All chemicals used in this study were of analytical grade and employed as received, without further purification. Methanol (99.8%), n-hexane, and sulfuric acid were acquired from Merck, while diethyl ether, petroleum ether, and acetic acid were purchased from Fluka. The feedstock for biodiesel production consisted of seeds from the "kikar" plant, which were sourced from agricultural areas in Bahawalpur, Pakistan. These seeds were initially separated from dried seed pods, and any impurities were meticulously removed. Subsequently, the seeds were washed with distilled water to eliminate dust particles. To ensure the removal of trapped moisture, the moist seeds were oven-dried at 110 °C for 12 hours. Finally, the dried seeds were finely ground using a commercial grinder to increase the surface area of the oil-containing material (Fig 2).



**Figure 2: (a) Acacia nilotica tree. (b) Acacia unripe seed pods (c) Acacia ripe seed pods (d) Acacia seeds (e) Powdered seeds**

### 2.2 Oil extraction from Acacia nilotica seeds

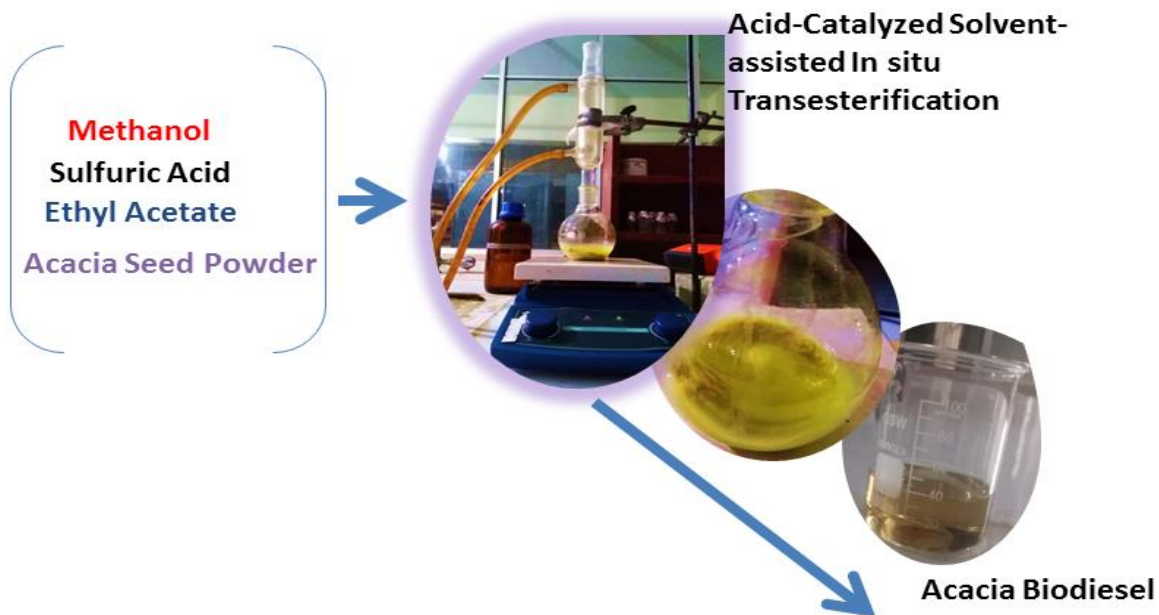
Acacia nilotica seeds oil was obtained by using a Soxhlet extraction technique. An 80 grams of dried seed powder was placed inside a thimble, and this thimble was inserted into a Soxhlet extractor. The Soxhlet setup consisted of a 1-liter round-bottom flask equipped with a reflux condenser and Iso-mantle was used as a heating source. Seeds oil was extracted with nonpolar n-hexane (350 mL) solvent at 70-75° C for 5 h. After oil extraction, oil was separated from the n-hexane solvent by utilizing a rotary evaporator at 65° C. Finally, oil was weighed. The oil content in seeds was determined by using equation (1) [23]. Physicochemical parameters of Acacia oil were determined according to the standard reported methods in Table S1

$$\text{Weight\%} = \frac{\text{Weight of oil produced}}{\text{Weight of the sample used}} \times 100 \quad (1)$$

## 2.5. Solvent-assisted Direct Transesterification

Solvent-assisted direct transesterification of acacia nilotica seeds was carried out in 500ml round bottom flask equipped with a reflux condenser and heated on a hot plate with constant magnetic stirring (Fig. 4). 25g of Acacia seeds powder, methanol, concentrated H<sub>2</sub>SO<sub>4</sub> as a catalyst, and 3% (by weight of seeds powder) ethyl acetate as co-solvent was added. According to the statistical design of the experiment (Table 2), the effects of different reaction parameters on biodiesel yield were examined. These parameters included reaction time (from 30 min to 300 min), molar ratio of methanol to oil (from 6:1 to 36:1), reaction temperature (from 40 °C to 70 °C), and catalyst amount (from 1 to 6%) based on oil weight. After the reaction, the biodiesel was filtered to separate the oil cake from biodiesel. No additional phase separation step was carried out because all of the glycerol produced was adsorbed onto de-oiled seed cake during reaction. Biodiesel yield was determined by using equation (2).

$$Yield = \frac{\text{wt.of biodiesel}}{\text{wt.of seed powder}} \times 100\% \quad (2)$$



**Figure 3: Experimental setup of solvent-assisted acid-catalyzed direct transesterification**

## 2.6. Analytical characterization

After the solvent-assisted acid-catalyzed direct transesterification, FAME content in the biodiesel sample was measured using gas chromatography (GC-FID) according to EN14103-11 standard method. The sample analysis revealed that the FAME content was approximately 98.7%. The primary functional groups in the biodiesel derived from Acacia nilotica seeds oil were identified through Fourier transform infrared spectroscopy (FTIR).

FTIR spectra were obtained from 400 to 4000  $\text{cm}^{-1}$  using a Bruker alpha FTIR spectrophotometer. The fatty acid composition of both *Acacia nilotica* seeds oil and its biodiesel derivative was analyzed using a gas chromatograph (Agilent Technologies 7890A model) system, which featured a capillary column and a flame ionization detector (FID). The samples were prepared by diluting them with n-hexane, and 1  $\mu\text{L}$  of the solution was introduced into the GC-FID system to quantify FAME content. A carrier gas, nitrogen, was employed with a flow rate of 1.5 mL/min, and the GC oven temperature was programmed to increase from 230  $^{\circ}\text{C}$  at a rate of 5 $^{\circ}\text{C}$  per minute.

The transesterification product (biodiesel) was qualitatively examined using thin-layer chromatography (TLC). The TLC analysis was executed with a silica gel 60 F254 plate, and the elution was carried out with a mobile phase comprised of a mixture of n-hexane, diethyl ether, and acetic acid in a ratio of 8:3:0.3 (v/v/v). Following the elution process, the TLC plate was dried, and the spots were subsequently identified and exposed to an iodine chamber to facilitate coloration and visualization of the individual components.

## 2.7. Statistical design of experiment and data collection

To enhance the production of *Acacia* biodiesel using the solvent-assisted direct transesterification method and investigate the influence of various independent variables (such as temperature, time, catalyst concentration, and methanol to seed mole ratio) on biodiesel yield, a response surface methodology was employed. This methodology was based on the Box-Behnken design. For optimization purposes, twenty-nine random experiments were conducted, as specified in Table 2. These experiments followed a 3-level and 4-factor Box-Behnken design, elaborated in Table 1. This design allowed for the assessment of the effects of the various factors on biodiesel yield.

**Table 1: experimental range and levels of the independent variable**

Factors	Symbols	Range and levels		
		Low	Medium	High
Temperature ( $^{\circ}\text{C}$ )	A	40	55	70
Time (min)	B	30	165	300
Catalyst Concentration (wt. %)	C	1	3.5	6
Molar ratio (methanol to seed)	D	1:6	1:21	1:36

**Table 2: The Box-Behnken design for optimizing solvent-assisted acid-catalyzed in situ transesterification**

Run	Factors				Response values %	
	A (Temperature of reaction)	B (Time of reaction)	C (Catalyst concentration)	D (Molar ratio)	Experimental	Predicted
1	55	165	3.5	221 21	96	96.6
2	55	165	1	6	95.5	94.95
3	55	165	3.5	21	96.7	96.62
4	55	165	6	6	96.7	96.05
5	40	165	6	21	91.5	91.82

6	40	165	3.5	6	92	92.51
7	55	300	6	21	98	96.41
8	55	165	6	36	94.5	94.00
9	55	30	3.5	36	93.5	94.14
10	55	300	3.5	36	93.5	93.27
11	70	165	1	21	93.5	93.74
12	70	165	6	21	94.5	94.94
13	70	300	3.5	21	96	97.90
14	55	30	3.5	6	92	92.79
15	55	165	3.5	21	96	96.62
16	40	300	3.5	21	91	89.49
17	55	300	1	21	95	95.51
18	55	300	3.5	6	97.5	97.42
19	70	165	3.5	36	94.5	93.47
20	70	165	3.5	6	94	93.97
21	55	30	6	21	94.1	94.08
22	70	30	3.5	21	90.5	89.97
23	40	165	1	21	92	92.12
24	55	165	1	36	94.6	94.20
25	55	30	1	21	94	94.08
26	55	165	3.5	21	95.2	96.62
27	40	165	3.5	36	89	90.21
28	55	165	3.5	21	96.2	96.62
29	40	30	3.5	21	94.6	93.65

### 3. RESULTS AND DISCUSSIONS

#### 3.1. Determination of statistical model

The utilization of Design-Expert software led to the suggestion of a quadratic response surface model. Equation 3, a coded second-order polynomial equation, was employed to demonstrate the association between four process variables and the output response, which is biodiesel yield in this context.

$$\begin{aligned} \text{\%Yield of biodiesel} = & 96.02 + 1.07A + 1.02B + 0.3917C - 0.6750D + 2.28AB + 0.3750AC \\ & + 0.8750AD + 0.7250BC - 1.37BD - 0.3250CD - 2.71A^2 - 0.6308B^2 - 0.1058C^2 - 0.93.8D^2 \end{aligned} \quad (3)$$

In equation 3, %yield represents biodiesel yield percentage while terms A, B, C, and D correspond to temperature, reaction time, catalyst concentration, and methanol to oil mole ratio, respectively. The quadratic response surface model described above was employed to predict the biodiesel yield based on four independent variables as outlined in equation 3. The specified ranges and levels of all process parameters are given in Table 1.

**Table 3: Analysis of variance (ANOVA) data for Adsorbant mediated transesterification.**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	107.85	14	7.70	10.11	< 0.0001	significant
A-Temperature	13.87	1	13.87	18.20	0.0008	
B-Time	8.84	1	8.84	11.61	0.0043	
C-Catalyst Conc.	0.6075	1	0.6075	0.7975	0.3869	
D-Mole Ratio	5.47	1	5.47	7.18	0.0180	
AB	20.70	1	20.70	27.18	0.0001	
AC	0.5625	1	0.5625	0.7384	0.4046	
AD	3.06	1	3.06	4.02	0.0647	
BC	0.2025	1	0.2025	0.2658	0.6142	
BD	7.56	1	7.56	9.93	0.0071	
CD	0.4225	1	0.4225	0.5546	0.4687	
A <sup>2</sup>	44.61	1	44.61	58.56	< 0.0001	
B <sup>2</sup>	4.13	1	4.13	5.42	0.0355	
C <sup>2</sup>	0.4817	1	0.4817	0.6323	0.4398	
D <sup>2</sup>	4.66	1	4.66	6.12	0.0268	
<b>Residual</b>	10.66	14	0.7618			
Lack of Fit	9.50	10	0.9497	3.25	0.1335	not significant
Pure Error	1.17	4	0.2920			
<b>Cor Total</b>	118.51	28				

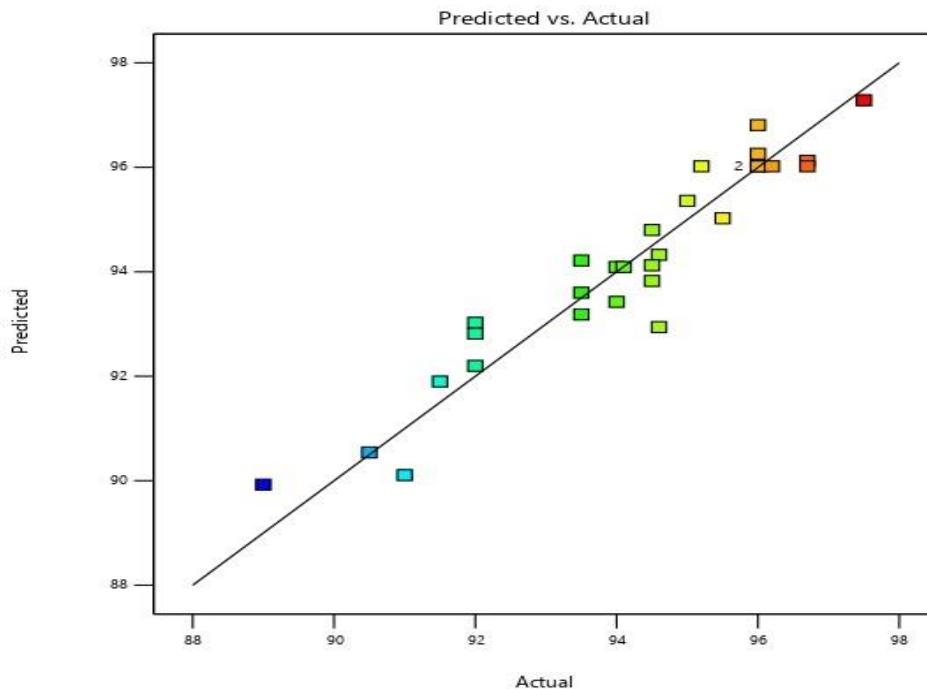
### 3.2.1. Seed Oil content

Oil content within *Acacia nilotica* seeds was assessed using the Soxhlet apparatus by using equation 1. The seeds were found to possess high oil content of 39%, suggesting that these seeds hold promise as a valuable resource for biodiesel production.

### 3.3. Model validation

To compare the experimental data with the predicted model, an Analysis of variance (ANOVA) technique was conducted and the results are summarized in **Table 3**. The ANOVA indicates that the probability value for the model is less than 0.0001, and it has a relatively high F-value of 21.52. Conversely, the F-value for lack of fit is 2.82, and its p-value is 0.1652, suggesting that it is not statistically significant. These findings confirm the excellent fit of the model to the experimental data. The ANOVA results indeed demonstrate that the model is statistically significant and temperature (A), reaction time (B), and molar ratio (D), while the most significant interaction terms are temperature-methanol to oil molar ratio (AD) and reaction time- methanol to oil molar ratio (BD) moreover quadratic terms A<sup>2</sup> and B<sup>2</sup> are also significant (p-value <0.05). The plot in Figure 4 and Supplementary Figure 1 illustrates the experimental and predicted biodiesel yield. It's evident that all data points closely align with the line of best fit, indicating a strong correlation between the predicted and experimentally measured biodiesel yield. This suggests that the model's predictions agree with the actual results.





**Figure 4: Predicted yields of biodiesel versus the actual yields of biodiesel.**

### **3.3.1. Response surface plots for the biodiesel yield**

**Fig. 5 and 6** depict three-dimensional response surfaces for process optimization, as per equation 3. These plots visually demonstrate the primary effects of individual independent process variables as well as the synergistic effects of pairs of process variables on biodiesel yield.

### **3.3.2. Interaction effects of temperature and molar ratio (AD) on product yield**

The ANOVA results are shown in **Table 3**, revealed that the interaction term of temperature-methanol to oil mole ratio (AD) is statistically significant ( $p$ -value  $< 0.05$ ). This indicates that both of these variables have a combined and meaningful impact on biodiesel yield.

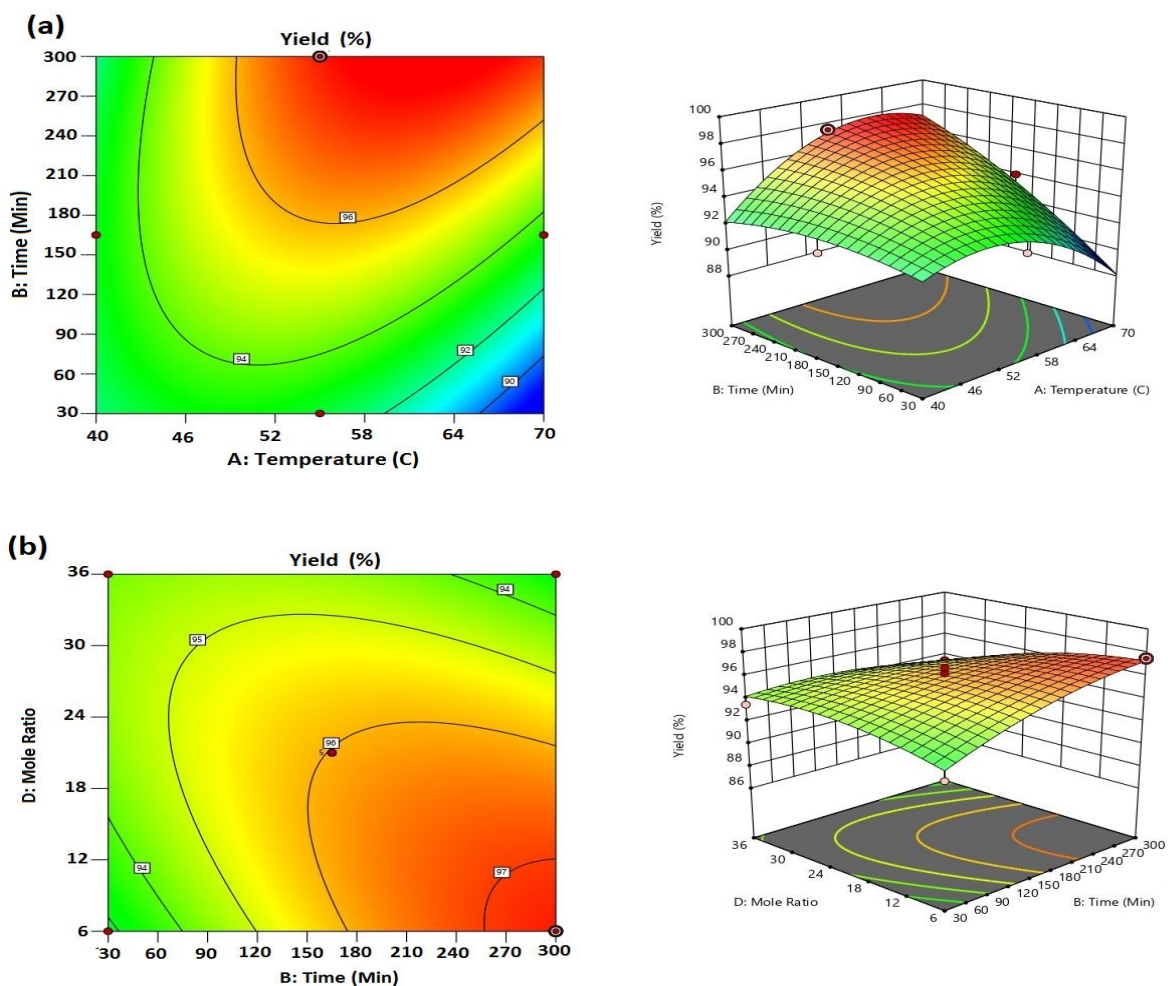
Response surface plot in **Fig. 5(a)** represents biodiesel yield versus temperature-time (AB) at constant catalyst concentration and alcohol to seed mole ratio. It shows that biodiesel yield is increased with the increase of reaction temperature and highest biodiesel yield (red colored zone) can be achieved at temperature  $55^{\circ}\text{C}$  and 300min of reaction time. After  $55^{\circ}\text{C}$ , temperature yield began to decrease. The ANOVA results presented in **Table 3** indicate that the interaction effect terms temperature-reaction time (AB) and reaction time-methanol to seed ratio (BD) are statistically significant (with  $p$ -values  $< 0.05$ ). This implies that these variable pairs have simultaneous impact on the biodiesel yield. 3D Surface plots with contour plots in **Figure 6a and 6b** illustrate simultaneous impact of temperature-reaction time (AB) and reaction time-methanol to seed ratio (BD) respectively on biodiesel yield. It shows that with the increase in

temperature and reaction time, yield also increased. Red colored zone indicates the combination of temperature and reaction time 55 °C and 300 min, respectively, at which the highest biodiesel yield is achieved, while after 55 °C temperature yield starts to decrease.

Transesterification is an endothermic reaction, so raising the temperature above 55°C triggers a significant loss in yield. In addition, transesterification is an equilibrium reaction therefore maximum yield could be achieved at 300 min when complete equilibrium state attain. Figure 5b represents the combine effect of reaction time and mole ratio (BD) on product yield at constant temperature and catalyst concentration. **Figure 6b** illustrate 3D response plots with its corresponding 2D contour plots showing the combined effect of reaction time with methanol to seed mole ratio (BD) at constant temperature (70 °C) and catalyst concentration (3.5%). According to which with the increase in mole ratio from 6:1 to 21:1 yield tends to increase which is due to in situ transesterification reaction which required more solvent but with further increase in mole ratio yield would not increase which is might be due to the hindrance with added co-solvent (DCM) while due to slow rate of reaction of acid-catalyzed transesterification maximum yield was achieved at maximum time (300 min). Due to substantial dilution of reactants and presence of polar hydroxyl group might instigate reverse reaction causing decrease in biodiesel yield When the molar ratio increases from 6:1 to 11:1, the yield tends to increase, but a further increase in the molar ratio causes a noticeable drop in yield due to the glycerol desorption process shifts the reaction backward. Therefore, mild temperature and molar ratio are ideal for achieving maximal yield, which is an economically advantageous situation.

### **3.3.3. Interaction effects of reaction time and molar ratio (BD) on biodiesel yield**

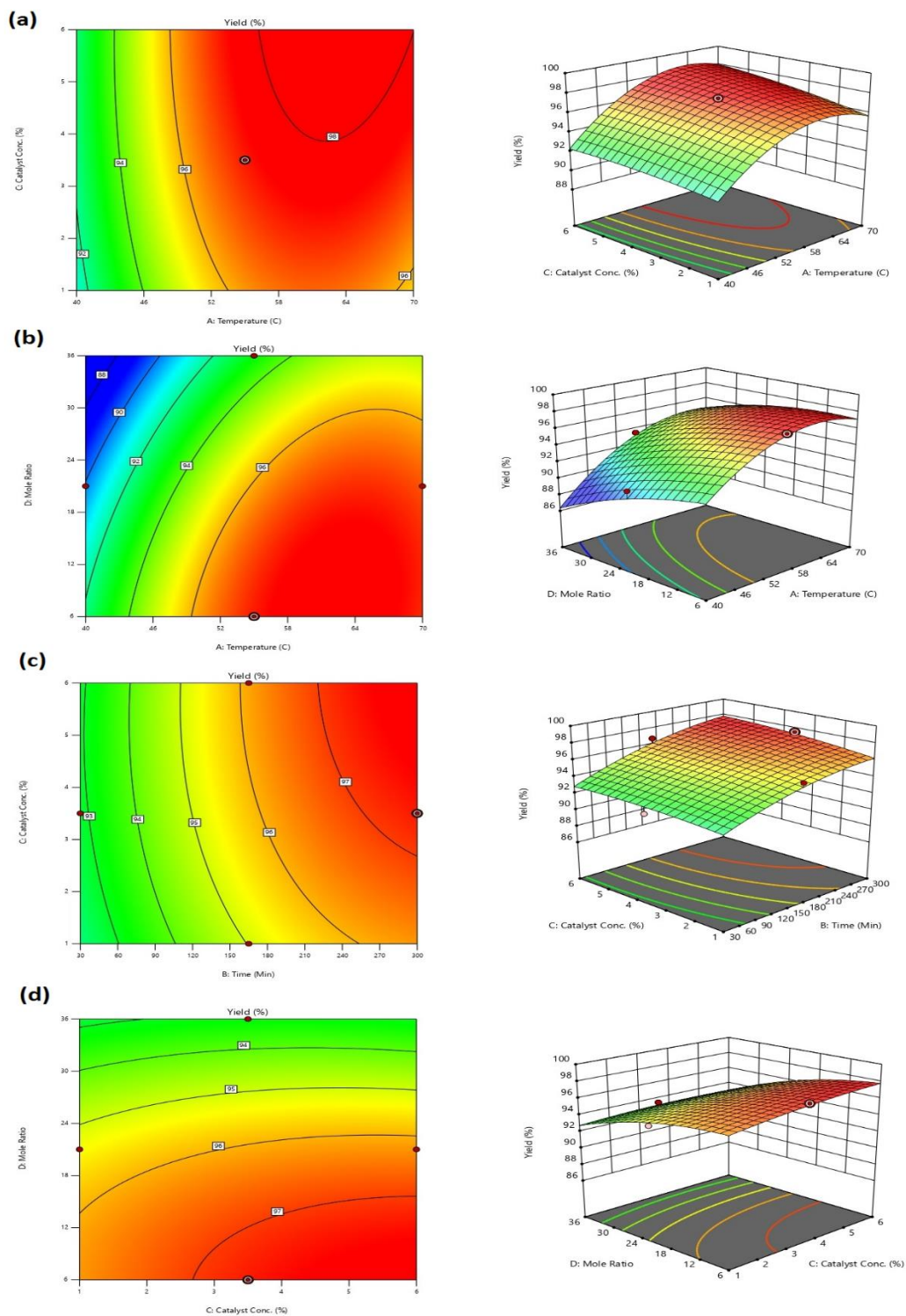
Analysis of variance in **Table 4** indicates the interaction effect of reaction time-molar ratio (BD) on biodiesel yield is quite significant, having p-value < 0.05 limit, which means both variables have a combined effect on yield. Response surface plot in **Fig. 5(e)** represents the mutual effect of reaction time-molar ratio (BD) at constant temperature and catalyst amount. Red colored zone showed maximum biodiesel yield, which can be achieved at a reaction time of 300 min and molar ratio 11:1. Due to the slow process of acid-catalyzed transesterification, the yield slowly increased and attained steady-state (96%) after 300 min. According to the equilibrium of the reaction, the increase in miscibility causes improved mass transfer, which will increase yield with the increase in a molar ratio from 6:1 to 11:1. However, a further increase in the molar ratio may cause the desorption of glycerol from dried powdered spent tea as adsorbent, which could shift the reaction backward.



**Figure 5: 3D surface graphs with 2D contour plots presenting the effect of significant reaction parameters on biodiesel yields**

### 3.3.4. Optimization process using response surface methodology (RSM) and confirmation

Based on the regression polynomial model obtained from response surface methodology described in equation 3, the model predicted the highest biodiesel yield and optimum values of all process parameters. This model allows you to optimize the process by identifying the conditions that would result in the maximum biodiesel yield. According to the predicted model optimum values of all studied process parameters including reaction time = 300 min, methanol to oil mole ratio = 21:1, reaction temperature = 55 °C, and catalyst concentration = 6%. These optimal conditions can help to achieve highest biodiesel conversion rate of 98%, which is very close to the experimental findings.



**Figure 5: 3D surface graphs with 2D contour plots presenting the effect of non-significant reaction parameters on biodiesel yields**

### 3.4. Characterization of biodiesel

#### 3.4.1. Fourier Transform Infrared Spectroscopy (FT-IR) analysis of biodiesel

The FTIR spectra of Acacia nilotica seeds oil and biodiesel are identical because both samples have similar functional groups with minor differences in band intensities and vibrational frequencies in **Fig. 6 (a-b)**. Most prominent peaks corresponding to the oil (triglycerides) sample are:  $2921\text{ cm}^{-1}$  ( $\text{sp}^2$  C-H stretching),  $2832\text{ cm}^{-1}$  ( $\text{sp}^3$  C-H stretching), and  $1732\text{ cm}^{-1}$  (C=O stretching),  $1627\text{ cm}^{-1}$  (C-H bending scissoring),  $1387\text{ cm}^{-1}$  (C-O stretching),  $1172\text{ cm}^{-1}$  (O-CH<sub>3</sub> stretching),  $722\text{ cm}^{-1}$  (C-H bending rocking) [25]. FTIR spectrum of biodiesel represents both samples have almost similar peaks between  $4000\text{ cm}^{-1}$  to  $2000\text{ cm}^{-1}$ . However, few characteristic peaks are observed in  $1750\text{ cm}^{-1}$  to  $1000\text{ cm}^{-1}$ , which are  $1738\text{ cm}^{-1}$ , [26] $1642\text{ cm}^{-1}$ , and  $1622\text{ cm}^{-1}$ . In the biodiesel sample, strong and sharp peaks at  $1738$  occur due to C=O stretching of the methoxycarbonyl group, while a larger peak at  $1627\text{ cm}^{-1}$  in oil has appeared into two peaks at  $1642\text{ cm}^{-1}$  and  $1622\text{ cm}^{-1}$  provides strong evidence of biodiesel formation [23].

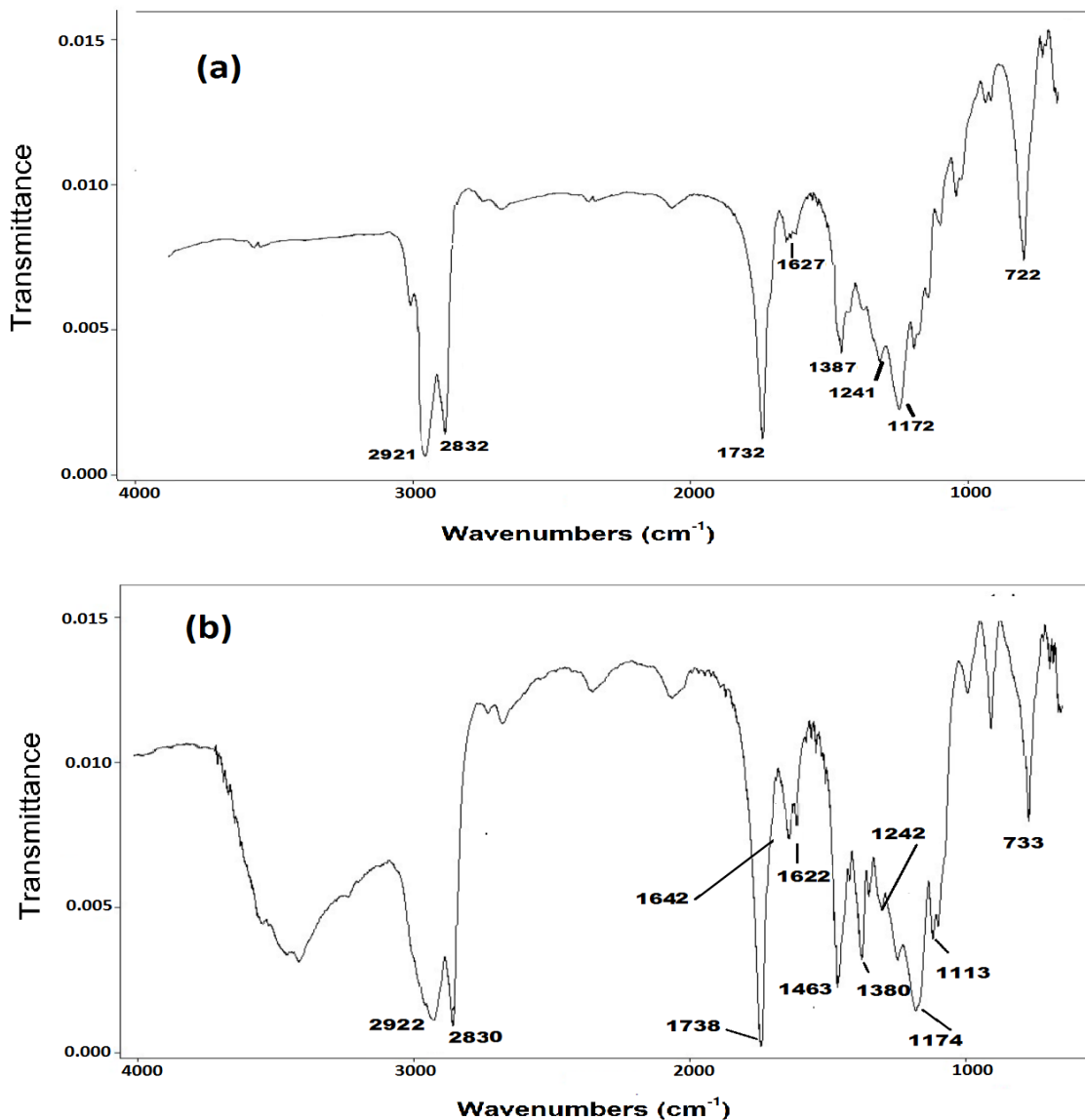
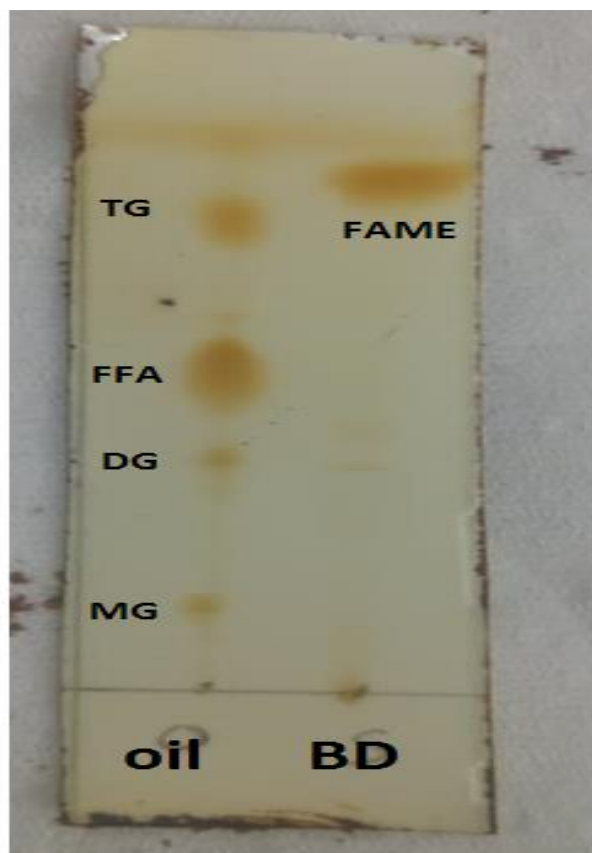


Figure 6: (a) FTIR of *Acacia nilotica* oil. (b) FTIR of *Acacia nilotica* biodiesel.

### 3.4.2. Thin-layer chromatographic analysis

The Thin-layer chromatography (TLC) analysis for *Acacia nilotica* oil indicate four distinct spots, corresponding to monoglycerides, diglycerides, free fatty acids, and triglycerides. Due to the elevated concentration of triglycerides, the spot appeared more pronounced and larger in **Fig. 7**. In contrast, the TLC analysis of *Acacia nilotica* biodiesel depicted a single, more substantial, and darker spot in **Fig. 7**. This observation suggests the presence of fatty acid methyl esters (FAME) and signifies the successful completion of the intensified acid-catalyzed transesterification reaction.



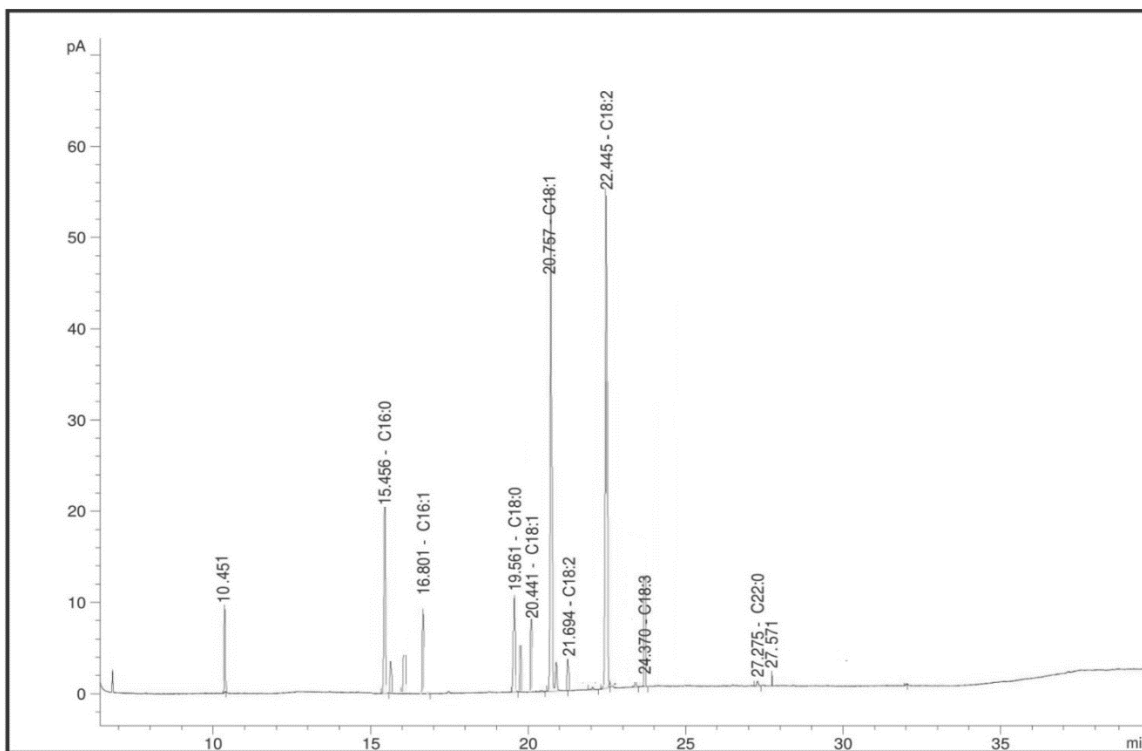
**Figure 7: TLC of *Acacia nilotica* oil and *Acacia nilotica* biodiesel.**

### **3.4.3. Gas chromatography analysis of *Acacia nilotica* seeds oil**

According to the fatty acid profile of ANO (Fig. 8), Linoleic acid was observed as the predominant fatty acid (31%), which is according to the reported value [27]. The ANO contained oleic acid (30%), linoleic acid (31%), Palmitic acid (16%), stearic acid (12%), and Linolenic acid (7%), which presents in **Table 4**. The combined contents were observed to be around 96%. Though *Acacia nilotica* seeds oil (ANO) has high unsaturated fatty acid content, our results highlight its potential as a biodiesel feedstock. Overall total unsaturated fatty acids were dominated by 68%, and saturated content was about 28%. Our findings have good agreement with the previous work of Abdullahi Muhammad, B. et al. [27].

**Table 4: Main fatty acids identified in the oil % (w/w)**

Fatty Acids	Systematic name	Structure	Weight%	Type of fatty acid
Palmitic acid	Hexadecanoic	C16:0	16	saturated
Stearic acid	Octadecanoic	C18:0	12	saturated
Oleic acid	Cis-9-Octadecanoic	C18:1	30	unsaturated
Linoleic acid		C18:2	31	unsaturated
Linolenic acid	Cis-9, Cis-12 Octadecadienoic	C18:3	7	unsaturated



**Figure 8: Gas chromatogram of Acacia nilotica biodiesel.**

#### 4. CONCLUSION

In the present research work, we investigated optimising biodiesel production acid-catalyzed solvent assisted direct transesterification approach. A computational optimization technique based on the Box-Behnken design of response surface methodology (RSM) was used to calculate the maximum product yield by optimizing process reaction parameters. After the optimization, reaction parameters such as reaction time of 300min, temperature of 55 °C, molar ratio of 21:1, and quantity of catalyst 6% wt. of H<sub>2</sub>SO<sub>4</sub> achieved 98% biodiesel yield. Analysis of variance indicated that temperature, time and mole ratio were most significant process parameters. Results of this study proved that use of ethyl acetate as a co-solvent can improve acid-catalyzed transesterification by improving miscibility of triglycerides in alcohol. The results showed



that Acacia seeds powder can't directly converted into its biodiesel by the acid-catalyzed process without extreme temperature and in a short time. We also minimize extra steps involved in biodiesel production, such as phase separation. This research proved that intensified transesterification is rapid, convenient, and single-step, which requires mild conditions and offers a maximum yield of biodiesel of 98%. The GC-FID analysis revealed that Acacia nilotica oil contained approximately 68% unsaturated fatty acids. Employing a solvent-assisted acid-catalyzed direct transesterification process with ethyl acetate (co-solvent) demonstrates significant potential as an economical and efficient method for producing high-quality biodiesel from non-edible feedstock.

### Acknowledgement

The authors gratefully acknowledge the Materials Chemistry Laboratory, Department of Chemistry, The Islamia University of Bahawalpur, Pakistan for the provision of research facilities.

### Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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