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Synthesis of *Persea Americana* Bio-Oil and Its Spectroscopic Characterization Studies

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ABSTRACT

The present investigation aims to evaluate the feasibility of using *Persea americana* (Avocado) biodiesel in compression ignition engines. *Persea americana* bio-oil was extracted through a soxhlet extraction process using *n*-hexane solvent after careful pre-processing of the feedstocks. Since the Free Fatty Acid content was 1.78% estimated through titration, single stage base-catalyzed transesterification technique was adopted using methanol and sodium hydroxide as catalysts in the molar ratio of 1:6. Gas Chromatography-Mass Spectrometry analysis revealed the presence of Oleic acid in major proportions. The Fourier transform Infra-Red analysis confirmed the presence of carbonyl group ester ions between 722.19 cm^{-1} and 1460 cm^{-1}. The ^{13}C NMR and ^{1}H NMR studies supported the successful transformation of triglycerides into Fatty Acid Methyl Esters with distinct peaks at 3.369 ppm and 48.147 ppm, respectively.

INTRODUCTION

The continuously rising human population and limited fossil fuel availability create an everlasting demand for energy supply. Depletion of petroleum reserves and upsurging transportation and Industrial pollution unveiled the need for renewable energy resources. Agricultural-based non-edible liquid fuel was one of the important alternatives to encounter the diminishing "Petro" products and the global environment and economic concerns. In European and American nations, the dependency on fossil fuel was greatly reduced by substituting it as the fuel source for animal fat and vegetable oil. Bio-diesel, a fuel source encompassing renewability, biodegradability, and non-toxicity, proved an eco-friendly substitute for "petrodiesel."

Literature report that bio-diesel-based fuel in compression ignition engine produces lesser and unborn hydrocarbons, carbon monoxide, and particulate matter comparatively. Few researchers reported higher levels of carbon dioxide emission. Still, its effects are minimized by the floral photosynthetic reactions at the ground levels. Thereby its effect on greenhouse gas is curtailed. The avocado fruit (*Persea americana*) is a native of Central America, found abundantly in Indonesia. It belongs to the order of Laurales, Lauraceae family, and the genes of *Persea*. The flesh of this fruit is highly nutritious and possesses a pleasant smell and flavor. The pulpy flesh of this fruit contains minerals and nutrients of human absorbable nature, which controls the blood's cholesterol level, thereby preventing cardio-vascular malfunctioning. The seeds of *Persea americana* is considered to be agriculturally based, which were found to have promising lipids and can be extracted by approximate methods. The processing of *Persea americana* seeds involves almost care, and suitable lipid extraction procedures like ultrasonication, enzymatic extraction, or solvent extraction method can be applied.

The transformation of *Persea americana* bio-oil into its biodiesel can be accomplished by many proven methods, including thermal cracking, catalytic and non-catalytic transesterification, biochemical fermentation, and others for converting the mono alkyl tri-glycerides into its fatty acid methyl esters (Macro et al. 2014). The standardization and quantification of the derived bio-diesel from non-edible feedstocks can be achieved by a series of spectroscopic studies like Gas Chromatography-Mass Spectrometry (GCMS), Fourier Transform Infrared Spectroscopy (FT-IR) and Nuclear Magnetic Resonance (NMR) studies. The literature reported numerous studies on the extraction and characterization of vegetable-based bio-oil, but fewer studies were reported on its standardization, quality, and authenticity.

Biodiesel from *Persea grastissima* was produced through a single-stage-based catalyzed transesterification process. Sodium Hydroxide was used as a catalyst at a major ratio of 1:6, with the reaction temperature and reaction time being 60^{o}C and 60 min, respectively. The transesterification efficiency was 84.56% (Rachimoellah et al. 2009). A comparative study in the transesterification process of Avocado and sesame seed oil and standardized them using Gas Chromatography and Mass Spectrometry. It was noticed that *Persea americana* seed oil contains a major proportional mono-unsaturated fatty acid in prominent quantity (Marwa et al. 2017). A hybrid methodology (Gas Chromatography Mass Spectrometry/ modified QuEcHERS) mass spectrometric analysis was employed on various edible and non-edible oils to identify the presence of prominent fatty acid methyl esters (Xiao et al. 2022, Hariram et al. 2017).

A non-invasive Fourier Transform Infrared Spectroscopy on Avocado fruit, including seeds, was performed practically. Lipid water and carbohydrates were estimated on the entire dry mass percentage of the *Persea americana* (Wedding et al. 2013). Estimating the presence of trans-fat and lipid profiles using FTIR and GC-FID studies with nitrogen-inert gas was performed. It was noticed that the cooking oil consisted relatively higher percentage of lipids than *Persea americana* seeds and other non-edible vegetable feedstocks (Sherazi et al. 2009). Detection and quantifying the presence of corn and soybean oil trends in *Persea americana* seed oil employing the multivariate calibration of Fourier Transform mid Infrared spectroscopic analysis with the root means the square value of soyabean oil and corn oil as 0.52% (V/V) and 0.2% (V/V) in the raw oil of *Persea americana* was estimated and was found to be accurate and deformation of authenticity (Fajar et al. 2015). A comparative Chemometric NMR analysis of *Persea americana* bio-oil with kennel, safflower, and olive oils was conducted to identify lipids, hydrolysis products, oxidation products, and steer oils. They have applied a novel NaOH super sequencing methodology in the traditional two-dimensional Nuclear Magnetic Resonance studies (Fenfen et al. 2021). A Chemometric analysis combined with compact, low field Nuclear Magnetic Resonance spectroscopy on *Persea americana,* grape seed, sesame, walnut, corn, linseed, and soybean bio-oils was conducted. The fatty acid methyl esters are estimated and compared with each other. The multivariate approach to understand analytical and statistical parameters on NMR outcomes with each other (Diego et al. 2021).

Most literature has concentrated on the chemometric analysis of *Persea americana* bio-oil. The objective of the present investigation is to evaluate the potential of *Persea americana* bio-oil and its bio-diesel to be used as the fuel for compression Ignition engines. Further, spectroscopic studies like Fourier Transform Infrared spectroscopy, Nuclear Magnetic Resonance Spectroscopy, and Gas Chromatography-Mass Spectroscopy leverage it's suitable to be used along with need diesel in the blended form without making any modification in the Compression Ignition engine.

Persea Americana Bio-Diesel Extraction

The raw *Persea americana* fruits were procured locally in Padur, Chennai, and Tamil Nadu, and seeds were removed from the flesh. The seeds were sundried for 72 hours, and the outer covers were carefully removed. The remaining seats were kept in a hot oven at 70 °C for four hours and later shuddered into small pieces, as shown in Fig. 1. For further process, the shuddered seed was powder in an automated motor and piston arrangement to make it into a powder form. Solvent bio-oil extraction techniques using Soxhlet apparatus as shown in Fig. 1. Fifty grams of dry *Persea americana* seed powder was filled in a suitable arrangement of the Soxhlet apparatus 200mL of n-hexane solvent was placed in the boiling flask as the extraction solvent upon heating the round bottom flask of the Soxhlet apparatus to 90 degrees Celsius the *n-hexane* solvent is vaporized. It occupies the upper condensation chamber of the Soxhlet apparatus, where the n-hexene solvent condenses and drops down into the extraction chamber and reacts with the thimble containing *Persea americana* seed powder.

At an elevated temperature above 45 to 50 degrees Celsius, the *n-hexane* solvent reacts with the cell wall membrane of the powder seeds. Thus, the remaining lipid content from the Persea americana seeds further drops into the solvent along with *n-hexane*. Currently, the boiling flask contains a magnet inducement of *n-hexane* and *Persea americana* seed oil. Further and contained heating, the boiling flask of the Soxhlet apparatus vaporizes the n-hexane solvent alone due to its lower boiling point, and the entire cycle is repeated thereafter. By employing the extraction methodology, 475mL *Persea americana* bio-oil was obtained for 27 batch cycles at a % extraction efficiency of 37.75%.

Persea americana Bio-Oil Transesterification

The free fatty acid content of *Persea americana* bio-oil was estimated by the titration process with potassium hydroxide and phenylethylene indicator, which was found to be 1.78%.

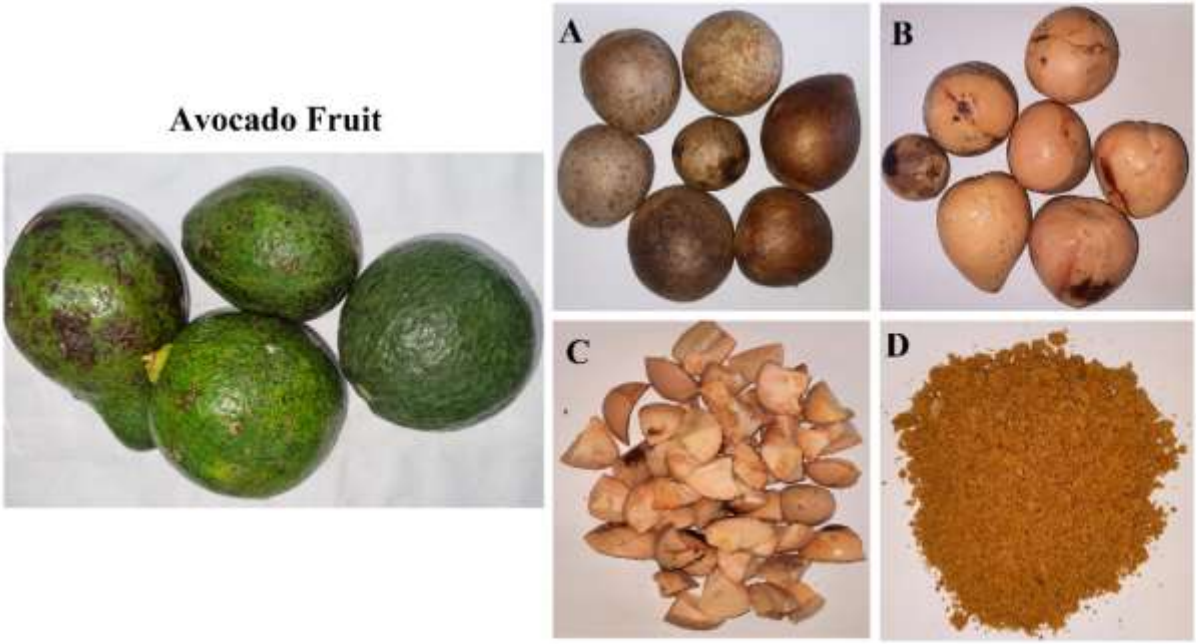

Fig. 1: *Persea americana* Fruit, Seeds (A), Ripening (B) and feedstock processing (C and D).

Hence the FFI contains a loss of less than 2%. The single state base catalyzes justification was a proven method per the literature. 240 mL of methanol was taken in a flat bottom conical flask to esterify 450 mL of *Persea americana* bio-oil at a molar ratio of 1:6. 1.72 grams of sodium hydroxide was thoroughly minimum with the methanol in the flat bottom conical flask at 60 degrees Celsius and 400 rpm of agitating speed for 45 minutes, eventually forming sodium methoxide solution.

Further, 450mL of *Persea americana* bio-oil was transferred into the flat bottom conical flask containing sodium net solution. The reaction temperature was elevated

Fig. 2: *Persea americana* (Avocado) bio-oil (A), Transesterification (B) and Biodiesel (C).

to 70 degrees Celsius for 120 minutes at 450 rpm agitation speed. After the reaction period, the entire content of the flat bottom conical flask was transferred into an inverted separating funnel under a cooling period of 4 hours. It was allowed to initiate the transesterification reaction process for converting tri Glyceride into glycerol and fatty acid methyl esters from Fig. 2. It was visible that airing formation took place in the separating funnel distinction the two layers of FAME as the upper layer and glycerol as the lower layer upon burning the rotating of carefully, glycerol was allowed to drop down in a beaker thereby crude *Persea americana* biodiesel was obtained. Five percent of hydrochloric acid diluted with double distilled water was used to remove impurities like a residual catalyst, unreacted triglycerides, glycerol, soaps, etc., from the crude biodiesel for washing the obtained crude biodiesel. This transesterification process yielded 375mL of *Persea americana* biodiesel at an efficiency of 83.33%.

Comparison of Physio-Chemical properties of Diesel, and *Persea americana* Bio-oil and Biodiesel

The physicochemical properties of *Persea americana* bio-oil and biodiesel were compared with commercial diesel in Table 1. The transesterification process considerably reduced the kinematic viscosity from 23.089 cSt to 2.789 cSt, thus making it more suitable for CI engine usage. The density of *Persea americana* biodiesel was slightly increased by 0.404% but was found to be within limits. The Gross calorific value showed significant appreciation up to 23.48% and a notable upsurge in oxygen content.

Spectroscopic Analysis

GCMS-Gas Chromatography-Mass Spectroscopic Analysis

Table 1. Physio-chemical properties of *Persea americana* – raw bio-oil, biodiesel, and diesel

Property	Diesel	*Persea americana* biodiesel	*Persea americana* bio-oil
Density [$kg.m^{-3}$]	842	851	847.56
Molecular formula	$C_{12}H_{22}$	-	C_{14}-C_{25}
Cetane number	48.5	44	37
Sulfur content [% vol]	0.04	0.22	0.31
Gross calorific value [$kJ.kg^{-1}$]	42700	41256	31568
Kinematic Viscosity (cSt)	2.82	2.798	23.089
Ash content	0	0.524	1.265
Flashpoint [^oC]	69	49	262-289
Oxygen content [% wt]	0	9.784	5.569

A single Quadrupole Agilent 8890 GC mass spectrometer was employed to identify various fatty acid methyl esters to estimate *Persea americana* bio-diesel. Agilent spectrometer works at over temperatures between 40°C to °450C. The chromatographic area repeatability and retention type repeatability are less than 0.5% and 0.8%, respectively, with an inlet split ratio of 7500:1. Heated hyperbolic monolithic quadrupole mass filter with chemical ionization with a mass range of 1.6 to 1050 amu was employed. The temperature of the ion source and the quadrupole was maintained between 150-350°C and 106-200°C respectively. Four µL of methanol was used as a pre-injection solvent, after which 10 µL of Avocado bio-diesel was injected into the spectrometer and the distant injection speed was maintained at 6000 $\mu m.m^{-1}$.

FTIR-Fourier Transform Infrared Spectrometry

A single reflection Attenuated Portal Interval Reflectance molecule Bruker- Alpha- Platinum- Instrument was employed to understand the transmittance range of *Persea americana* bio-diesel. This FT-IR instrument uses deuterated tri-glyceride sulfate as a detector under the spectrometer from $500cm^{-1}$ to $400cm^{-1}$ with a resolution of 2 cm^{-1}. Infrared light radiation will enter the crystal cavity and diffract into internal reflections. The resultant incident angle fall between the crystal and the sample is shown as the transmittance range of *Persea americana* bio-diesel.

NMR-Nuclear Magnetic Resonance

Bruker Avance 3 500 MHz non-invasive nuclear magnetic spectrometer was used to understand the 1H and 13C nuclei NMR spectrometer in the *Persea americana* bio-diesel. The equipment comprises a 5.4 cm long holed standard bore with an 11.7 Tesla shielded superconducting magnet with temperature shins (34 channel) and cryoshims. The RF console of the Bruker Avance 3 spectrometer has low heat dissipation due to its gradient shining with Deuterium solvent. The face resolution and the frequency resolution of the RF console are 0.1° and 0.1Hz, respectively. ^1H de-coupling was observed using a 5 mm broadband gradient prob, and the 13C de-coupling was observed using a 5 mm quadruple inverse probed gradient.

RESULTS AND DISCUSSION

Gas Chromatography-Mass Spectrometry

The Gas Chromatography-Mass Spectrometry was carried out to evaluate the presence of fatty acid methyl esters in the presence of *Persea Americana* biodiesel. It was derived through base catalyst transesterification using methanol. In prominent quantities, unsaturated and saturated fatty acids were present in the *Persea americana* biodiesel. It was observed that oleic acid (9112 - Octadecadienoic acid (Z, Z)

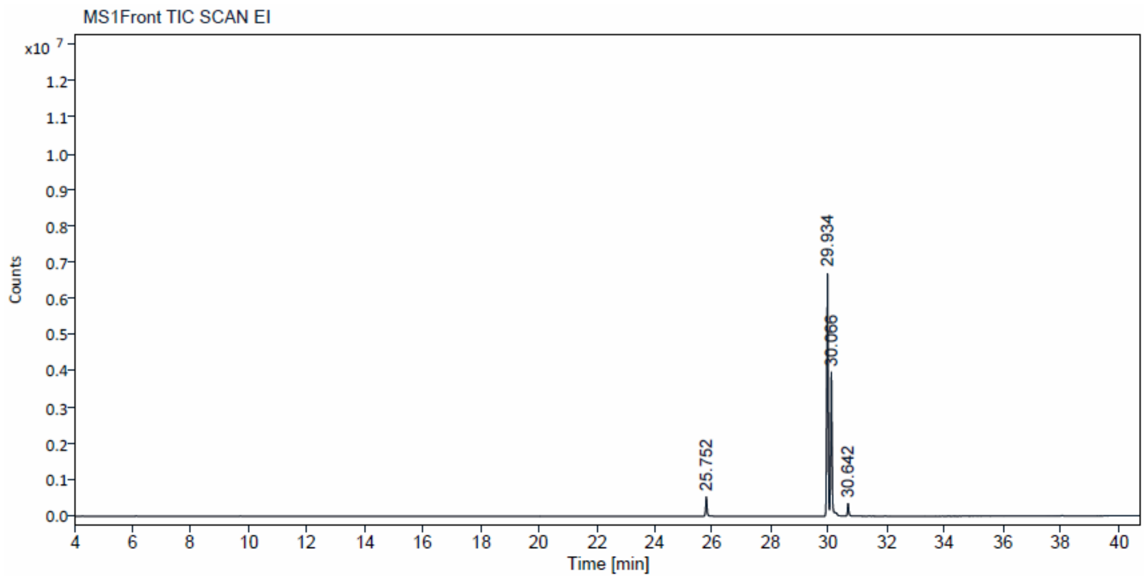

Fig. 3: *Persea americana* (Avocado) – GC MS mass chromatogram.

methyl ester) constituted a significant portion, accounting for up to 43.7% of the total composition of fatty acid methyl esters (FAME). At the retention time of 29.934 min. The other relevant fatty acid methyl ester such as palmitic acid (hexadecanoic acid methyl ester), linoleic acid (trans - 13 - octadecanoic acid methyl ester), and stearic acid (octadecanoic acid methyl ester) in notable proportions (Fig. 3).

Fourier Transform Infrared Spectrometry (FTIR)

The Fourier Transform Infrared spectroscopic analysis on the *Persea americana* biodiesel sample showed the stretching and bending vibration between 524.73 cm^{-1} and 3008.40 cm^{-1} of wave number as the percentage of transmittance. Stretching vibrations between 722.19 cm^{-1} and 1460.81 cm^{-1} confirmed the presence of carbonyl group ions (C = O) (Fig.

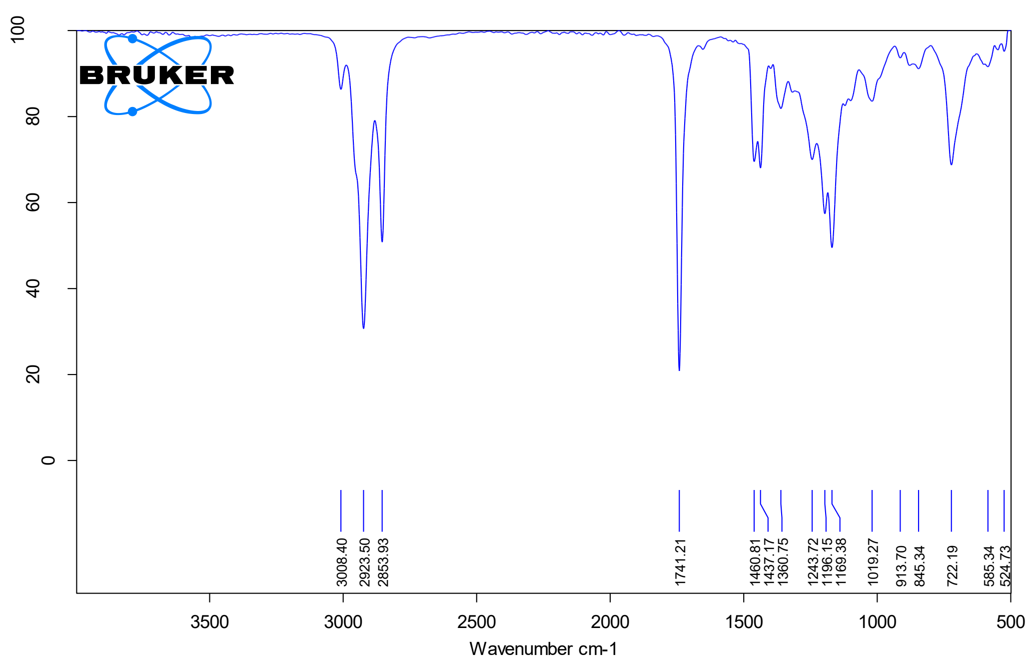

Fig. 4. *Persea americana* (Avocado) – FT IR spectrum transmittance.

4). A strong signal at 1714.21 cm^{-1} indicated the presence of fatty acid methyl esters in *Persea americana* biodiesel. The combination of stretching and bending vibration between 2853.93 cm^{-1} and 3008.40 cm^{-1} is also supported as evidence for the presence of FAMES in the biodiesel sample. A strong stretching signal at 722.19 cm^{-1} and 1196.15 cm^{-1} indicated the presence of a C-H bond in the biodiesel. The absence of signals and vibrations between 1741.21 cm^{-1} and 2853.93 cm^{-1} showed that the effectiveness in the transesterification reaction for converting the bio-oil of *Persea americana* feedstock into its biodiesel was more than 80%.

Nuclear Magnetic Resonance (NMR)

The derived *Persea americana* biodiesel was characterized using ^{1}H NMR (proton NMR) and ^{13}C NMR carbon in Bruker Avance 3 500 MHz equipment.

Fig. 5 shows the typical ^{1}H NMR spectrum of the *Persea americana* biodiesel. A strong characteristic singlet peak was noticed at 3.369 ppm, which is the distinctive feature indicating the presence of methoxy proton. 2 triplet peaks at 5.39 ppm and 2.08 ppm confirmed the presence of fatty acid methyl ester in *Persea americana* biodiesel. The absence of singlet and triplet peaks beyond 5.411 ppm indicated the absence of oleic and aliphatic acid hydrogen in the transesterified *Persea americana* biodiesel. Furthermore, a strong singlet peak at 4.899 ppm indicated the presence of a methanol group in the hydrocarbon chain. Several weak signals (singlet and doublet peaks) at one point (6.24 ppm and 3.333 ppm) were also noted in the 1H NMR spectrum, possibly due to the minimal hydroxy and amine group.

Fig. 6 shows the NMR spectrum at ^{13}C (carbon) belonging to the *Persea americana* biodiesel at 48.147 ppm. A characteristic singlet peak was noticed, which indicates the presence of fatty acid methyl esters in *Persea americana* biodiesel. A clustered peak between 26.785 ppm and 33.443 ppm was formed, which may be due to the existence of (-COO) and (C-O) carbonyl groups. A singlet terminal carbon peak was also noticed at once 17 ppm, with may be due to the presence of the methylene group. Multiple singlet peaks were seen between 127.681 ppm and 129.556 ppm, possibly due to the long-chain hydrocarbon formed due to the transesterification process. The formation of a weak signaled singlet peak at 179.40 pm may be due to hydroxy ions in the *Persea americana* biodiesel.

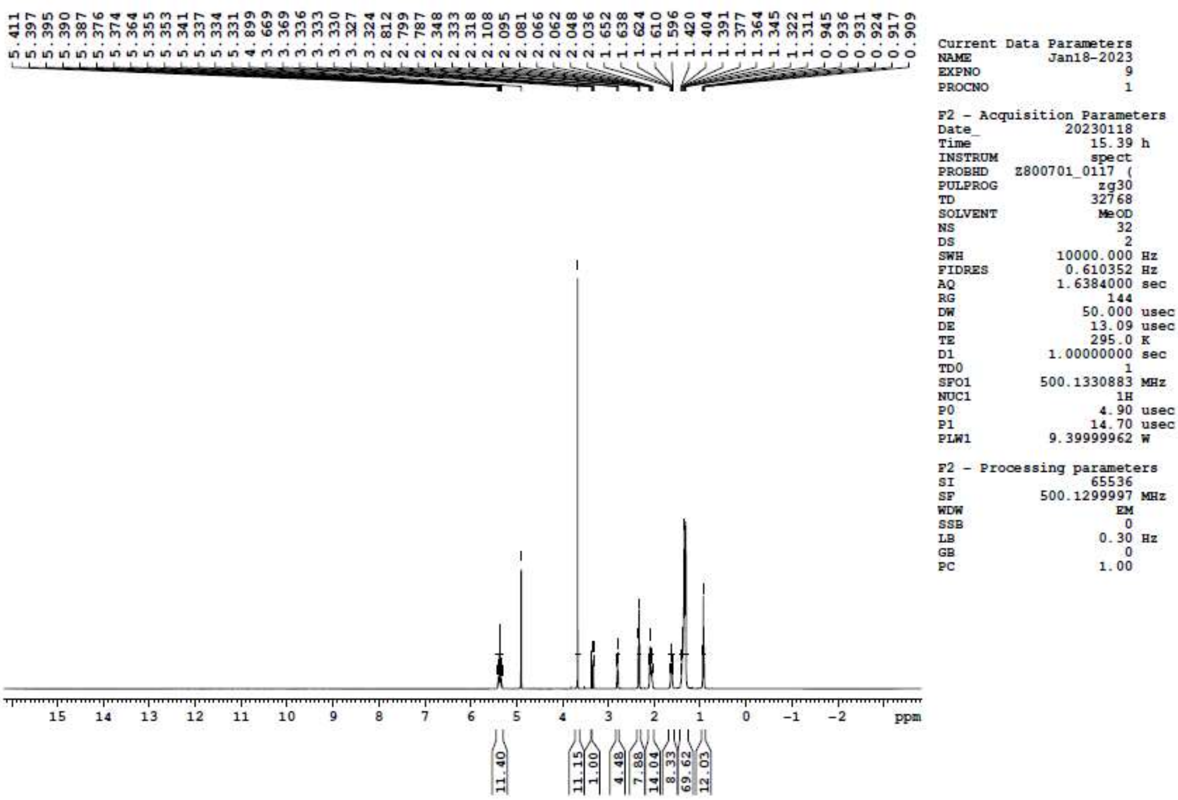

Fig. 5: *Persea americana* (Avocado) – ^{1}H NMR spectrum.

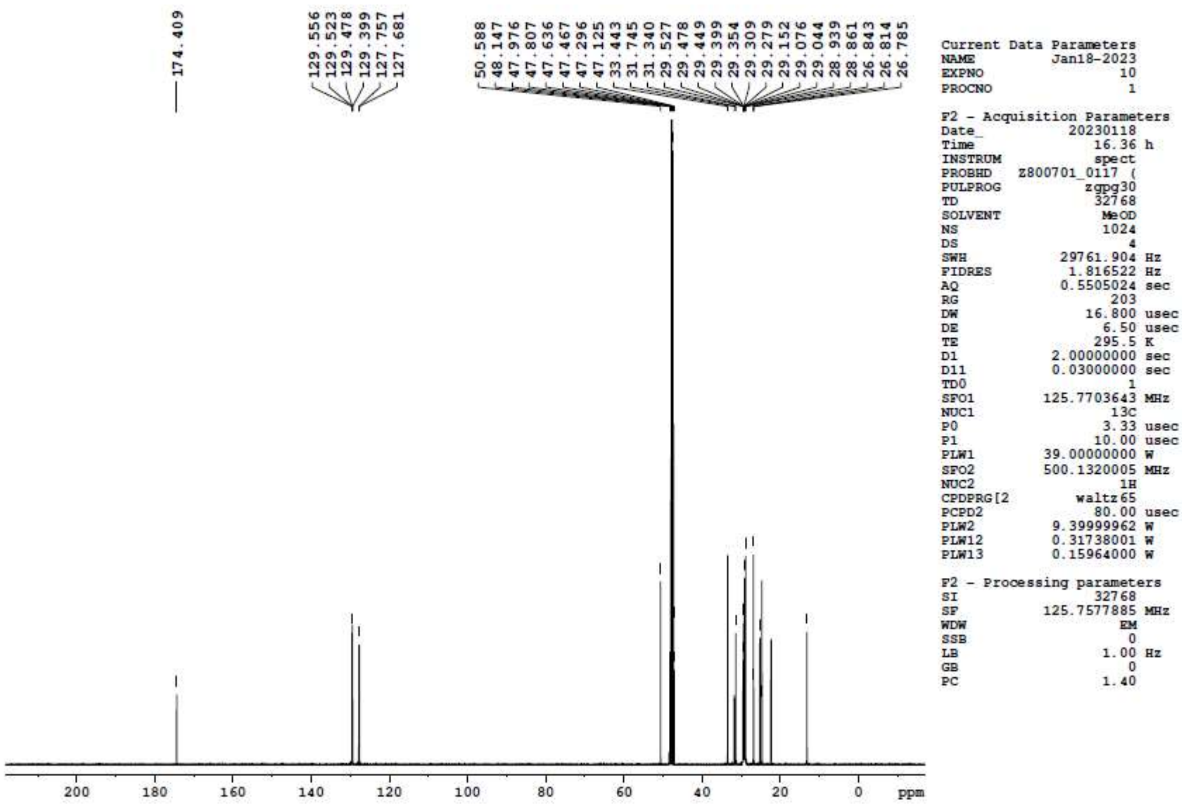

Fig. 6: *Persea americana* (Avocado) – ^{13}C NMR spectrum.

CONCLUSION

The present investigation evaluates the feasibility of using *Persea americana* biodiesel blends in compression ignition engines. The following conclusions were drawn.

1. The pre-processing of *Persea americana* seeds and a Soxhlet extraction technique with 200 mL of n-hexane in 27 batches isolated 475 mL of *Persea americana* bio-oil at an extraction efficiency of 37.5%.
2. A single-stage base-catalyzed transesterification process with methanol and NaOH at a molar ratio of 1:6 was employed to produce *Persea americana* biodiesel with a reduced FFA content (less than 1.78%). The process yielded 375 mL of biodiesel with an esterification efficiency of 83.33%.
3. Significant reduction in kinematic viscosity, flash point, and ash content was evidenced along with appreciation in density, gross calorific value, and oxygen content of the derived *Persea americana* biodiesel.
4. GC-MS analysis identified the presence of Oleic acid in prominent proportions.
5. FT-IR spectroscopic analysis confirmed the presence of carbonyl ester ions by revealing a stretching vibration between 722.19 cm^{-1} and 1460.81 cm^{-1}. Additionally, a strong signal at 1196.15 cm^{-1} attributed to the above outcomes.
6. A singlet peak at 3.369 ppm in the 1H NMR spectrum and strong cluster peaks between 26.785 ppm and 33.443 ppm in the 13C NMR evidenced the transformation of triglycerides into FAMEs during the transesterification reaction.

Therefore, it can be concluded that the biodiesel derived from *Persea americana* feedstock can be utilized as a substitute fuel for commercial diesel in blended form without making any modifications to the existing compression ignition engine.

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