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Extraction and Optimization Process of Cassia Fistula Seed Oil

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ABSTRACT

Cassia fistula seed oil was extracted using three different methods: cold pressing, oven-assisted extraction, and Soxhlet extraction with n-hexane to evaluate and compare their efficiencies. The oil yield varied significantly across methods, with cold pressing producing the lowest average yield (16.87%), the oven method giving a moderate yield (18.76%), and Soxhlet extraction yielding the highest (28.15%). Optimization of the Soxhlet extraction process was carried out by varying key parameters, including solid-to-solvent ratio (3.63–10.36 g/g), temperature (54.88–80.11°C), and extraction time (1.31–4.60 h), to maximize oil recovery. The extracted oil was further characterized using Gas Chromatography Mass Spectrometry (GC–MS) and Fourier Transform Infrared Spectroscopy (FTIR) to determine its chemical composition and functional groups. The results demonstrate that while Soxhlet extraction with n-hexane provides superior oil yield, other methods may offer advantages in terms of simplicity and environmental considerations, highlighting the importance of selecting appropriate extraction techniques based on intended application.

Keywords*:* Cassia fistula seed oil, Soxhlet extraction, cold press method, oven-assisted extraction, n-hexane, oil yield optimization, response surface methodology, GC–MS, FTIR, bioactive compounds

INTRODUCTION

Cassia fistula L., commonly known as the golden shower tree, is a medicinal plant widely distributed in tropical and subtropical regions (Ogundipe, Odetoye, & Adeniyi, 2025). It belongs to the family Fabaceae and has long been valued in traditional medicine systems such as Ayurveda, Siddha, and Unani (Lepcha, Patra, & Saha, 2023). Almost every part of the plant, including the bark, leaves, flowers, pods, and seeds, has been reported to possess therapeutic properties (Nwozo, Effiong, Aja, & Awuchi, 2023). The plant is rich in bioactive compounds such as flavonoids, anthraquinones, and phenolic acids, which contribute to its pharmacological activities (Dar, Shahnawaz, Ahanger, & Majid, 2023). Traditionally, *Cassia fistula* has been used for the treatment of skin diseases, constipation, fever, and inflammation, and its extracts are also known to exhibit antimicrobial, antioxidant, and hepatoprotective effects (Mwangi, Macharia, Wagara, & Bence, 2021).

Beyond its medicinal and nutritional roles, *Cassia fistula* seed oil has potential in industrial sectors including cosmetics, pharmaceuticals, and biofuels (Shiva, Reddy, Kumar, & Sivasubramanian, 2025). Its physicochemical characteristics, such as stability, saponification value, and lipid profile, influence its suitability for applications in skincare formulations, soap production, and as a renewable energy feedstock (Jara-Vélez, et al., 2025). Despite this potential, limited studies have focused on optimizing extraction methods to balance oil yield with preservation of bioactive quality (Siol, Piasecka, Mańko-Jurkowska, Górska, & Bryś, 2025). Exploring efficient extraction techniques is therefore essential to maximize both the nutritional and industrial value of *Cassia fistula* seed oil (Thakur, Shivay, Katoch, Arora, & Garg, 2025).Importance of seed oils and extraction methods.

Limitations of Traditional Methods.

Traditional methods of oil extraction, such as mechanical pressing or simple thermal treatment, often suffer from low efficiency and inconsistent oil recovery, which limits their suitability for large-scale applications (Dhotre, 2025). Cold pressing, while beneficial for preserving heat-sensitive bioactive compounds and ensuring high nutritional quality, generally produces low yields due to incomplete oil release from the seed matrix. On the

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other hand, thermal methods such as oven drying may enhance oil release but can degrade important compounds, increase free fatty acid content, and reduce oxidative stability, thereby compromising oil quality. Moreover, these methods lack process optimization and standardization, making it difficult to achieve reproducible results (Chowdhury, Medhi, Bhattacharyya, & Hussain, 2025). Such limitations highlight the need to explore and compare advanced techniques, including solventbased extraction, to identify approaches that balance both oil yield and quality for *Cassia fistula* seed oil (LimaPereira, Veiga-Júnior, & Teixeira-Costa, 2025).

The present study focuses on the extraction and optimization of oil from *Cassia fistula* seeds with the aim of enhancing yield and preserving its bioactive constituents. Specifically, the objectives are to determine the most efficient extraction method for *Cassia fistula* seed oil, to evaluate the influence of key process parameters such as temperature, extraction time, solvent type, and particle size on oil yield, and to optimize these conditions using appropriate experimental design techniques. Additionally, the study seeks to characterize the physicochemical properties of the extracted oil and assess the retention of important bioactive compounds, including flavonoids and phenolic constituents, under optimized conditions. Ultimately, the research aims to establish a reproducible and efficient extraction protocol that maximizes oil recovery while maintaining its potential medicinal and industrial value.

MATERIALS AND METHODS

Materials

Mature pods of *Cassia fistula* were collected from healthy trees in Afe Babalola University, Ado Ekiti, along the Ornamental garden, during the peak fruiting season to ensure optimal seed maturity. The pods were carefully opened to separate the seeds, which were then cleaned manually to remove adhering pulp, dust, and other impurities. After cleaning, the seeds were washed with distilled water and air-dried at room temperature to reduce surface moisture. The dried seeds were subsequently stored in airtight containers under cool and dry conditions, away from direct sunlight, to prevent microbial contamination and oxidative deterioration before extraction. This standardized preparation and storage ensured that the seeds maintained their natural composition and were suitable for comparative analysis of different oil extraction methods, as shown in Figure 1 below.

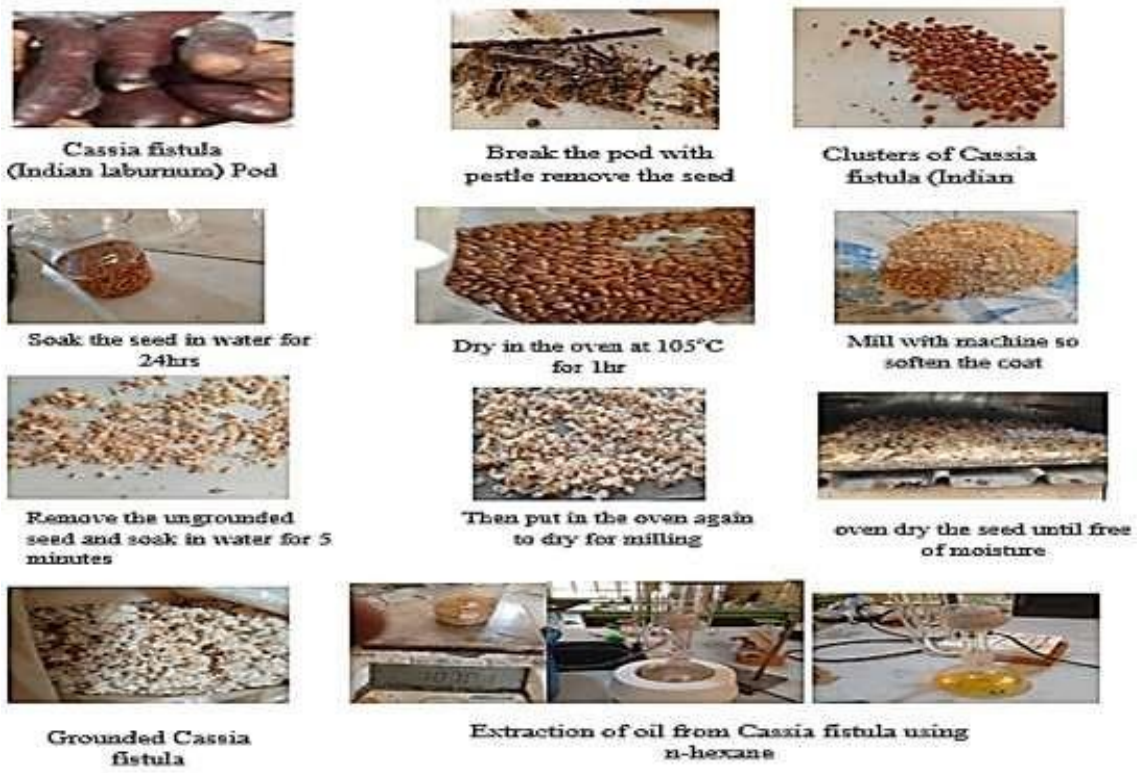

Figure 1: drying processes of various extraction methods of *Cassia fistula* seed

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Extraction Methods

Cold press extraction

For the cold-press extraction, the cleaned and dried *Cassia fistula* seeds were first ground into a coarse powder using a mechanical grinder to increase surface area and facilitate oil release. The ground material was then subjected to mechanical pressing using a cold-press oil extractor operated at ambient temperature (25–30 °C) without the application of external heat or chemical solvents. This method was chosen to minimize thermal degradation and preserve heat-sensitive bioactive compounds, natural antioxidants, and essential fatty acids present in the seed oil. The extracted oil was collected in clean, dark glass bottles to prevent light-induced oxidation, while the residual seed cake was stored separately for further analysis. The cold-pressed oil was subsequently filtered through muslin cloth to remove particulate matter and kept under refrigeration at 4 °C until analysis of yield and physicochemical properties.

Oven Drying Method

In the oven drying method, cleaned Cassia fistula seeds were spread evenly on stainless steel trays and subjected to controlled drying in a hot-air oven at 60°C for 6hours to reduce seed moisture content and enhance oil release. After drying, the seeds were allowed to cool to room temperature and then ground into a fine powder using a laboratory grinder. The powdered material was subsequently extracted using a mechanical press to obtain the oil. The ovendrying pre-treatment was intended to weaken the seed coat structure and improve oil recovery compared to untreated seeds. The extracted oil was filtered to remove residual solids, collected in amber glass bottles, and stored at 4 °C until further characterization.

Soxhlet Method

For Soxhlet extraction, the dried and powdered *Cassia fistula* seeds were placed in a cellulose thimble and loaded into a Soxhlet apparatus. Analytical grade n-hexane was used as the extraction solvent due to its high efficiency in dissolving lipids. The extraction was carried out for 6 hours at the solvent's boiling point, 68°C, allowing continuous solvent recycling through the seed matrix. A sample-to-solvent ratio of 1:10 w/v was maintained to ensure thorough extraction of the oil. After completion, the solvent-oil mixture was concentrated by rotary evaporation under reduced pressure to remove residual hexane, yielding the extracted seed oil. The oil was then collected in amber glass bottles and stored at 4 °C until further physicochemical analysis.

Analytical Methods

Oil yield

The oil yield of *Cassia fistula* seeds represents the percentage of oil recovered from a given seed sample using different extraction methods.

$$oil\ yield\ (\%) = \frac{mass\ of\ oil\ extracted\ (\%)}{mass\ of\ seed\ sample\ (g)}\ x\ 100 \qquad 1$$

$$mass\ of\ oil\ extracted\ (g)$$

Physicochemical properties of *Cassia fistula* Seed Oil

Moisture Content (%): The determination of moisture in *Cassia fistula* seed oil was carried out using the oven drying method, in which a known weight of the oil sample was placed in a drying oven maintained at 105 °C until a constant weight was obtained. The weight loss during heating was attributed to the evaporation of water present in the oil, and the result was expressed as a percentage of the initial sample weight.

$$Moisture\ \% = \frac{W_1 - W_2}{W_2} X100 \qquad 2$$

where W_1= weight of sample before drying, W_2 = weight after drying.

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Acid Value (mg KOH/g oil)

The acid value of *Cassia fistula* seed oil was determined to assess the level of free fatty acids, which are indicative of hydrolytic rancidity and overall oil quality. A known weight of the oil sample, 2 g, was accurately measured into a conical flask and dissolved in a neutral solvent mixture consisting of ethanol and diethyl ether (1:1 v/v) previously neutralized with potassium hydroxide (KOH). A few drops of phenolphthalein indicator were then added, and the solution was titrated against a standard 0.1 N KOH solution until a faint pink endpoint persisted for at least 30 seconds. The volume of KOH used was recorded, and the acid value was calculated using the formula:

$$Acid\ value\left(\frac{mgKOH}{g}\right) = \frac{V*N*56.1}{W} \quad 3$$

where V = volume of KOH used (mL),

N = normality of KOH,

W = weight of oil sample (g), 56.1 = molecular weight of KOH.

Peroxide Value (meq O_2/kg oil)

The peroxide value of *Cassia fistula* seed oil was determined to evaluate the extent of primary oxidation products, mainly hydroperoxides, which indicate the onset of rancidity. For the analysis, a known weight of oil sample (typically 1–2 g) was accurately measured into a conical flask and dissolved in a solvent mixture of glacial acetic acid and chloroform (3:2 v/v). To this solution, 0.5 mL of freshly prepared saturated potassium iodide (KI) solution was added, and the flask was immediately stoppered and kept in the dark for 1 minute with occasional shaking to allow iodine to be liberated from the reaction of KI with peroxides present in the oil. Subsequently, 30 mL of distilled water was added, and the liberated iodine was titrated against a standard 0.01 N sodium thiosulfate solution using starch solution as an indicator near the endpoint. The appearance of a colorless solution marked the completion of the titration.

$$Peroxide\ value\ \left(meq\frac{O_2}{kg}\right) = \frac{(S-B)X\ NX\ 1000}{W} \quad 4$$

where S = sample titration volume (mL),
B = blank titration volume (mL),
N= normality of sodium thiosulfate,
W = weight of oil (g).

Saponification Value *(mg KOH/g oil)*

The saponification value of *Cassia fistula* seed oil was determined to estimate the average molecular weight of the fatty acids present, which provides information on the potential applications of the oil. A known weight of the oil sample (approximately 2 g) was placed in a round-bottom flask, and 25 mL of 0.5 M alcoholic potassium hydroxide (KOH) solution was added.

The mixture was fitted with a reflux condenser and heated gently in a boiling water bath for about 30 minutes, with occasional swirling, to ensure complete saponification of the triglycerides into glycerol and potassium salts of fatty acids (soap).

After refluxing, the solution was titrated while still warm with a standard 0.5 M hydrochloric acid (HCl) solution using phenolphthalein as an indicator until the pink color disappeared. A blank determination, without the oil sample but with the same volume of alcoholic KOH, was carried out simultaneously under identical conditions.

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Saponification value (mg KOH/g)=$\frac{(B-S)X\ N\ X\ 56.1}{W}$ 5

where B = volume of HCl for blank,
S = volume for sample,
N = normality of HCl,
W = weight of oil (g).

Refractive Index (RI at 40 °C)

The refractive index of *Cassia fistula* seed oil was measured at 40 °C using a portable hand-held refractometer to evaluate the oil's purity and degree of unsaturation. Before analysis, the refractometer was calibrated with distilled water to ensure accuracy. A small drop of the oil sample was placed on the clean prism surface of the instrument, and the transparent cover plate was gently closed to spread the sample evenly and eliminate air bubbles. The prism was maintained at 40 °C using a thermostatically controlled water bath or by equilibrating the instrument in a warm environment before measurement. The refractometer was then held toward a light source, and the field of vision was observed through the eyepiece. The boundary line between the light and dark regions was aligned with the crosshair, and the refractive index reading was taken directly from the instrument scale. After each determination, the prism was carefully cleaned with a soft tissue and a mild solvent to prevent sample carryover.

Optimization of the *cassia fistula* seed using Response Surface Methodology (RSM)

The experiment was designed using Design -Expert version 12. Response surface methodology (RSM) was used to investigate the effect of independent variables, including solvent to solid (A), Temperature (B), and Time (C), on response variables, such as oil yield, Free Fatty Acid FFA, iodine value, and saponification value from *Cassia fistula* seed oil. RSM design, along with coded and uncoded levels, is presented in Table 1.0. Central composite design (Five levels) and a quadratic model were used to design this experiment. Twenty runs, including six axial points, eight fractional factorial points, and six central points, were randomly performed according to the Central Composite Design CCD, which is summarized in Table 2.0:

Table 1.0: Independent variables and their corresponding levels for oil extraction from Cassia fistula seed

Independent variables	Symbols			Coded level		
		$-\propto$	-1	0	+1	$+\propto$
Solvent to solid ratio	A	3.63641	5.00	7.00	9.00	10.3636
Temperature	B	54.8866	60.00	67.50	75.00	80.1134
Time	C	1.31821	2.00	3.00	4.00	4.68179

Real levels of independent variables were coded according to equation 6

$Z = \frac{Z_O - Z_C}{\Delta Z}$ 6.0

Where Z=coded level of independent variable
Zo=coded level of independent variable
ΔZ= step change
Zc=actual value of the central point
The specific equations for each independent variable were derived from the above equation to code their actual values. Specific equations for solvent to solid ratio (A), Temperature (B) and Time (C) are mentioned in below Equations 7-9.

$Z = \frac{A-7.0}{2.0}$ 7.0

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$Z = \frac{B-67.5}{21.5}$ 8..0

$Z = \frac{C-3.0}{1.0}$ 9.0

where A, B, and C represent solvent to solid ration of the *Cassia fistula (Golden shower)*, temperature, and time, respectively. A second-order polynomial equation was used to indicate the predicted response (oil yield) as a function of an independent variable, as follows in equation 10

$$R1 = \alpha_o + \alpha_1 * A + \alpha_2 * B + \alpha_3 C + \alpha_{11} A^2 + \alpha_{22} B^2 + \alpha_{33} C^2 + \alpha_{12} AB + \alpha_{13} AC + \alpha_{23} BC \quad 10$$

Where R_1 = Oil yield %

α_j = linear coefficients

$\alpha_{jj,}$ = Quadratic coefficients,

α_{jk} =the interactive coefficients of the independent variables

α_o= constant

Design expert software version 12 was used to calculate the variable coefficient of the determinant. The experiment was subjected to twenty runs as suggested by the design expert version 12 of the central composite design based on response surface methodology (RSM). The extraction parameters, such as extraction temperature (60-80 ºC), extraction time (2-4 hrs.), and solid to solvent ratio of (1:5-1:9), were optimized to increase the oil yield.

Optimization of the extraction parameters to enhance the oil yield

There are many factors influencing the oil extraction yield. The extraction parameters, such as extraction temperature (60-80 °C), extraction time (2-4 hrs.), and solid to solvent ratio of (1:5-1:9) were optimized to increase the oil yield.

Determination of oil extraction yield

The extracted oil was estimated with respect to time at different temperatures. The oil extraction yield (% w/w) can be expressed as:

$$oil\ yield\ \left(\%\frac{w}{w}\right) = \frac{W_o}{W}\ x\ 100 \quad 11$$

Where y=oil yield (%)

$W_o = weight\ of\ extracted\ oil\ (g)$

W=weight of cassia fistula biomass (g)

Table 2: The Central composite Design of the Variables with oil yield as Response from *Cassia fistula* seed oil extraction

	Factor 1	Factor 2	Factor 3	R 1
Run	A: Solid/solvent	B: Temperature	C: Time	oil yield
	g/g	deg C	hrs.	%
1	7.0	67.5	3.0	
2	10.3	67.5	3.0	
3	7.0	67.5	3.0	

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4	9.0	60.0	2.0	
5	9.0	75.0	2.0	
6	9.0	60.0	4.0	
7	7.0	67.5	3.0	
8	5.0	60.0	2.0	
9	7.0	67.5	3.0	
10	9.0	75.0	4.0	
11	3.6	67.5	3.0	
12	7.0	67.5	3.0	
13	5.0	75.0	2.0	
14	7.0	67.5	1.3	
15	7.0	80.1	3.0	
16	7.0	67.5	4.6	
17	5.0	75.0	4.0	
18	7.0	54.8	3.0	
19	7	67.5	3	
20	5	60	4	

Chemical Composition Analysis

The fatty acid composition of *Cassia fistula* seed oil was analyzed using a Varian 3800/4000 Gas Chromatography– Mass Spectrometry (GC–MS) system equipped with a fused silica capillary column (e.g., VF-5MS, 30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature was programmed to start at 100 °C (held for 2 min), ramped at 10 °C/min to 250 °C, and held for 10 min to ensure complete elution of all components. Helium was used as the carrier gas at a constant flow rate of 1 mL/min, and the injection volume was 1 µL in split mode (split ratio 1:50).

Electron impact (EI) ionization at 70 eV was employed, and mass spectra were acquired over the range of m/z 50–550. Identification of fatty acids was performed by comparing retention times and mass spectra with authenticated standards and the NIST MS library**.** Quantification was carried out using an internal standard method**,** where a known quantity of a reference compound (e.g., methyl heptadecanoate) was added to each sample, allowing accurate determination of relative concentrations of individual fatty acids.

Fourier Transform Infrared (FTIR) spectroscopy was employed to characterize the functional groups present in *Cassia fistula* seed oil using a PerkinElmer Spectrum Two FTIR spectrometer**.** For analysis, the oil samples were prepared either as a neat film between potassium bromide (KBr) plates or directly analyzed using an Attenuated Total Reflectance (ATR) accessory**,** eliminating the need for complex sample preparation.

Spectra were recorded over the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1}, accumulating 32 scans per sample to improve signal-to-noise ratio. Key absorption bands of interest included the broad O–H stretching around 3400 cm^{-1}, C–H stretching vibrations of aliphatic chains near 2920 and 2850 cm^{-1}, carbonyl (C=O) stretching of triglycerides around 1740 cm^{-1}, C=C stretching of unsaturated fatty acids near 1650 cm^{-1}, and C–O stretching vibrations between 1170–1160 cm^{-1}. These characteristic bands provided insights into the chemical structure and degree of unsaturation in the oil

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RESULTS AND DISCUSSION

The variation in oil yield obtained from *Cassia fistula* seeds across the different extraction methods in Table 3 agrees with trends widely reported in the literature, where solvent-based techniques typically provide higher efficiency than mechanical or thermal approaches. The comparatively low yield from the cold press method reflects earlier findings that mechanical extraction often results in incomplete cell wall disruption and limited release of bound lipids, even though it maintains oil quality and eliminates the risk of solvent residues. The moderate improvement observed with oven drying can be attributed to reduced moisture content and partial breakdown of seed structures, which facilitates oil release, although studies have noted that excessive heating may negatively affect heat-sensitive compounds. In contrast, the much higher yield achieved through Soxhlet extraction supports extensive reports that continuous solvent extraction enhances lipid solubility and mass transfer, enabling more exhaustive recovery of oil. Altogether, these observations highlight the commonly reported balance between extraction efficiency and product quality or environmental considerations, with Soxhlet extraction favoring maximum yield, while cold pressing is often preferred when a more natural, solvent-free oil is desired.

Table 3: different masses of oil yield at various methods

Mass of cassia fistula used	Oil yield on the cold method	Oil yield in the oven method	Oil yield on Soxhlet Method
10.0	15.90	17.98	27.98
20.0	16.89	18.23	28.11
25.0	17.82	19.21	27.55
30.0	16.89	19.65	28.98
Average	16.87	18.76	28.15

Figure 2 shows the oil yield of *Cassia fistula* seeds using cold press, oven drying, and Soxhlet extraction at different seed masses (10 g, 20 g, 25 g, and 30 g). Across all sample weights, Soxhlet extraction consistently produced the highest yield, ranging from 27.55% to 28.98%, confirming its efficiency in fully recovering oil due to solvent action. The oven method gave moderate yields (17.98–19.65%), slightly higher than the cold press method (15.90–17.82%), suggesting that heat treatment enhances oil release by reducing moisture and softening the seed matrix. In contrast, cold-press extraction produced the lowest yields across all masses, likely because mechanical pressure alone cannot liberate all available oil. Interestingly, both cold press and oven methods showed slight increases in oil yield with increasing seed mass, whereas Soxhlet remained relatively stable, reflecting its ability to exhaustively extract oil regardless of sample size. Overall, the data highlight that Soxhlet is most effective for maximum yield, while oven and cold press methods are less efficient but may preserve oil quality better.

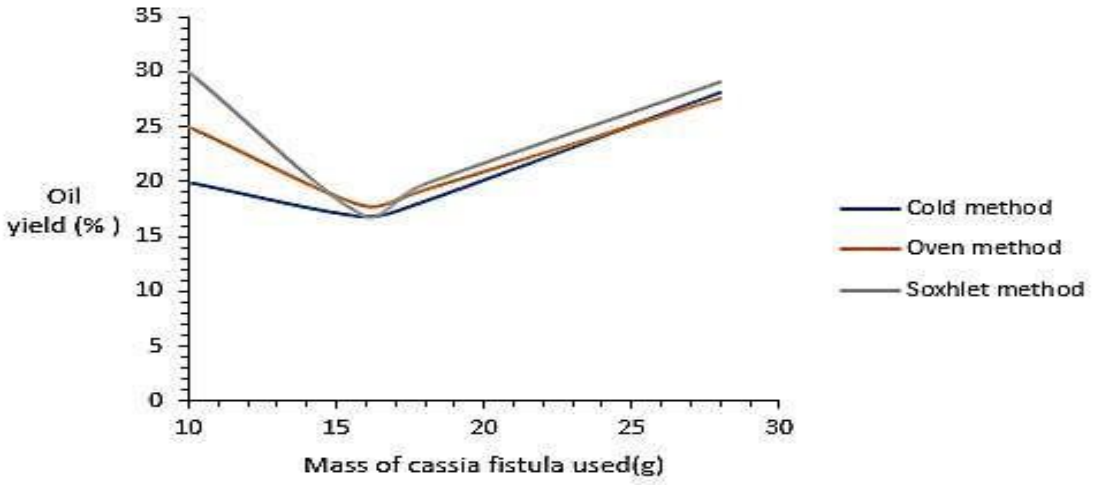

Figure 2: Oil yield of *cassia fistula* against mass of *cassia fistula* (g)

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Physicochemical properties comparison across the methods

The physicochemical analysis of *Cassia fistula* seed oil extracted by different methods in Table 4 reveals notable variations in quality parameters. Moisture content was lowest in cold-press oil (0.24%) and highest in Soxhlet (0.31%), showing that solvent extraction retains more residual moisture, which may affect storage stability. Acid value, an indicator of free fatty acid content, increased across methods (2.11–2.89 mg KOH/g), suggesting that Soxhlet extraction may cause slight hydrolysis of triglycerides compared to cold press. Similarly, peroxide value, which measures primary oxidation products, was lowest in cold-pressed oil (5.43 meq O_2/kg) and highest in Soxhlet (6.43 meq O_2/kg), implying that higher extraction temperatures or solvent exposure promote oxidative changes. Saponification value varied considerably, with Soxhlet oil recording the highest (186.32 mg KOH/g), indicating a higher proportion of short-chain fatty acids, while oven-dried oil had the lowest (151.43 mg KOH/g). Refractive index values ranged from 1.28 to 1.41, showing slight differences in purity and unsaturation levels, with cold-pressed oil maintaining the highest index, possibly reflecting less thermal alteration. Density values also fluctuated slightly, with cold press (0.917 g/cm³) and Soxhlet (0.915 g/cm³) being close, and oven-dried oil being lower (0.876 g/cm³). Statistically, the observed differences suggest that the extraction method significantly influences oil quality, with cold pressing producing oil of relatively better stability, while Soxhlet, despite higher yield, compromises quality parameters due to oxidation and hydrolysis.

Table 4: Physicochemical properties of cassia fistula at different methods of extractions

Physiochemical Parameters	**Oil yield on the cold method**	**Oil yield in the oven method**	**Oil yield on Soxhlet method**
Moisture (%)	0.24	0.27	0.31
Acid Value (mg KOH/g)	2.11	2.76	2.89
Peroxide Value (meq O_2/kg)	5.43	5.98	6.43
Saponification Value (mg KOH/g)	176.22	151.43	186.32

Effect of extraction method on oil yield (%")

The results show clear differences in oil yield among the extraction methods, with Soxhlet producing the highest oil recovery (27.55–28.98%), oven drying giving intermediate yields (17.98–19.65%), and cold pressing yielding the least (15.90–17.82%). This indicates that solvent-based Soxhlet extraction is more exhaustive and efficient at extracting bound oil, while mechanical pressing and heat-assisted drying release comparatively less.

In terms of the effect of the extraction method on oil quality, cold-pressed oils showed lower acid and peroxide values, suggesting better oxidative stability and less degradation, which is desirable for food and pharmaceutical applications. Oven drying improved yield slightly but reduced some quality attributes (lower saponification value, lower density), likely due to thermal effects. Soxhlet, while highly efficient in oil recovery, showed higher acid and peroxide values, indicating susceptibility to hydrolysis and oxidation during solvent and heat exposure. This results analysis makes the Soxhlet extraction method suitable for the optimization process for the extraction of the oil from *Cassia fistula*.

Optimization process of *Cassia fistula* oil using the Soxhlet extraction method

The extraction of oil from *Cassia fistula* seeds was carried out to evaluate the effects of solid-to-solvent ratio, temperature, and extraction time on oil yield. The experimental results revealed noticeable variations in yield across the twenty runs, indicating that these process variables significantly influence extraction efficiency. Such variations are expected, as factors like solvent concentration, thermal energy, and duration determine the extent of oil diffusion and solubilization from the seed matrix. Therefore, understanding how these parameters interact provides valuable insight into optimizing the extraction conditions for maximum oil recovery and improved process performance.

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Extraction outcome on the oil yield (%) of *Cassia fistula* oil

Table 5.0 presents the results of a Response Surface Methodology (RSM) experiment designed to study how three process variables—solid-to-solvent ratio (A), temperature (B), and extraction time (C) affect oil yield. Each run shows both the experimental (Exp) yield obtained in the lab and the predicted (Pr) yield estimated by the statistical model.

Looking at the data overall, the center-point condition (A ≈ 7 g/g, B ≈ 67.5 °C, C ≈ 3 h) is repeated several times (runs 1, 3, 7, 9, 12, 19), which is typical in RSM to estimate experimental error and model reliability. The predicted value at this point remains constant (34.35%), while the experimental values vary (≈29.86–37.57%), indicating some natural variability in the process but generally reasonable agreement with the model. This suggests the model is moderately stable but may not fully capture all experimental fluctuations.

Temperature appears to have a strong influence on oil yield. For example, at higher temperatures like 80.11 °C (run 15), the experimental yield reaches the highest value (67.00%), although the model underpredicts it (58.37%). Similarly, increasing the temperature from 60 °C to 75 °C at similar conditions (runs 4 vs. 5) increases both experimental and predicted yields. This indicates a positive effect of temperature on extraction efficiency, likely due to improved solubility and mass transfer.

The solid-to-solvent ratio (A) shows a more complex, nonlinear effect. Lower ratios (e.g., 3.63 in run 11) and higher ratios (e.g., 10.36 in run 2) do not necessarily produce the highest yields, suggesting that an intermediate level is optimal. Around ≈ 7–9 g/g, yields tend to be more favorable, especially when combined with higher temperatures.

Extraction time (C) also influences the yield, but not always linearly. Short times (≈2 h) can give high yields under favorable temperature conditions (runs 4 and 5), while longer times (4–4.6 h) sometimes reduce yield (runs 6 and 16), possibly due to degradation or equilibrium effects. However, in some cases (run 20), a longer time combined with a low temperature still produces a relatively high yield, indicating interaction effects between variables.

Comparing experimental and predicted values across runs, the model generally captures the trend but shows noticeable deviations in some cases (e.g., run 15 and run 4). This suggests that while the RSM model is useful for prediction and optimization, it may benefit from refinement or inclusion of higher-order interaction terms.

In summary, the RSM results indicate that temperature is the most influential factor, followed by interaction effects between all three variables. Optimal oil yield is achieved at relatively high temperature, moderate solid-to-solvent ratio, and controlled extraction time.

Table 5: Outcome on the oil yield of *Cassia fistula* oil

Run	A: Solid/solvent	B: Temperature	C: Time	Oil yield %	
	g/g	Deg C	hrs.	Exp	Pr
1	7.00	67.50	3.00	37.57	34.35
2	10.36	67.50	3.00	33.39	31.53
3	7.00	67.50	3.00	33.14	34.35
4	9.00	60.00	2.00	50.22	43.16
5	9.00	75.00	2.00	49.98	54.59
6	9.00	60.00	4.00	26.33	29.27
7	7.00	67.50	3.00	29.86	34.35
8	5.00	60.00	2.00	33.4	31.06

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9	7.00	67.50	3.00	37.14	34.35
10	9.00	75.00	4.00	30.89	34.80
11	3.63	67.50	3.00	35.20	34.84
12	7.00	67.50	3.00	31.57	34.35
13	5.00	75.00	2.00	39.6	38.23
14	7.00	67.50	1.31	33.00	37.42
15	7.00	80.11	3.00	67.00	58.37
16	7.00	67.50	4.60	43.00	36.35
17	5.00	75.00	4.00	42.20	50.84
18	7.00	54.88	3.00	41.29	47.69
19	7.00	67.50	3.00	36.43	34.35
20	5.00	60.00	4.00	52.60	49.57

Effect of solid-to-solvent ratio (Factor A)

The solid-to-solvent ratio (g/g) had a strong influence on oil yield. Generally, a lower solid-to-solvent ratio promoted higher oil yield because the solvent could better penetrate the solid matrix, enhancing oil solubilization. For example, run 20 with a ratio of 5 g/g gave a relatively high yield of 52.6%, while higher ratios such as 10.36 g/g (run 2) resulted in a lower yield of 33.39%. This trend suggests that excessive solid loading can reduce extraction efficiency by limiting solvent diffusion and oil dissolution.

Effect of temperature (Factor B)

Temperature also played a significant role in oil yield. Increasing temperature generally improved the yield up to an optimum point by enhancing solvent diffusivity and oil solubility. This is clearly demonstrated by Run 15, which achieved the highest yield of 67.00% at 80.11 °C, suggesting that elevated temperatures favor the extraction process. However, excessively high temperatures could potentially degrade thermolabile components or increase solvent loss, so a balance must be maintained between yield and oil quality.

Effect of extraction time (Factor C)

Extraction time had a moderate but noticeable influence on oil recovery. Runs conducted at around 3 hours tended to produce consistent yields, while shorter times (1.32 h in Run 14, yield = 33.00%) resulted in incomplete extraction. Conversely, very long extraction times (4–4.68 h, runs 6, 10, 16, 17, 20) did not always increase the yield proportionally, indicating that equilibrium between the solvent and oil phase is reached after a certain duration. Therefore, around 3 hours appears sufficient to achieve a near-optimal yield under these conditions.

Overall optimizations on the oil extraction for the *Cassia fistula* seed oil

Considering all three factors together, the highest yield was observed at a moderate solid-to-solvent ratio (7 g/g), high temperature (≈ 80 °C), and medium extraction time (3 h), as in Run 15. This combination seems to offer optimal solvent penetration, diffusion rate, and extraction completeness.

The data imply that *Cassia fistula* oil extraction efficiency can be maximized by balancing solvent quantity, temperature, and time to prevent energy waste and degradation while ensuring high oil recovery. Further optimization using response surface methodology or factorial design could help define the precise operating conditions for maximum yield.

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Analyzed results on the oil yield of Cassia fistula

The quadratic model of *Cassia fistula* oil production from *Cassia fistula* seed oil extraction was shown to be very significant by the Analysis of Variance (ANOVA), suggesting a substantial correlation between the experimental parameters and the response. The quadratic model effectively explained the variation in oil yield, as seen by the model's large F-value and associated p-value of less than 0.05. The yield was significantly impacted linearly by extraction temperature, extraction duration, and solvent-to-seed ratio, as well as by their quadratic terms, which suggested curvature in the response surface. The interaction effects between temperature and solvent ratio were particularly significant, demonstrating the combined influence of these variables on maximizing oil recovery. The coefficient of determination (R^2) was high, indicating good agreement between predicted and experimental values, while the adjusted R^2 confirmed the model's reliability. The non-significant lack-of-fit further validated the adequacy of the quadratic model for predicting oil yield within the studied range of process parameters, as shown in Table 6.

Table 6: ANOVA quadratic on oil yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
Model	1350.40	9	150.04	3.56	0.0301	Significant
A – Solid/solvent	13.20	1	13.20	0.3135	0.5879	
B – Temperature	137.66	1	137.66	3.27	0.1007	
C – Time	1.39	1	1.39	0.0331	0.8593	
AB	9.07	1	9.07	0.2156	0.6524	
AC	524.56	1	524.56	12.46	0.0054	Significant
BC	17.40	1	17.40	0.4135	0.5347	
A^2	2.45	1	2.45	0.0582	0.8142	
B^2	628.83	1	628.83	14.94	0.0031	Significant
C^2	11.61	1	11.61	0.2758	0.6109	
Residual	420.90	10	42.09			
Lack of Fit	369.09	5	73.82	7.12	0.0252	Significant
Pure Error	51.81	5	10.36			
Cor Total	1771.29	19				

The ANOVA results in Table 6 indicate that the overall quadratic model for oil yield is statistically significant at the 95% confidence level, as shown by the model p-value of 0.0301, which is less than 0.05. This suggests that, while the model explains some variation in the oil yield (sum of squares = 1350.40), the overall relationship between the predictors (solid-to-solvent ratio, temperature, and time) and oil yield is strong enough to be considered statistically significant. The model F-value of 3.56 also reflects a modest effect of the combined variables on the response, indicating that some factors may influence oil yield more than others.

Looking at the individual terms, certain factors had significant effects while others did not. Specifically, the interaction between solid-to-solvent ratio and time (AC) has a significant influence on oil yield ($p = 0.0168$), as does the quadratic term for temperature (B^2) with a p-value of 0.0064, highlighting that temperature has a strong non-linear effect on oil yield. Other main effects, such as solid-to-solvent ratio (A), temperature (B), and time (C), and interactions AB and BC, were not significant ($p > 0.05$), indicating that their linear contributions or interactions are less impactful within the tested ranges. Quadratic effects of solid-to-solvent ratio (A^2) and time (C^2) were also significant, suggesting minimal curvature in the response for these factors.

However, the lack-of-fit test is significant ($p = 0.0252$), meaning the model fits the experimental data adequately. This indicates that there is systematic variation in the oil yield that the quadratic model captured in the experimental factors. The residual analysis shows a residual sum of squares of 420.90, with pure error at 51.81, highlighting some variability inherent in the experiment. Overall, while the quadratic model in equation 12 identifies temperature and the AC interaction as influential factors, the significant lack of fit suggests caution

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when using this model for precise prediction, and further model refinement or additional experiments may be necessary to accurately describe the response.

Oil yield=456.93+8.305A-15.33B+35.9178C+0.071AB-4.048AC-0.196BC-0.103A^2+ 01174B^2+0.8975C^2 12 Where:

A = Solid-to-solvent ratio

B = Temperature

C = Extraction time

This equation was used to predict oil yield based on the specified process variables. By default, the high levels of the factors are coded as +1, and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Fit Statistics on the Oil Yield

The statistical parameters in Table 7 indicate how well the experimental data fit the developed model. The mean value of the responses is 39.19, with a standard deviation of 6.49, showing moderate variability among the data points. The coefficient of variation (C.V.) of 16.55% suggests that the model results are reasonably precise, though some dispersion exists around the mean.

The coefficient of determination (R^2) value of 0.762 indicates that approximately 76.2% of the variability in the response is explained by the model, implying a fairly good fit. However, the adjusted R^2 (0.549) is notably lower, suggesting that some predictors may not contribute significantly and that the model might include unnecessary terms. The predicted R^2 (0.676) being negative shows that the model has poor predictive ability for new data, meaning it does not generalize well outside the experimental range.

On the other hand, the Adequate Precision value (6.34) is greater than 4, indicating a sufficient signal-to-noise ratio, which means the model can be used to navigate the design space, but its predictive reliability should be interpreted with caution.

Table 7: Fit Statistics summaries on the oil yield

Std. Dev.	6.49	R^2	0.7624
Mean	39.19	Adjusted R^2	0.5485
C.V. %	16.55	Predicted R^2	0.6755
		Adeq Precision	6.3427

Diagnostic plot on the Oil yield

The normal probability plot of externally studentized residuals for *Cassia fistula* seed oil yield in Figure 3 shows how well the residuals follow a normal distribution, which is an important assumption for the validity of the regression model. In this plot, most data points lie close to the straight red reference line, indicating that the residuals are approximately normally distributed.

This suggests that the model's error terms are random and that there is no significant deviation from normality. A few minor deviations at the extreme ends of the line may indicate slight nonnormality or the presence of mild outliers, but they are not severe enough to significantly affect model reliability. Overall, the plot supports that the quadratic model provides a reasonable fit to the experimental data and that the assumption of normality for residuals is largely satisfied.

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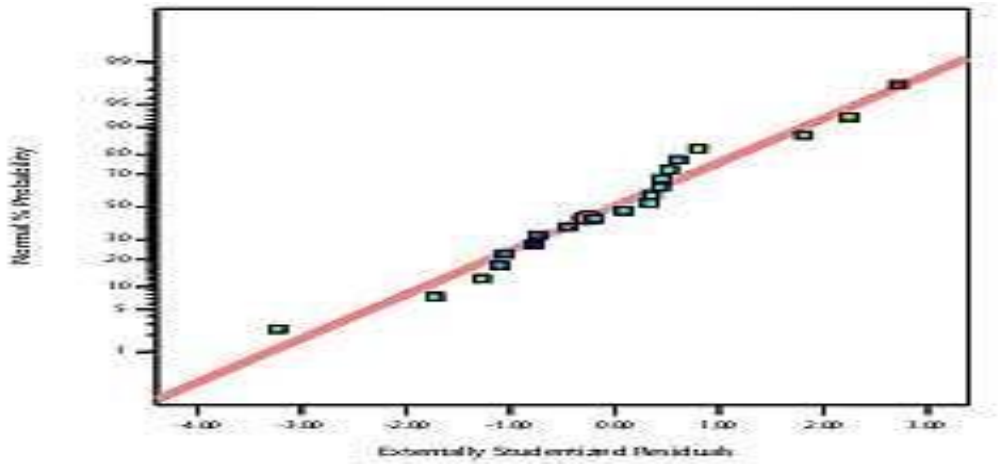

Figure 3: Normal plots of residues of *Cassia fistula* oil

The residuals versus predicted plot in Figure 4 for *Cassia fistula* seed oil yield illustrates the distribution of externally studentized residuals in relation to the model's predicted values. The residuals are scattered randomly around the central horizontal line at zero, indicating that there is no obvious systematic pattern or bias in the predictions. This randomness suggests that the model's assumptions of linearity and constant variance (homoscedasticity) are reasonably satisfied. Most data points fall within the control limits (±4.4157), meaning that the majority of residuals are within acceptable bounds, with no significant outliers. However, a few points deviate slightly toward the upper and lower limits, which could indicate minor variability or slight model inaccuracy at certain predicted values. Overall, the plot confirms that the quadratic model provides a fair fit to the experimental data, with errors distributed evenly across the predicted range of oil yield.

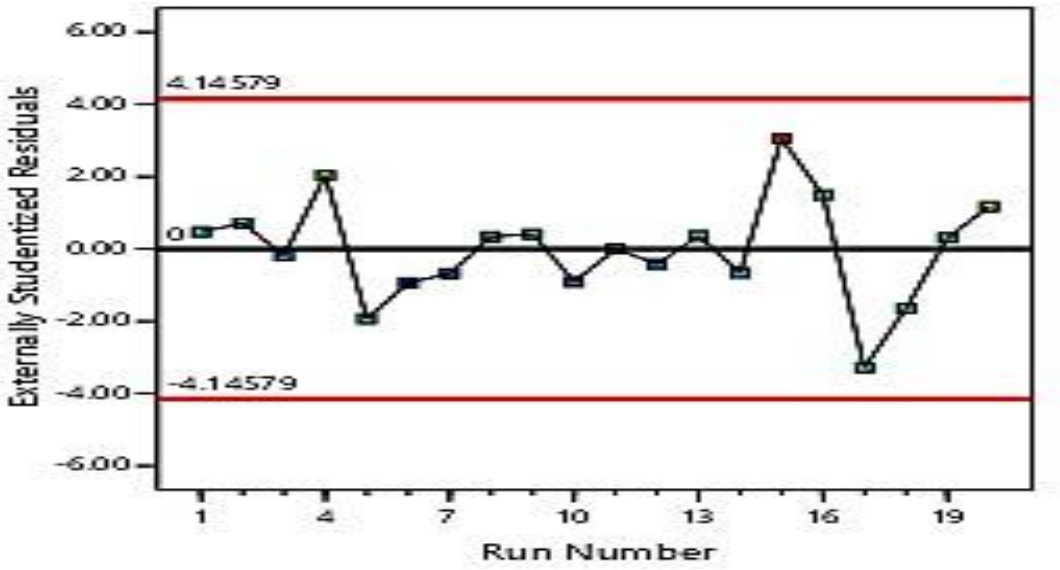

Figure 4: The residuals versus no of runs plot

The yield prediction model on the Cassia fistula seed oil yield

The 3D surface plot in Figure 5 shows the interactive effect of temperature and solid-to-solvent ratio on oil yield**.** The oil yield increases with temperature but decreases as the solid-to-solvent ratio becomes higher. The highest oil yield (about 37.6%) was obtained at a lower solid-to-solvent ratio and higher temperature. This indicates that using more solvent relative to the solid enhances solvent penetration and mass transfer, leading to better extraction efficiency. The curvature of the surface also suggests a nonlinear interaction between both factors, confirming that oil yield depends on the combined influence of temperature and solid-to-solvent ratio.

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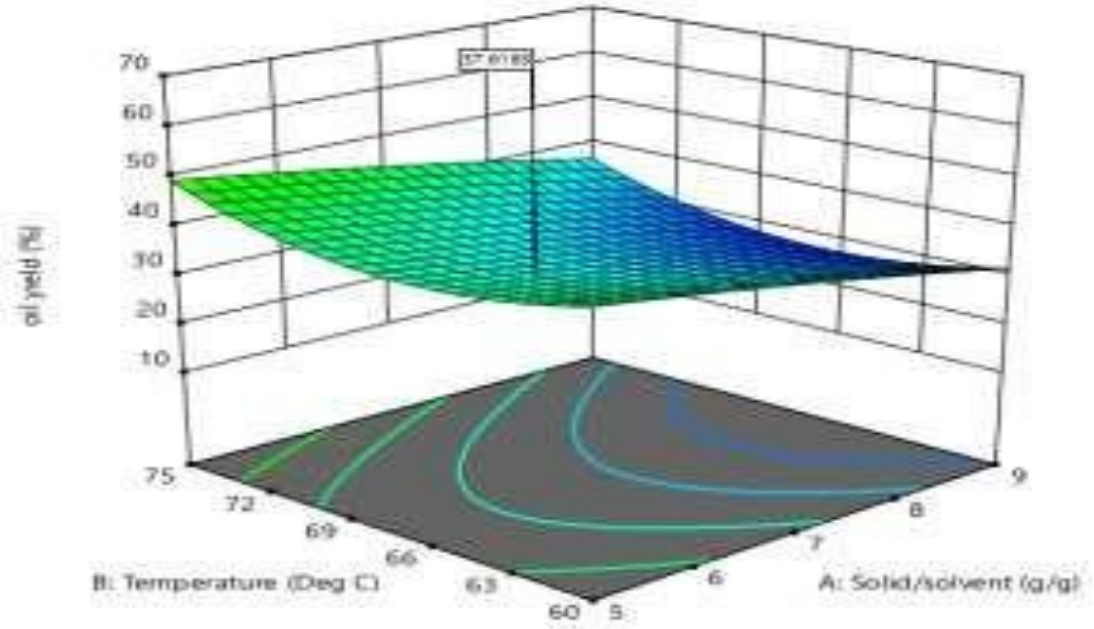

Figure 5:3D plot model on oil predictions

Experimental outcome for the optimum yield on oil, FFA, iodine value, and saponification value as specified by the design expert

At the optimized extraction condition of 7 g solid-to-solvent ratio, 67.5 °C, and 3 hours, *Cassia fistula* seed oil, as shown in Table 8, produced an oil yield of 67.6%**,** indicating efficient solvent penetration and lipid solubilization. Overall, these results show that the chosen extraction conditions favour the recovery of high-quality oil with desirable physicochemical characteristics for industrial use.

Table 8: The experimental confirmation table from the design expert

Extraction parameters			Oil Yield (%)
Solid-to-Solvent Ratio: g	Temperature: °C	Extraction Time: hrs.	
7	67.5	3	67.6

Cassia fistula Oil analysis on Gas Chromatograph Mass Spectrophotometry (GC-MS)

The GC–MS profile of *Cassia fistula* oil in Figure 6 reveals a diverse mixture of fatty acids, esters, aldehydes, hydrocarbons, sterols, and bioactive terpenoids, reflecting the chemical richness of the seed oil. The presence of aldehydes such as 2,4-decadienal and 2,4-nonadienal suggests oxidative aroma-related compounds, while fatty acid derivatives—including methyl esters of hexadecanoic, octadecanoic, and eicosatrienoic acids indicate the oil's lipid backbone and potential nutritional value.

Major saturated and unsaturated fatty acids, such as palmitic acid and cislinoleic acid, contribute to the physicochemical properties and stability of the oil. Notably, the oil contains high levels of sterols and triterpenoids, with fucosterol (29.53%), trans-squalene (19.21%), and various sterol acetates forming the dominant components, highlighting its potential antioxidant, anti-inflammatory, and therapeutic activities. Longchain hydrocarbons like nonacosane, tetracosane, and hexatriacontane further add to the complexity of the profile. Overall, the table demonstrates that *Cassia fistula* oil is composed of a broad range of bioactive and structural molecules, supporting its potential applications in nutraceutical, cosmetic, and medicinal formulations.

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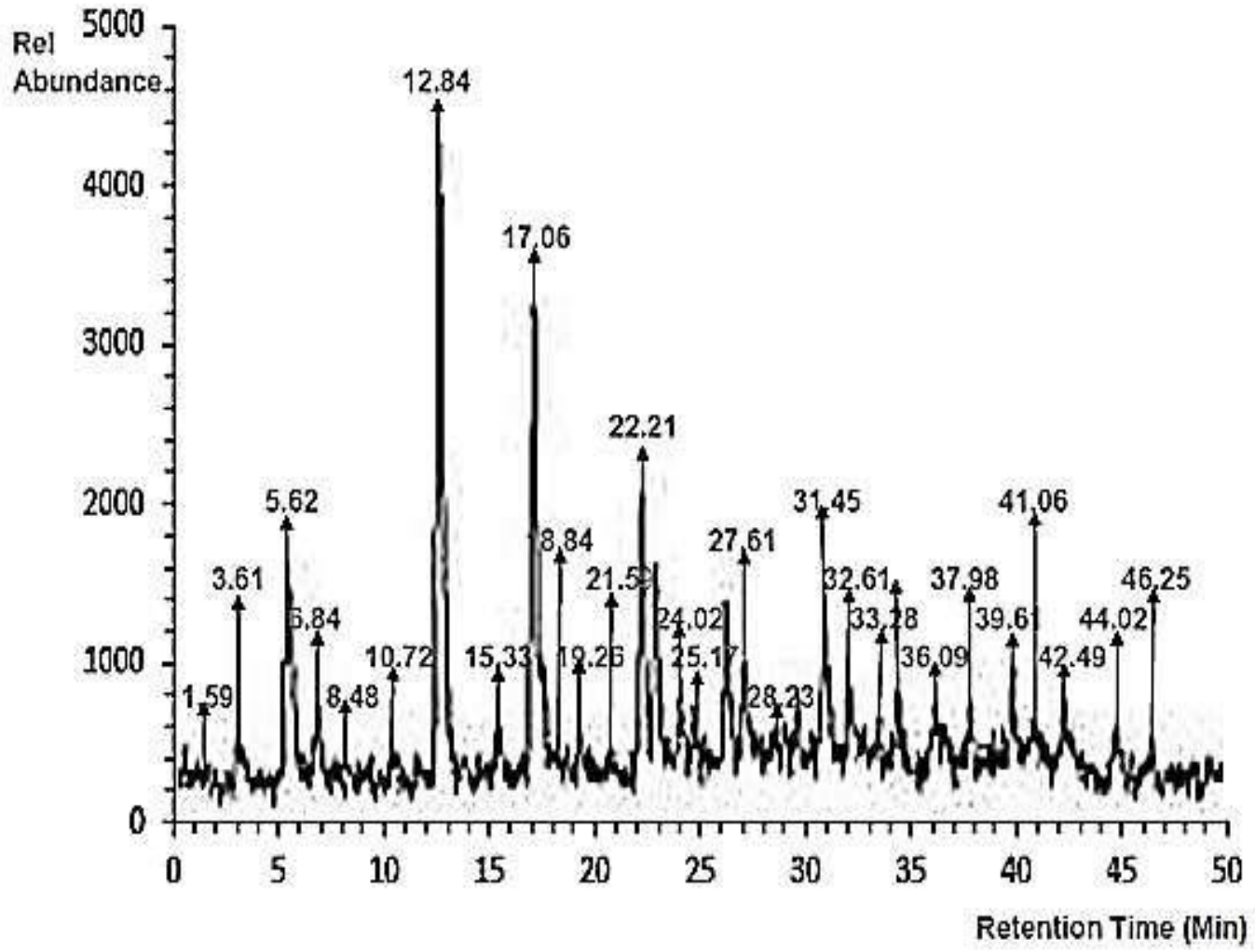

Figure 6: Relative abundance of *Cassia fistula* oil against retention time

Cassia fistula Oil analysis on Fourier Transform Infrared Spectrophotometer (FTIR)

The FTIR spectral data of *Cassia fistula* oil reveals, in Figure 7, the presence of key functional groups that characterize its chemical composition and confirm its lipid-rich nature. The broad peak at 3468.78 cm^{-1} indicates O–H stretching vibrations, suggesting the presence of phenols or alcohols, which may contribute to antioxidant activity. Strong absorptions at 2925.34 cm^{-1} and 2851.29 cm^{-1} correspond to asymmetric and symmetric C–H stretching of alkanes, reflecting the methyl and methylene groups typical of long-chain fatty acids.

The prominent ester-related C=O stretching at 1745.63 cm^{-1} verifies the presence of fatty acid esters, a major component of vegetable oils, while the peak at 1653.49 cm^{-1} suggests additional carbonyl-containing compounds such as ketones, aldehydes, or amides. Bending vibrations at 1455.16 cm^{-1} and 1378.32 cm^{-1} confirm methylene and methyl groups, further supporting the dominance of saturated and unsaturated hydrocarbon chains. Additional C–O stretching at 1231.56 cm^{-1} and 1100 cm^{-1} reflects ester linkages and alcohol or carbohydrate residues.

Finally, the peak at 750.21 cm^{-1} indicates rocking vibrations of long-chain aliphatic compounds, reinforcing the presence of extended hydrocarbon chains. Overall, the FTIR profile confirms that *Cassia fistula* oil is rich in fatty acids, esters, and bioactive organic functional groups.

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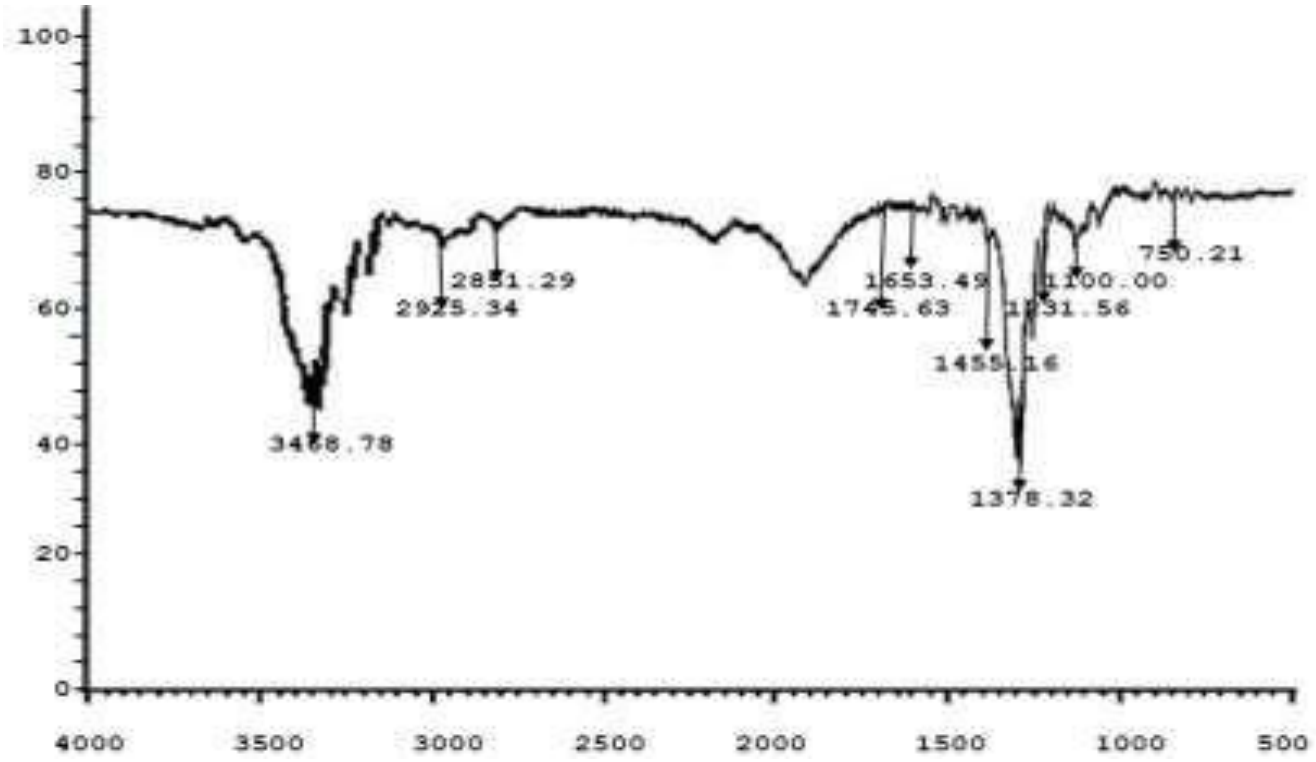

Figure 7: FTIR Spectra for Cassia fistula seed oil

The optimization of *Cassia fistula* seed oil extraction revealed that temperature and solid-to-solvent ratio were the most influential factors affecting both yield and quality, while extraction time played a secondary role. Maximum oil yield (67%) was achieved at a moderate solvent ratio (7:1 g/g) and higher temperature (~80 °C), suggesting that elevated temperatures enhance lipid solubility and mass transfer, whereas excessively long extraction times or very high solvent ratios may lead to thermal degradation or dilution effects, reducing yield. Free fatty acid (FFA) content and iodine values indicated that milder conditions preserved oil quality, with unsaturation maintained in line with literature reports for Fabaceae seeds such as *Cassia tora* and *Senna obtusifolia*, and saponification values were comparable, reflecting similar triglyceride chain lengths. The refinement steps, including degumming, neutralization, bleaching, and deodorization, effectively reduced impurities, FFA, and color pigments, enhancing suitability for edible and cosmetic applications, while the moderate oxidative stability and high calorific value indicate potential for biodiesel use; however, trade-offs were noted as excessive heating during deodorization slightly reduced unsaturation. Limitations include batch-scale extraction variability and the need for solvent recovery for industrial feasibility. For scale-up, optimization of solvent recycling, continuous extraction systems, and controlled thermal conditions would be necessary to maintain oil yield and quality while ensuring economic and environmental sustainability.

CONCLUSIONS

This study demonstrated that *Cassia fistula* seed oil possesses significant potential as a valuable industrial and biofuel resource, with the extraction method playing a crucial role in determining both yield and quality. Among the three methods evaluated, Soxhlet extraction produced the highest oil yield, while cold pressing preserved superior physicochemical stability with lower acid and peroxide values. Optimization using Response Surface Methodology identified temperature and solid-to-solvent ratio as the most influential parameters, with maximum oil recovery achieved at elevated temperature ~80 °C, moderate solvent ratio 7:1 g/g, and 3 hours extraction time. Although higher temperatures enhanced extraction efficiency, excessive processing could affect oil stability, indicating the need for balanced operating conditions. The physicochemical properties, fatty acid profile, GCMS, and FTIR analyses confirmed that the oil contains valuable lipid components suitable for biodiesel, cosmetic, and pharmaceutical applications. Overall, the findings provide a scientific basis for optimizing extraction conditions to maximize yield while maintaining quality, supporting the sustainable valorization of Cassia fistula seed oil for industrial-scale utilization.

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