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Synthesis and Characterization of Hydroxylated Plant Oil from Thevetia Peruviana Seed

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Abstract

Oils derived from oil-bearing seeds or certain fruit pieces are known as plant oils or vegetable oils. Using the Soxhlet extraction technique and n-hexane as the solvent, *Thevetia peruviana* seed oil was extracted from the seed flour. Its physicochemical parameters were determined using standard analytical methods. The hydroxylation process was done through the vigorous stirring of the oil and formic acid with the slow addition of peroxide. The hydroxylated oil was analyzed for functional groups present using Fourier Transform Infrared Spectrometer. The results of the physiochemical parameters showed that *Thevetia peruviana* oil has acid value (3.88 mg KOH/g), iodine value (93.5 Wij's), peroxide value (30.0 meq/kg), kinetic viscosity (1.6cp) and saponification value (121.76 mgKOH/g) while the hydroxylated oil has acid value (0.77 mg KOH/g), iodine value (81.40 Wij's), peroxide value (7.85 meq/kg), kinetic viscosity (7.43cp) and saponification value (162.05 mgKOH/g). The FTIR results showed that both non-hydroxylated and hydroxylated plant oils had the following functional groups OH, CH, C=C, CH_2, and C=O. The results showed that the bands were shortened in the hydroxylated oil spectrum except (OH) which became broad indicating the formation of the hydroxyl group by the consumption of the C=C band in the unhydrolyzed oil. The hydroxylation of plant oils using *Thevetia peruviana* shows the potential of the plant oil to be used as a precursor for the production of industrial intermediates which can replace the dependence on petroleum products.

Keywords: Hydroxylation, Fatty acids, Hydroxyl, Oil, Spectra.

1. INTRODUCTION

Oils that are obtained from seeds or other fruit pieces are known as plant oils or vegetable oils (fats). Vegetable fats are triglyceride combinations, just like animal fats [1]. Among the fats from seeds are soy oil and cocoa butter. Fats from different portions of fruits include rice bran oil and palm oil. Vegetable fats that are liquid at room temperature are the only types of vegetable oil that are commonly used. [2]. They serve as a means for cooking or frying, making them crucial ingredients in the processing of food. While the bulk of vegetable oils (often referred to as edible oils) are safe for cooking, some vegetable oils are hazardous and should not be eaten by humans [3]. These oils, which include Karanja oil and Jatropha oil, which can be a good precursor for materials to replace some petroleum-derived products, are classified as non-edible oils. Glycerol and fatty acids referred to as triglycerides make up the chemical foundation of vegetable oil. As seen in Figure 1.1, triglycerides are an ester created when one molecule of glycerol, organic alcohol, chemically bonds with three fatty acids, an organic acid. Triglycerides are a form of fat (lipid) that may be found in human blood as well as in vegetable oils. Their purpose is to store extra calories and turn them into energy. Fatty acids are normally long unbranched hydrocarbons attached to hydrogen and other groups and the chain terminates with a carboxylic acid.

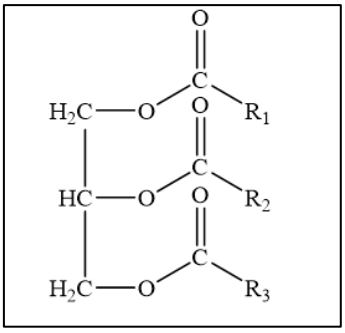

Fig. 1.1: Structure of Triglycerides (R1, R2, and R3 are the fatty acids) [4].

1.1 Hydroxylation Of Plant Oils

A hydroxyl group (-OH) is added to an organic compound by the method known as hydroxylation. The amount of OH groups in a molecule is described as the level of hydroxylation. The placement of hydroxyl groups on a molecule is referred to as the structure of hydroxylation [5].

1.2 Synthetic Hydroxylations

Different metal catalysts can have an impact on adding hydroxyl groups to organic molecules. Numerous of these catalysts are biomimetic or designed to resemble enzymes like cytochrome P450 [6]. Some processes insert OH groups into unsaturated molecules, whereas many hydroxylations add O atoms to C-H bonds. Such a process is the Sharpless dihydroxylation, which turns alkenes into diols. Hydrogen peroxide, which reacts across the double bond of alkenes, supplies the hydroxy groups [7].

1.3 Biological Hydroxylation

The hydroxylase family of enzymes frequently aids hydroxylation processes. When an oxygen atom is inserted into a C-H bond, the result is alcohol. The following reactions are typical for the hydroxylation of a basic hydrocarbon:

$$2R_3C - H + O_2 \rightarrow 2R_3C - OH$$
$$R_3C - H + O_2 + 2e^- + 2H+ \rightarrow 2R_3C - OH + H_2O$$

Catalysts are needed to speed up the process and add selectivity because O_2 is a sluggish and unselective hydroxylating agent [8]. In the air, the first stage in the breakdown of organic molecules is frequently hydroxylation. Since hydroxylation changes lipophilic substances into hydrophilic (water-soluble) molecules that are more easily reversed by the kidneys or liver and expelled, it is crucial for detoxification. By hydroxylation, several medications (such as steroids) are triggered or inhibited [9]. Cytochrome P-450, which exists in a vast variety and is the primary hydroxylation promoter in life, has hundreds of different forms. Alpha-ketoglutarate-dependent hydroxylases and a few diiron hydroxylases are other hydroxylating substances [10].

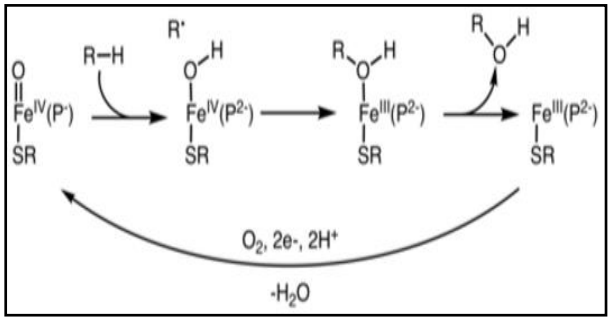

Fig. 1.2: Following are the mechanisms in an oxygen rebound process that describes numerous hydroxylation's that are iron-catalyzed: Alcohol decomplexation, oxygen rebound, and H-atom abstraction [8]

2.0 MATERIALS AND METHODS

2.1 Apparatus and Reagents Used

Retort stand, Isomantle, Soxhlet apparatus, Analytical weighing balance, Filter paper, Thread, Blending machine, Oven, Beakers, Cotton wool, Razor Blade, Tap, Separation funnel, Thermometer, Magnetic stirrer and plate, Spatula, Hand gloves, 3-necked flask, Measuring cylinder, Ice bath, distilled water, n-Hexane, Formic acid, Hydrogen peroxide, Diethylether, Sodium chloride, Sodium bicarbonate, Sodium sulfate (solvents used were analytical grade).

2.2 Sample Collection

Thevetia peruviana fruits were harvested from Thevetia plantations grown at a residence used as hedges along Housing Road Ado-Ekiti, Ekiti State, Nigeria. The kernels were carefully removed from the collected Thevetia fruits using a steel knife. Thevetia kernels were sun-dried for five hours straight to make it easier to separate the seeds from them. The kernels were separated from the seed, then processed into a powder using a blender, and then preserved for additional extraction.

2.3 Extraction of Oil

A filter paper was used to wrap 30g of the crushed seed sample and put it into a soxhlet extractor making a complete batch of 60g inside the thimble. A condenser was firmly placed at one end of the extractor and 250 ml of n-hexane was put into a 500 ml round bottom flask attached to the end of the device. For three hours, the temperature was fixed at 60 °C. The clean vapor rose through a by-pass as the solvent boiled in the boiler, reaching the top of the Soxhlet vessel (thimble), which held the sample to be extracted. The vapors condensed in the condenser and dripped into the thimble holding the sample. The liquid containing the extracted material is siphoned back into the boiler when the liquid level is at the same level as the top of the siphon. To get oil free of solvent, the extracted oil-solvent mixture was obtained, recaptured, and distilled. The aforementioned process was done multiple times until a sufficient amount of oil was collected, at which point the percentage oil yield was determined.

2.4 Hydroxylation of Extracted Oil

A measuring cylinder was used to pour 25 ml of *Thevetia peruviana* seed oil into a three-necked flask that had a mechanical shaker and a thermometer. 25 ml of 97% formic acid was added. The stirrer was positioned in the oil, and the three-necked flask and its contents were placed in a water bath on a magnetic plate. To obtain a uniform mixture, it was continually swirled at a temperature of 10-15℃ Hydrogen peroxide (H_2O_2) at a concentration of 30% was measured, added cautiously and gradually while maintaining a temperature range of 10 to 15 ℃. H_2O_2 was then added, and the mixture was vigorously stirred for the following 24 hours at room temperature. The emulsion was extracted with diethyl ether after the reaction mixture

had been placed into a vacuum flask to cool. Water, $NaHCO_3$, and saturated sodium chloride solution were used to wash the ether layer, respectively. Over Na_2SO_4, the resultant ether layer was dried.

2.5 Physiochemical Characterization of Hydrolyzed Oil

2.5.1 Oil Yield Percentage Calculations

The following formula was used to compute the percentage oil yield;

$$\text{Oil yield} = \frac{X_1 - X_2}{X_1} \times 100\%$$

X_1 = Actual Weight of the Sample before extraction
X_2 = Final Weight after extraction

2.5.2 Iodine Value Evaluation

A dry 250 ml conical flask was filled with a 0.2 g oil sample that had been weighed on a small square of aluminum foil. Carbon tetrachloride (CCl_4) 10 ml was added and the flask was shook-up to mix the oil. A fume cupboard flask was filled with 20 ml of Wijs solution, and the flask was sealed with cotton wool that had been wet and immersed in potassium iodide (KI) solution. The flask was left to stand in the dark for 30 minutes. 100 ml of distilled water was added to 10 ml of 5 percent (v/v) potassium iodide (KI) to create a blank. Using starch solution as a marker, the iodine freed was back titrated with 0.1M sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$).

$$\text{Iodine Value} = \frac{M(X_1 - X_2) \times 12.69}{Sample's\ weight}$$

V_1 = Blank titre value
V_2 = Sample's titre value
M = Sodium thiosulphate's molarity
12.69 = molecular weight of iodine

2.5.3 Relative Density Determination

This is the weight of the analyte in grams relative to the weight of a similar amount of water. A density bottle was weighed (W1) with its stopper on, then filled with the oil and sealed with the stopper (W2). The density bottle underwent a thorough cleaning, draining, filling, and weighing (W3).

$$\text{Relative Density} = \frac{W_2 - W_1}{W_3 - W_1}$$

2.5.4 Viscosity Analysis

Using a syringe and needle, the sample oil was injected to the upper calibration mark into the upper glass bulb of a jacket-mounted viscometer, whose temperature was kept at 40°C. To halt the flow till the upper bulb was filled, the gadget had a rubber bung stopper at the end close to the lower bulb. The rubber bung was taken off after taking note of the temperature, and a stopwatch was started at the same time. The amount of liquid needed to discharge from the upper bulb into the lower bulb in both the upper and lower calibration markers was determined in seconds. The time elapsed was used in conjunction with the formula below supplied by the manufacturer of the viscometer bulb to determine the viscosity in centipoises.

$$\text{Viscosity} = \frac{1.002 \times flowtime(mins) \times oilrelativedensity}{17.186}$$

2.5.5 Assessment of the Peroxide Value

A dry boiling tube containing 1g of the oil sample, 1g of KI powder, 20ml of mixed glacial acetic acid (CH_3COOH), and 30 ml of chloroform was filled. The tube was then brought to a boil for 60 seconds. The mixture was added to the titration flask, which already had 25 ml of distilled water and 20ml of a 5% Potassium iodide (KI) solution. So, utilizing starch indicator, the content was titrated with 0.002 M sodium thiosulphate.

$$\text{Peroxide Value} = \frac{(V_2 - V_1)ml \times M \times 1000}{Sample's\ weight}$$

M = Molarity of sodium thiosulphate
V_1 = Volume of the blank
V_2 = Titre value

The acid value determined was used for the determination of free fatty acid.

2.5.6 Estimation of the Acid Value

A conical flask was filled with 5 g of an oil sample. Phenolphthalein indicator of 1 ml and 25 ml of 95% (v/v) alcohol were added. 0.1M Potassium hydroxide (KOH) was used to titrate the solution.

$$\text{Value of Acid} = \frac{V \times M \times 56}{Sample's\ weight}$$

The molar mass of KOH = 56
V= Titre value
M = Molarity of potassium hydroxide used.

2.5.7 Estimation of Free Fatty Acid (FFA)

The amount of free fatty acid was calculated using the determined acid value:

$$\text{Acid Value} = 2 \times Free\ Fatty\ Acid\ (FFA)$$

2.5.8 Saponification Value Estimation

A conical flask containing 2g of seed oil sample was weighed, and alcoholic potassium hydroxide of 25ml solution was added. For 30 minutes, while sometimes shaking, the flask was heated in a bath of boiling water. The solution was delivered 1ml of phenolphthalein indicator, and 0.5 M hydrochloric acid was used to titrate it while it was still hot. There was a blank titration performed.

$$\text{Saponification Value} = \frac{(a - b)ml \times M \times 56V}{Sample's\ weight}$$

a = Titre value
b = Blank titre value
M = Molarity of acid used

2.5.9 Fourier Transform Infrared Spectroscopy (FTIR)

Potassium bromide (KBr) salt was used to assess the samples' infrared spectra. Weighing the

sample at 1 mg and triturating it in 100 mg of potassium bromide (KBr) produced the sample (IR grade). A disc was filled with the triturate, which was then squeezed at a hydraulic pressure of 10 tons weight. The pellet that resulted was applied to the IR spectrum.

3.0 RESULT AND DISCUSSION

3.1 Physiochemical Properties

The results of the physiochemical properties of the hydroxylated and non-hydroxylated oil are presented in the table below:

Table 3.1: Physiochemical properties of hydrolyzed seed oil of Thevetia peruviana and unmodified seed oil of Thevetia peruviana

Properties	TPO	HTPO
Oil yield %	64.33	--
Acid value (mg KOH/g)	3.88	0.77
Iodine value (mg KOH/g)	93.5	81.40
Saponification value (mg KOH/g)	121.76	162.05
Peroxide value (meq/kg)	30.0	7.85
Kinetic viscosity (cp)	1.6	7.43
Colour	Golden yellow	Bright yellow

NB: TPO = *Thevetia peruviana* oil, HTPO = Hydrolyzed *Thevetia peruviana* oil

3.1.1 Oil Yield

Thevetia oil was discovered to have a 64.33% oil yield. The oil contents of the Yellow Oleander seed (64.33%) obtained in this work compare favorably with the oil yields of Yellow Oleander seeds (48.8%-60.0%) [11]. This value also compares favorably with those from other oil-bearing plants such as Jatropha kernel (52.4%-56.5 %) as reported by [12]. This result is within the allowed range for the *Thevetia peruviana* seed's oil yield (52–65%). [13]. However, the oil yields are higher than those of *Azadirachta indica* (44.5%), *Pongamia pinnata* (33%), and *Ziziphus mauritiana* (34%) [14]. The result of the oil yield showed that the *Thevetia peruviana* plant can be classified as an oil seed and thus, can be used commercially for the production of biodiesel [12].

3.1.2 Iodine Content

Using Wij's technique, the iodine content was obtained. Experimental results showed that the iodine value was 81.40 mg KOH/g in HPTO and 93.5 mg KOH/g in TPO. As a result of the oxirane group in HTPO functional groups replacing carbon to carbon double bonds, TPO has a larger degree of unsaturation than HTPO, which lowers its iodine value [14]. The drop demonstrates that C=C double bonds have changed into hydroxyl groups [15].

3.1.3 Saponification Value

The saponification value was found to increase from 121.76 mg KOH/g in TPO to 162.05 mg KOH/g in HTPO. Due to the great extent of decrease in the unsaturation character of the TPO oil caused by the hydroxylation process, HTPO has a relatively high saponification value than TPO [16].

3.1.4 Acid Value

The acid content, which is typically indicative of deterioration, shows the concentration of free fatty acids as a result of enzymatic activity. The peroxide value was reduced to 0.77 meq/g from 30.0 mg KOH/g and the acid value from 3.88 mg KOH/g in TPO to 0.49 mg KOH/g in HTPO. [16].

3.2 Fourier Transform Infrared Spectrometer (FTIR)

In Figs. 3.1 and 3.2, respectively, the FT-IR spectra of raw (undisturbed) and hydrolyzed *T. peruviana* oil are displayed. The table for the functional groups is presented in table 3.2.

Table 3.2: Summary of significant bands appearing in Figures 3.1 and 3.2

Unmodified seed oil		Hydroxylated seed oil	
Wavenumber (cm^{-1})	Bond	Wavenumber (cm^{-1})	Bond
3469.50	OH	3466.65	OH
3006.11	=C-H	NP	NP
2917.75	CH_3	2921.32	CH_3
2852.39	C-H	2853.48	C-H
2727.8, 2679.32	H-C=O	2729	H-C=O
2030.69	C≡C	NP	NP
1746.71	C=O	1730-1746	C=O
1654.55	C=C	1654	C=C
1417.48, 1455.68	CH_2	1454	CH_2
1117.86, 1162.08, 12	C≡C-C-O	1378, 1416.48	C-H

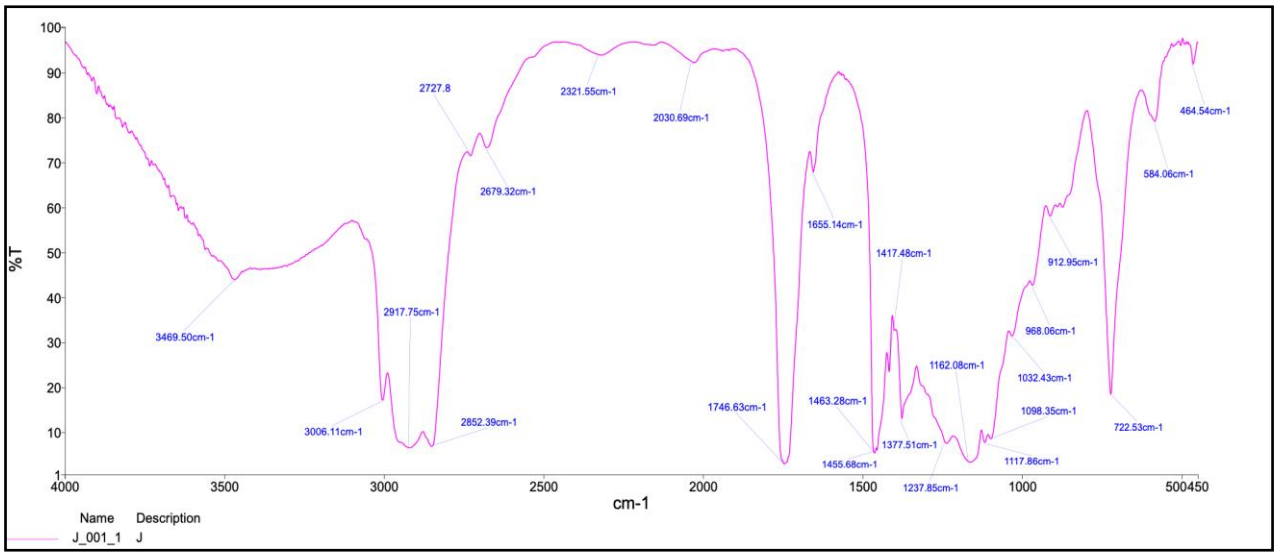

Fig. 3.1: FT-IR spectrum of unmodified Thevetia peruviana oil

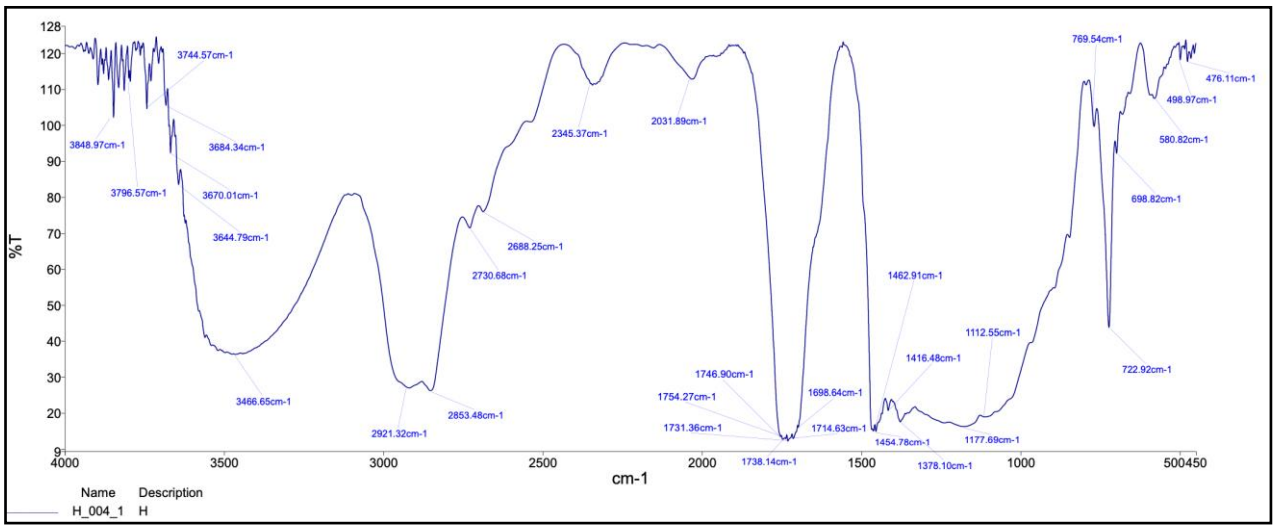

Fig. 3.2: FT-IR spectrum of 8hrs hydrolyzed Thevetia peruviana oil

In Fig. 3.1, the vibration at 3469.50 cm^{-1} is assigned to OH stretching. The occurrence of some H_2O in the sample is probably what allows the OH group to show up in this band. According to a widely held belief [16], the vibration at 3005.91 cm^{-1} is identical for the majority of vegetable oils and is caused by CH stretching associated with the =C-H bonding. This band is a crucial factor to take into account in this study because it is this double bond that is broken to create an epoxy group, which is then depleted to create a hydroxyl group. The following three bands are attributed to methyl (CH_3) and methylene (CH_2) group C-H stretching. The vibration at 2917.75 cm^{-1}, which results in only a broad weak peak, is caused by the asymmetric stretching of a CH_3 group. Typical triglyceride oil spectrums have two very noticeable bands that follow at vibrations 2852.81 cm^{-1} and 2727.8 cm^{-1}. Both of them are the outcomes of symmetric and asymmetric stretching vibrations in CH_2. The alkyne group, C=C stretch, is visible as faint bands in the spectra at vibrations 2030.69 cm^{-1} and 2321.55 cm^{-1}. The ester groups' C=O stretching vibration, which causes a strong band to form at vibration 1745 cm^{-1}, is related. This group can be found right where the fatty acid chain connects to the glycerol molecule and is found in all fatty acid chains. At vibration 1655.14 cm^{-1}, a crisp band is visible. A stretching vibration of the form C=C is attributed to this band. This is supported by the fact that this band does not show up on the spectra of saturated vegetable oils, where the C=C bond does not exist [17].

The CH_2 scissors compression vibration is allocated to a prominent band at 1465 cm^{-1}. There is no evidence about whether the presence of the CH_2 group in the glycerol, fatty acid, or both parts of the triglyceride molecule is specifically responsible for this phenomenon. The spectra show a noticeable band at vibration 1162.08 cm^{-1}. There are two weaker bands on either side, before and after at vibrations of 1237.85 cm^{-1} and 1117.86 cm^{-1}, respectively. This band pattern is attributed to the stretching vibration C=C-C-O. The

CH_2 rocking mode is attributed to a significant band that appears at vibration 722.5 cm^{-1}.

It is obvious from fig. 3.2 that the peak associated with O-H stretching at vibration 3466.65 cm-1 is a sign of the hydrolytic cleavage of the minor portion of oxirane groups to the hydroxyl group. This finding is evidence that TPO has been hydroxylated [18].

Significant broad twin peaks were observed at vibrations of 2921.32 cm^{-1} and 2853.48 cm^{-1}, which are caused by C-H stretching. O-H stretching and C=O stretching are attributed to the two tiny peaks at 2730.68 cm^{-1} and 1714.63 cm^{-1}, respectively. Carboxylic acid is present, as indicated by this band. Between 820 cm^{-1} and 843 cm^{-1}, there was no peak. The refinement, characterization, and modification of *Thevetia peruviana* seed oil for its enhanced potential for industrial use, provides reassurance that all the epoxy groups were absorbed to create the hydroxyl group during the hydroxylation [19]. At vibration 1722.92 cm^{-1}, a very distinct band becomes visible and doesn't change with the unaltered TPO.

The other bands in all spectra share features with the C-H bond's predominate bands at 1112.55 cm^{-1}, 1378 cm^{-1}, and 1416.48 cm^{-1}. The C-H_2 rocking band was visible at 1454 cm^{-1} and 1462.91 cm^{-1}, and the carbonyl group (C=O) was present in all of the spectra thanks to the peak at 1730 cm^{-1}-1746 cm^{-1} [20].

3.3 CONCLUSION

The hydroxylation of plant oils using *Thevetia peruviana* shows the potential of the plant oil to be used as a precursor for the production of industrial intermediates which can replace the dependence on petroleum products. The higher yield of the seed oil makes it a good source for large-scale production.

3.4 RECOMMENDATION

Thevetia peruviana seed has a very good oil yield. Contrary to what has been said in the past, there haven't been many scientific studies on this plant that are relevant to medicine. However, further pharmacological and clinical research must be done to fully comprehend the therapeutic benefits of this plant oil.

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