Utilization of Microwave Energy for Bio-Oil Extraction from *Bauhinia Variegata* Seeds: A Short-Time Technology

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ABSTRACT: *The oil extraction from Bauhinia variegata seeds to produce biodiesel was performed using microwave-assisted extraction. About, 9 combinations of solvents were employed and found that hexane-acetone (80:20 mL) gave a better yield, and the maximum bio-oil yield was found to be 29.03±0.61% at the optimal condition of 9 min of processing time with a supply of 240 W and 12 mL/g of solvent volume. The presence of functional groups in the Bauhinia variegata seed oil was identified using FT-IR analysis and the fatty acid composition was analyzed using GC-FID. The results reveal a predominant fatty acid (C18:2) present in the group was linoleic (about 43.4%). From scanning electron microscopy analysis it was observed that the microwave treatment promoted rapid extraction of oil, as a structure of raw materials was ruptured. The physicochemical properties of the oil were determined as per ASTM D6751 and found that a free fatty acids value of less than one can be directly treated for the transesterification process. The results from this study suggest that MAE is an efficient and eco-friendly method to extract oil from Bauhinia variegata seeds in bio-diesel production.*

KEYWORDS: *Microwave-assisted extraction; Optimization; Bauhinia variegata; Fatty acid composition; Physicochemical properties.*

INTRODUCTION

An exponential increase in industrial development and population has increased energy depletion, eventually creating a drift on hydrocarbon deposits (fossil fuels) which are a major energy source of late [1,2]. However; present studies reveal that the feedstock may last for only the next 100 years if the depletion continues [3, 4]. Thus, the exploitation of these resources cannot be subjected to prolonged usage, since it leads to scarcity and poses a threat to the environment. The utilization of renewable energy resources has been gaining a global thrust for the past few decades [5-7]. As oilseeds play a vital role in an agricultural economy, in recent times, the focus has shifted

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towards it [8]. Also, the credibility of non-edible oilseeds has gained momentum among researchers since there is an increasing demand for non-food resources as feedstock for biofuels [9,10]. The bio-diesel production from oil seeds would be the potential option for alternate fuel production. Generally, the volume of non-edible oilseed plants is enormous and is given much less importance. One such feed is *Bauhinia variegata Linn*. Commonly known as 'Camel's foot or Orchid tree', belongs to the *Leguminosae* family. It is mostly found in tropical countries, frequently used for treating diabetes, infections, pain, inflammation, etc. [11]. *Bauhinia variegata Linn*. is an 8m tall, deciduous tree with dark brown, smooth bark. This plant may serve as a potential source of low-cost proteins. Thus, it can be inferred that *Bauhinia variegata* seeds are a potential source for oil extraction [12,13].

The selection of extraction method with the concern of yield, time, energy, and product quality is still a bottleneck of the process. Numerous techniques have been used to extract oil based on its chemical characteristics of which soxhlet is a common method and it has a huge disadvantage of larger solvent consumption and higher extraction time. Some of the other methods such as ultra-sonication, microwave irradiation, and high-pressure extraction are extensively used nowadays in order to improve their efficiency [14]. A microwave uses electromagnetic wave frequencies (from 300 MHz to 300 GHz) to generate heat energy by penetrating with the molecules in a very shorter time compared to other advanced extraction techniques [15,16]. An increase in temperature above the boiling point leads to the rupture of cell walls. Lipids present in seeds are more susceptible to this radiation due to their low specific heat which ultimately results in higher yield [17,18]. Microwave-Assisted Extraction (MAE) has been found to minimize the time of extraction and requires less energy compared to conventional methods [18,19]. Maximum oil yield, better extraction rate, and obtaining good quality oil make a microwave system is a good alternative [20].

Another important aspect of the extraction processing is "optimization" which consumes a lot of trial experiments in order to find the best combinations of variables to obtain the maximum yield. Response Surface Methodology (RSM) is a mathematical/statistical tool used in recent studies for the optimization process. It is helpful to evaluate the interaction between the process variables on the response as well as it can be used to reduce the number of experiments [10].

The *Bauhinia variegata* is an important sustainable feedstock for producing biodiesel. Therefore, the present work is the first attempt to focus on the utilization of the microwave extraction technique with minimum solvent usage and shorter extraction time for the production of bio-oil from naturally available non-edible seed material *Bauhinia variegate* seeds. In addition to that, the MAE process variables were optimized using RSM and interaction effects have also been studied.

EXPERIMENTAL SECTION

Sample preparation

Fresh *Bauhinia variegata* seeds were collected locally from Chennai, India. Seeds are then cleaned thoroughly with water to separate the dust adhering to them. The seeds are dried in a hot air oven and the temperature was maintained at 50°C until getting constant weight then it was pulverized. The crushed seeds are meshed (No.35-ASTM) to obtain an even powder which is then used for microwave exaction and analysis. All chemicals and reagents used for this study are of analytical reagent (AR) grade procured from Sigma Aldrich - Merck, Bangalore, India.

Microwave-Assisted Extraction (MAE)

Microwave extraction was performed using a reactor that has power ranging from 0 to 800 W at a constant frequency of 2.45 GHz. The solid matrix was mixed with corresponding solvents and was irradiated in a microwave reactor in a batch mode. The extraction process was carried out under various conditions of time ranging from 90 to 300 seconds, power from 150 W to 180 W, and solvent solid ratio of 90:10, 80:20, and 70:30 mL concentration. After each irradiation, the sample is allowed to cool at room temperature. The samples were filtered with Whatman No. 1 filter paper and were stored for further analysis. After filtration, the solvent and oil sample were fed into a rotary evaporator which separates oil and solvent under vacuum conditions. The schematic representation of the microwave extractor is shown in Fig. 1.

Characterization

The fatty acid composition of bio-oil was determined using Gas Chromatography (TRACE-1110, Thermo Scientific

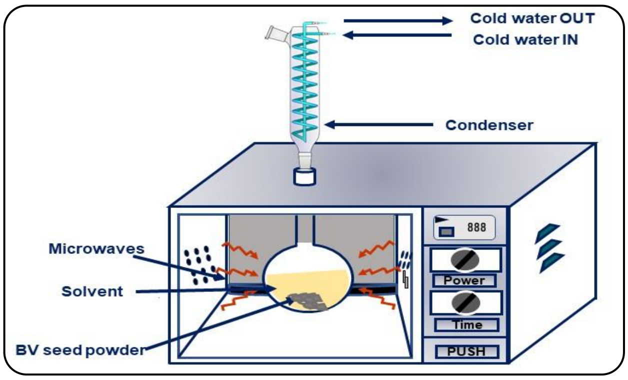

Fig. 1: The schematic representation of the microwave extractor

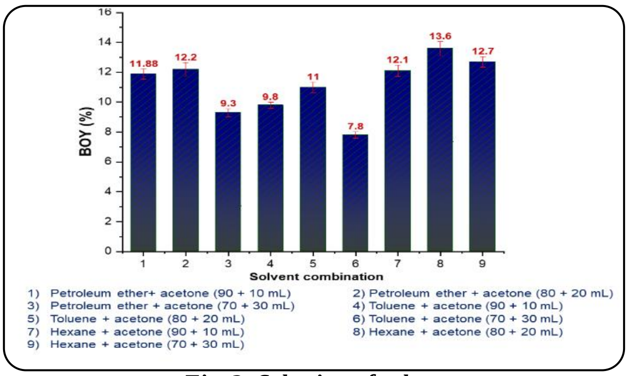

Fig. 2: Selection of solvent

and India) whereas Flame Ionization Detector (FID) was used and the standard was procured from Sigma Aldrich - Merck. The HP-88 capillary column was used with dimensions of 100 m length × 0.2 µm width × 0.25 mm ID. The temperature of the oven program was maintained as 75°C/5 min, 210 °C/10 min, and 250°C/15 min. Nitrogen gas was used as a carrier gas for the vapor phase of the oil. The presence of a functional group in the oil was examined using an FT-IR spectrophotometer (Jasco 6600, Japan). The impact of the microwave on the solid surface and the untreated sample was analyzed using SEM analysis with a model of the Tescan Vega3-SBU (CZECH Republic country) scanning system. Physicochemical characteristics of oil were determined as per ASTM standard methods.

Response surface methodology

The software Design–Expert (version. 13.0.5.0) was used for Bio-Oil Yield (BOY %) from *Bauhinia variegata* seeds experimental design, data analysis, and predictive model development. The second-order empirical model with individual and interaction terms of the process variables was developed as a polynomial equation and it is given below (Eq. (1)): [21,22].

$$Y=\beta_0+\sum_{i=1}^{k}\beta_i X_i+\sum_{i=1}^{k}\beta_{ii} X_i^2+\sum_{i<j}^{k}\sum_{i=1}^{k}\beta_{ij}X_iX_j+\varepsilon \qquad (1)$$

Where Y denotes the response (BOY %); β_0 denotes the model intercept coefficient; β_j, β_{ii}, *and* β_{ij} the interaction coefficients of linear, quadratic, and second-order terms, respectively; X_i and X_j denote independent variables [23, 24].

RESULTS AND DISCUSSION

Solvent selection

In order to identify a suitable solvent for maximum oil recovery, different solvent combinations were tried in this study. Vegetable oils that are extracted from oil-bearing seeds and/or fruits are generally considered non-polar (lipophilic systems). Non-polar molecules are inert to dielectric loss. This nature of the non-polarity of the seeds is the reason behind its high flash point and a discerning dissolving power with non-polar solvents [25]. The selection of solvent is of utmost importance for the MAE process. The extraction solvent in MAE cannot be selected simply based on conventional extraction. The solvent should be selected in such a way that it has the ability to absorb microwave energy so that the extraction process becomes more effective [26]. Polar molecules absorb microwaves since an effective microwave absorption result in a higher boiling point called the “superheating effect” [27].

Low boiling temperature and low corrosive nature make hexane, the most predominantly used solvent in oil extraction. Other non-polar solvents such as petroleum ether, pentane, toluene, ethyl acetate, and cyclohexane are also used in vegetable oil extraction. Polar solvent absorbs the microwave energy and non-polar solvents dissolve with oil; hence extraction occurs with higher efficiency. Hence, the mixture of polar and non-polar solvents can be effective in the MAE process to obtain a better yield [28]. The present study aimed to evaluate the effectiveness of solvents, namely hexane, toluene, petroleum ether, and along with acetone in different ratios studied using MAE. To obtain maximum BOY (%) various solvent combinations were carried out at microwave power of 180 W for 5 min time with 50 mL solvent volume per 5 g of sample weight (5:1 ratio) and their results are shown in Fig. 2. It can be seen that the maximum BOY of 13.6% was obtained with the solvent of hexane + acetone (80 + 20 mL)

Table 1: Experimental and predicted data of BOY (%)

S. No	Type	Microwave power (W)	Time (min)	Solvent volume (mL/g)	Bio-oil yield (%)		
		(X_1)	(X_2)	(X_3)	Y_{Act}	Y_{Pre}	Residual
1	Center	240 (0)	10 (0)	10 (0)	26.77	26.53	0.24
2	Factorial	300 (1)	10 (0)	15 (1)	24.13	24.58	-0.45
3	Factorial	240 (0)	15 (1)	15 (1)	23.76	23.51	0.25
4	Factorial	300 (1)	5 (-1)	10 (0)	17.43	17.13	0.30
5	Factorial	240 (0)	15 (1)	5 (-1)	12.09	12.24	-0.15
6	Factorial	300 (1)	15 (1)	10 (0)	20.34	20.14	0.20
7	Factorial	240 (0)	5 (-1)	5 (-1)	10.22	10.47	-0.25
8	Center	240 (0)	10 (0)	10 (0)	27.01	26.53	0.48
9	Center	240 (0)	10 (0)	10 (0)	26.23	26.53	-0.30
10	Factorial	180 (-1)	10 (0)	5 (-1)	13.61	13.16	0.45
11	Center	240 (0)	10 (0)	10 (0)	25.98	26.53	-0.55
12	Factorial	180 (-1)	5 (-1)	10 (0)	15.32	15.52	-0.20
13	Factorial	240 (0)	5 (-1)	15 (1)	19.17	19.02	0.15
14	Factorial	300 (1)	10 (0)	5 (-1)	14.29	14.34	-0.05
15	Factorial	180 (-1)	10 (0)	15 (1)	22.81	22.76	0.05
16	Factorial	180 (-1)	15 (1)	10 (0)	18.46	18.76	-0.30
17	Center	240 (0)	10 (0)	10 (0)	26.68	26.53	0.15

compared to other combinations. This could be due to the solubility of vegetable oil in hexane as well as the absorbing microwave energy of acetone. The combination of all non-polar and polar solvents (80:20) gives better BOY was observed. It can be inferred that the ratio might affect the oil yield since an excess amount of polar solvent (above 20 mL of acetone) absorbs more energy. Moreover, the actone's dielectric constant value of 21.01 gives better microwave absorption to enhance the rapid heat generation inside the reactor. The critical part of the process is to maintain the acetone volume to absorb the microwave energy and the hexane volume to solubilize the oil present in the solid matrix. However, an insufficient volume of a non-polar solvent (below 80 mL *n*-hexane) may not be able to solubilize the maximum amount of oil which ultimately leads to lower BOY (%). Hence, further studies of MAE optimization were carried out with a solvent ratio of *n*-hexane + acetone (80+20 mL).

RSM experiments of model fitting

In the present study, three factors and three levels of Box–Behnken Designs (BBD) were employed to produce maximum BOY (%) from *Bauhinia variegata* seeds. The different experiment combinations (17 runs) and their results are given in Table 1.

Analysis of variance (ANOVA) was used to analyze the adequacy and fitness of the predictive model. The second-order polynomial equation of the predicted model with regression coefficient coded variables is given in Eq. (2). Results of ANOVA show (Table 2) that a high F-value (261.11) and lower *p*-value (less than 0.0001) exhibited the developed model was highly significant. R^2adj values (0.9932) were found to be equal to R^2 (0.9970). A better correlation between the experimental and predicted values was revealed through the very high value of the correlation coefficient [29,30].

$$\text{BOY (\%)} = +26.53 + 0.7487X_1 + 1.56\,X_2 + 4.96\,X_3 - 0.057\,X_1X_2 + 0.16\,X_2X_3 + 0.68\,X_2\,X_3 - 3.12\,X_1^2 - 5.52\,X_2^2 - 4.70\,X_3^2 \quad (2)$$

The lack of fit and its associated *p*-value (0.3283) was not significant which showed the fitness of the proposed statistical models (Table 2).

The Coefficient of Variance (CV) is the ratio of the standard value of model prediction error to an average value of the experimental values and <10 % ascribed

Table 2: Analysis of Variance (ANOVA)

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remarks
Model	513.97	9	57.10	261.11	< 0.0001**	significant
X_1-Microwave power	4.48	1	4.48	20.50	0.0027*	
X_2-Time	19.56	1	19.56	89.44	< 0.0001**	
X_3-Solvent to solid ratio	196.61	1	196.61	898.95	< 0.0001**	
$X_1 X_2$	0.013	1	0.013	0.060	0.8128 ^{ns}	
$X_1 X_3$	0.10	1	0.1024	0.468	0.5158 ^{ns}	
$X_2 X_3$	1.84	1	1.8496	8.456	0.0227*	
X_1^2	41.07	1	41.07	187.79	< 0.0001**	
X_2^2	128.44	1	128.44	587.28	< 0.0001**	
X_3^2	93.04	1	93.04	425.39	< 0.0001**	
Residual	1.530	7	0.21			
Lack of Fit	0.828	3	0.27	1.57	0.3283^{ns}	not significant
Pure Error	0.70	4	0.17			
Cor Total	515.50	16				
Std. Dev.	0.47			R^2	0.9970	
Mean	20.25			Adj R^2	0.9932	
C.V. %	2.31			Pred R^2	0.9722	
PRESS	14.35			Adeq.Pre	44.789	

**- highly significant, *- significant, ^{ns} – not significant.

To better reproducible models. The C.V value (2.31) of the present study showed the good reproducibility of a regression model. Greater than 4 of adequate precision is a good signaling impact on the model, the obtained value of 44 shows better signaling effects [29,31].

Influence of MAE variables on BOY (%)

The independent variable influence on the BOY (%) was studied using 3D surface plots (Fig. 3). Higher microwave radiation leads to rising in temperature in the extraction process by allowing the oil to diffuse out and dissolve in the solvent. Since power can be measured by the amount of energy transmitted per unit of time.

An increase in microwave power usually raises the heat, resulting in a higher BOY (%) (Shown in Fig. 3a). So, when the power is higher, larger will be the yield of oil [32,33]. When the microwave power was raised from 180 W to 240 W, the BOY (%) increased from 13.61 to 23.76 %, and BOY (%) yield started to decrease 17.43% at 300 W since a high microwave power caused solvent loss also, increasing microwave radiation power causes the extraction system to overheat, causing the solvent to evaporate [28] (shown in Fig. 3a). Extraction time is an important factor influencing the extraction yield. The production cost depends on the processing time is long and exposure to microwave radiation [34]. The influence of extraction time on BOY (%) is shown in Fig 3a,c. and is observed that when the extraction time is increased from 5 to 15 minutes, the BOY (%) increases rapidly due to the rapid dissolution of oil solutes emanating from the surface. When the cells are ruptured, their porosity increases and more oil is brought to the cell surface. Hence the overall rate of extraction could be enhanced [27]. The highest BOY (%) was 26.77 ± 0.16%, which was observed at 10 min. After that, the oil yield remained constant. The solvent volume plays a vital role in the extraction of oil. Fig. 3b,c shows the BOY (%) using various solvent-to-solid ratios from 5:1 to 15:1 mL/g. The BOY (%) was found to be the highest (27.01 %) with a solvent-to-solid ratio of 10:1 mL/g and as the solvent-to-solid ratio was increased it remained constant. This is because, a certain amount of solvent is desirable for oil extraction, and excessive solvent might result in higher costs [35]. Moreover, washing with fresh solvent created a high concentration gradient preventing the attainment of equilibrium and facilitating a higher extraction rate. The experimental and predicted values were highly significant and there was not much residual error obtained (Fig. 3d).

Optimization

MAE from *Bauhinia variegata* seeds was determined using numerical optimization for maximum BOY (%) and the optimal extraction conditions are found to be: 8.6

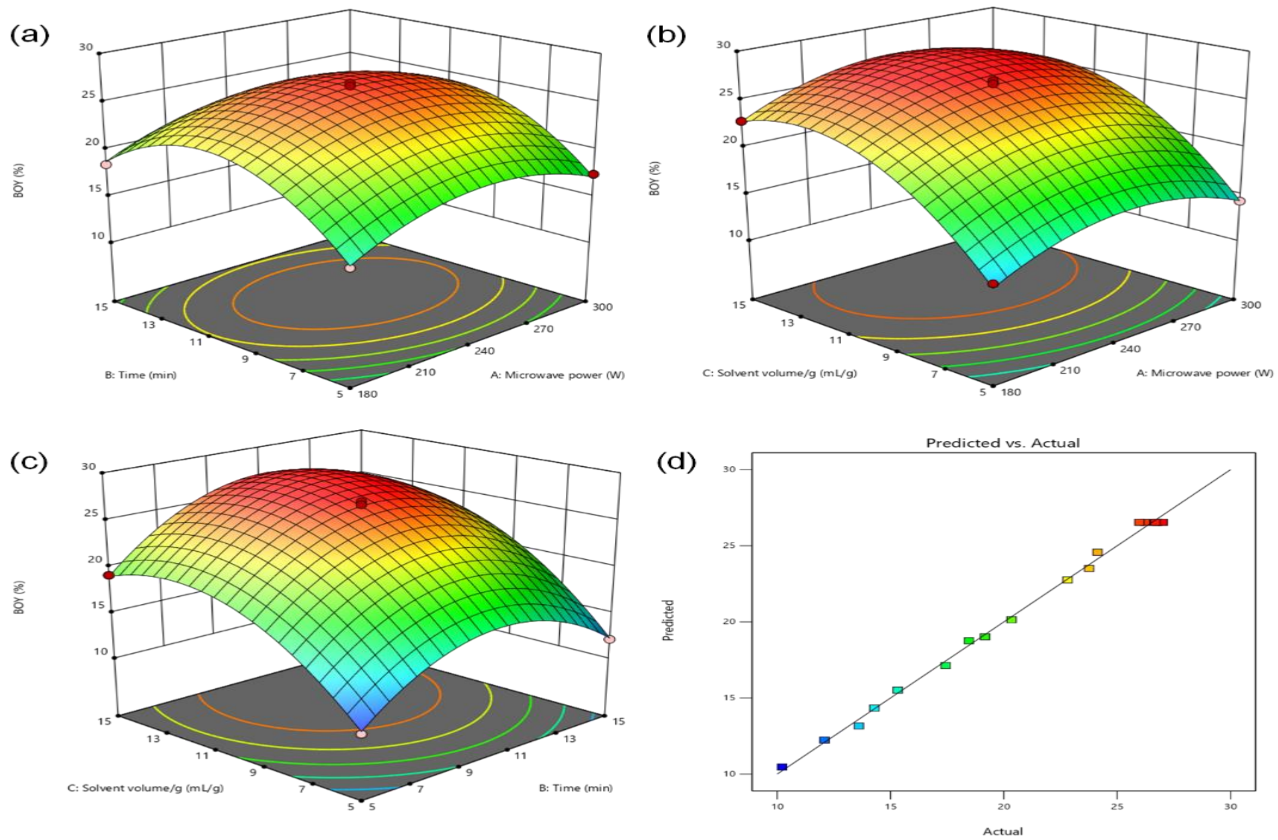

Fig. 3: Influence of MAE process variables on BOY (%)

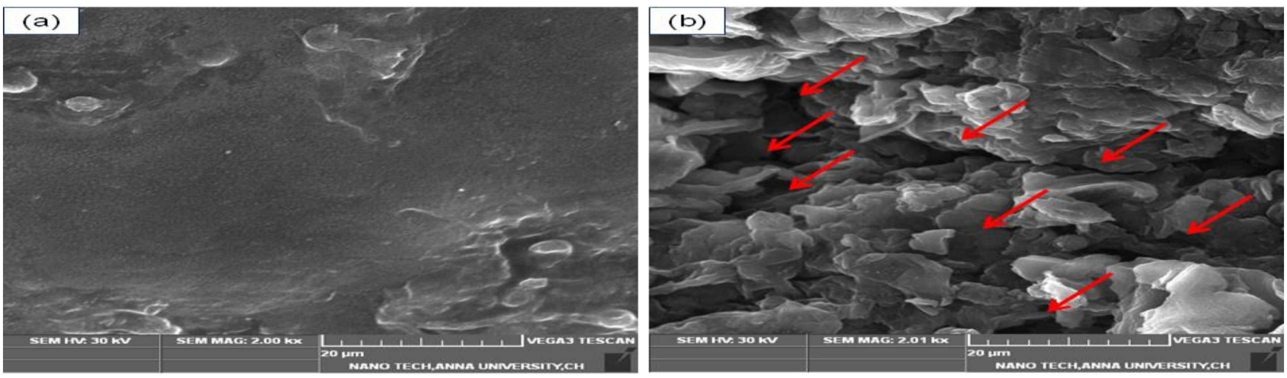

Fig. 4: SEM images of (a) raw material (b) MAE-treated sample of Bauhinia variegata seed

min of time with 238 W power and 11.5 mL/g solvent volume. Under the optimum condition, the obtained predictive yield was 30.45 % with a desirability value of 0.9877. For the operability of experimental values, the optimal condition was slightly modified to 9 min of processing time with a supply of 240 W and 12 mL/g of solvent volume. The experiments were carried out in triplicates and the maximum BOY was found to be 29.03±0.61 %.

The experimental value was well in agreement with RSM's predicted value with high desirability (>0.9) which is close to 1. The present study results are compared with previous oil extraction from *Bauhinia variegata* seeds and it is shown in Table 3. It can be seen that the present study results gave better BOY % with a shorter duration of time at minimum solvent usage. This phenomenon was clearly shown in SEM images of untreated and treated solid matrix (shown in Fig. 4). Fig. 4a shows SEM images of raw

Table 3: Previous results of BVO extraction

S. No	Extraction method and condition	Yield (%)	Reference
1	Soxhlet extraction Condition Solvent: n-hexane	16	[36]
2	Soxhlet extraction Condition Solvent: n-hexane Time: 6 h	18	[13,17]
3	Soxhlet extraction Condition Solvent: n-hexane Time: 4.5 h Temperature: 68ºC	28.43	[9]
4	Soxhlet extraction Condition Solvent: n-hexane Time: 4 h Temperature: 70ºC	24	[37]
5	MAE Condition Solvent: n-hexane+acetone Power: 240 W Time: 9 min Solvent to solid ratio: 12 mL/ g	29.03	Present study

Table 4: Physico-chemical properties of BVO

S. No	Properties	ASTM Standard	Value
1	Kinematic viscosity (mm^2/s)	ASTM D445	30.2
2	Density (kg/m^3)	ASTM D4052	896
3	Acid value (mg KOH/g)	ASTM D1980	1.12
4	Free fatty acids (% mass)	ASTM D5555	0.56
4	Calorific value (kJ/kg)	ASTM D240	38521
5	Flash point (ºC)	ASTM D93	204
6	Cloud point (ºC)	ASTM D2500	5
7	Pour point(ºC)	ASTM D97	7
8	Ash content (%)	ASTM D874	0.014
9	Carbon residue (%)	ASTM D4530	1.0

material where the surface is found to be smooth before the extraction process and Fig. 4b shows SEM images of a treated sample where the surface is ruptured and porous. This clearly indicates that the probability of penetration solvent is higher which results in higher oil yield in a shorter time (10 min).

Physico-chemical properties

The physicochemical properties of BVO are determined as per ASTM standards and shown in Table 4. The main important parameters in oil are acid value and free fatty acids which determine whether the oil can treat the transesterification process. The free fatty acid value and acid value have to be less than one and two, respectively for an efficient transesterification process. Otherwise, the reaction creates a lot of soap formation rather than methyl esters [33]. The present study's BVO value of free fatty acids (0.56) and acid number (1.12) clearly shows that BVO can be treated for the transesterification process.

The other properties such as viscosity (30.2 mm^2/s) and thermal properties such as flash (204 ºC), cloud (5ºC)

Table 5: Bauhinia variegata seed oil fatty acid composition

S. No	Fatty acids	Composition (%)			
		Present study	[28][a]	[36][b]	[37][c]
1	Palmitic acid C16:0	22.7	22.1	19.52	21.36
2	Palmitolic acid C16:1	0. 1	0.4	-	-
3	Stearic acid C18:0	18.0	17.5	16.95	12.22
4	Oleic acid C18:1 *cis9*	14.0	13.4	26.14	13.52
5	Oleic acid C18:1 cis7	0.2	0.5	-	-
6	Linoleic acidC18:2	43.4	42.1	36.84	51.56
7	Linolenic acid C18:3 n-3	0.1	0.6	0.55	0.46
8	Linolenic acid C18:3 n-6	-	0.5	-	-
9	Archidic acid C20:0	1.0	1.3	-	0.88
10	Behenic acid C22:0	0.2	0.5	-	-
11	Neronic acid C24:1	0.2	0.6	-	-

[a,b,c]- soxhlet extraction using *n*-hexane.

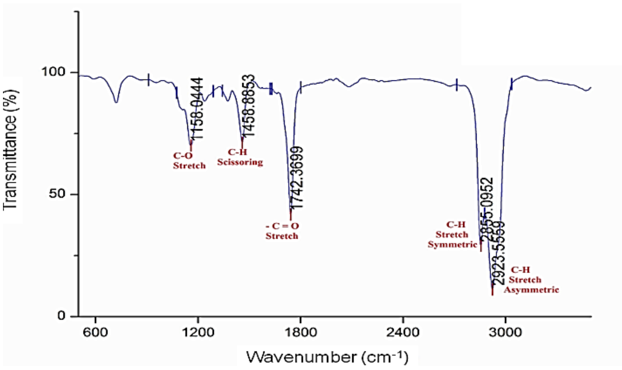

Fig. 5: FTIR spectra of Bauhinia variegata seed oil

and pour point (7 ºC) need to be improved *via* transesterification to meet the biodiesel specification (ASTM D6751).

FT-IR Analysis

The FT-IR analysis was carried out to identify the functional groups present in the *Bauhinia variegata* seed oil and it is shown in Fig. 5. The peak of 2917 cm^{-1} indicates the presence of alkane groups and the peak of 2884 cm^{-1} suggests the presence of C=O antisymmetric stretch of methylene groups in the ester's chain of lipid molecules. The same bandgap was observed in *Swietenia macrophylla* seed oil [34] and *Maesopsis eminii* seed oil [38], which are potential sources of biodiesel production. The spectrum showed that a strong peak of 1741 cm^{-1} indicating the ester carbonyl (C=O) which is present in the ester groups. The peaks of 1461 cm^{-1} and 1307 cm^{-1} were an indication of CH_2 and CH_3 asymmetric bending respectively. In addition to that, the peaks of 1135 cm^{-1} and 1112 cm^{-1} exhibited the ester group of C-O stretch [38,39].

GC- FID Analysis

The fatty acid composition of *Bauhinia variegata* seed oil was analyzed using GC-FID and the results are shown in Fig. 6 and Table 5. The standard FAME was procured from Sigma-Aldrich, Bangalore, India. The predominant fatty acid (C18:2) present in the group was linoleic (about 43.4%). *Bauhinia variegata* seed oil carbon chain length can be observed from C16 to C24. The high content of monounsaturated fatty acid in the oil is highly preferable for biodiesel production. This leads to better storage stability in fuel characteristics [40,41]. In addition, higher monounsaturated content in the oil helps in better flow properties and oxidative stability of the fuel. In the transesterification process, this linoleic acid could be converted into linoleic methyl ester as a FAME component. Other minor fatty acids such as palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1 *cis9),* and arachidic acid (C20:0) are 22.7%, 18.0%, 14.0%, and 1.0%, respectively.

This fatty acid composition would be better performance in FAME production from oil [36,37]. The present study results were compared with previous studies and found a slight variation in fatty acid composition through a similar extraction method and solvent. This could be due to seed collected from different geographical locations, processing time, and solvent volume utilized for the extraction.

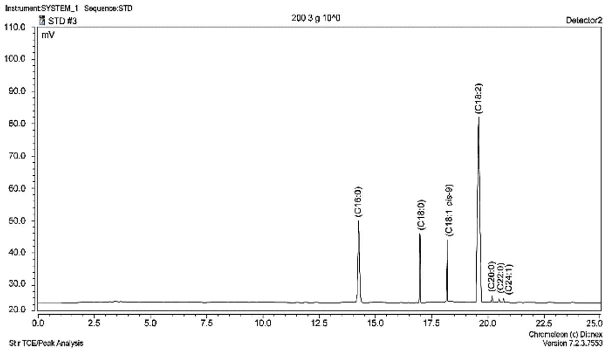

Fig. 6: GC-chromatogram of Bauhinia variegata seed oil

CONCLUSIONS

In the present study, bio-oil was extracted from *Bauhinia variegata* seeds using microwave energy for biodiesel production. The crucial point of solvent selection for MAE was carried out and *n*-hexane+actone combination gave a better yield in out of 9 combinations. The optimum condition was determined using response surface methodology and the maximum BOY was 29.03% at 8.6 min of time with 238 W power and 12 mL/g solvent volume. From the GC-FID analysis linoleic (C18:2) was found to be the predominant fatty acid about 43.4%. Characterization of bio-oil has shown a better resource for biodiesel production. Moreover, the shorter oil extraction time provides an energy and time-saving process which directly implies the production cost of bio-oil and biodiesel production.

Nomenclature

ANOVA	Analysis of variance (ANOVA)
ASTM	American Society for Testing and Materials
BOY	Bio-Oil Yield
BVO	*Bauhinia variegata* oil
CV	Coefficient of Variance (CV)
FAME	Fatty acid methyl esters
FTIR	Fourier transform infrared spectroscopy
GC	FID - Gas Chromatography/Flame Ionization Detector
MAE	Microwave-assisted extraction
RSM	Response Surface Methodology
SEM	Scanning Electron Microscope

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