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MILK BUSH (*THEVETIA PERUVIANA*) SEED OIL AS FEED STOCK FOR BIO-POLYMER PRODUCTION

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Abstract: To increase the safety of our environments, the selection of proper building block for bio-polymer synthesis becomes vital. This research focused on determining the degree of unsaturation and reactivity of unsaturated milk bush (*Thevetia peruviana*) oil (TPO). The oil was extracted from *Thevetia peruviana* seed by Soxhlet extraction method and was further purified. Structural elucidation and characterization of purified TPO was carried out using Proton NMR spectrophotometer, Fourier transform infrared (FTIR) spectrophotometer and Gas chromatography mass spectrometer. The results revealed that the non-edible oil has a triglyceride structure, with 3.16 carbon – carbon double bonds per triglyceride which can be used as starting material to synthesized bio-polymer.
Keywords: bio-polymer, seed oil, extraction, biodegradable and building block

1. INTRODUCTION

Non-biodegradable petroleum-based polymeric materials have raised serious environmental concerns [1]. The growing demands for such nonrenewable and indestructible materials have increased our dependence on crude oil. Thus, biodegradable polymeric materials, especially those prepared from readily available, renewable and inexpensive natural resources have become increasingly important and focus of considerable recent scientific research [2].

Oil and its derivatives are used in the production of paints, varnishes, lacquers, hydraulic fluids, soaps, printing inks, linoleum, oil cloth, lubricants and grease. They are also used as raw material in the manufacturing of various chemicals like sebacic acid and undecylenic acid which are used in the production of plasticizer and Nylon [3]. Nuts oils, seed oil and oils of fruits and vegetables are receiving growing interest due to their high concentration of bioactive lipids components, such as polyunsaturated fatty acids and phytosterols, which are extensively used in cosmetics, pharmaceuticals, oleochemicals and food industry [4]. However, relatively little work has been done on the conversion of their fats and oils to produce high molecular weight polymers [5].

Thevetia peruviana (TP) is an ever–green ornamental dicotyledonous shrub that belongs to Apocyanaceae family [6]. It is commonly found in the tropics and sub – tropics regions but it is native to Central and South America. It grows to about 3 – 7 m high; its leaves are spirally arranged, linear and about 13 – 15 cm in length. There are two varieties of the plant; one with yellow flowers, yellow oleander, and the other with purple flowers and nerium oleander. Both varieties of flower and fruit provide a steady supply of seeds all the year round. Grown as hedges, they can produce between 400 – 800 fruits per annum depending on the rainfall pattern and plant age. The flowers are funnel-like with petals that are spirally twisted. The fruits are somewhat globular, with fleshy mesocarp and have a diameter of 4 – 5 cm. The fruits are usually green in colour and become black on ripening. Each fruit contains a nut which is longitudinally and transversely divided. The fruit contains between one to four seeds in its kernel, and the plants bears milky juice in all organs.

In Nigeria, TP has been grown for over fifty years as an ornamental plant in homes, schools and churches by missionaries and explorers, all parts of the plant are toxic, due to the presence of glycosides and the seed contains 52 – 65 % oil; depending on the method of extraction and solvent used [7 - 9]. Despite the fact that there is high level of oil and protein in the seed, it still remains non – edible because of the presence of cardiac glycoside [7]. Therefore, this research was carried out to determine the degree of unsaturation of the TP oil, its reactivity and consequently the establishments of its use as source of raw material for bio-polymer production.

2. MATERIALS AND METHOD

— Materials

Thevetia peruviana (TP) fruits shown in Figure 1c, were locally sourced from Akure, Ondo State, Nigeria. *Akure* is situated at 7.25° North latitude, 5.19° East longitude in the map of Nigeria. The good seeds were washed with water and air dried for 3 days, the nuts shown in Figure 1d; were cracked with stone in order to remove the peels (Figure 1e) from the meats (Figure 1e), the meats were oven dried at temperature of 103° C to remove moisture and latter milled using C & N Junior Laboratory mill size 5 (Christy and Norris Limited Engineers, Chemlsford, UK). *Thevetia peruviana* oil (TPO) were extracted by Soxhlet method using normal hexane as solvent, which was purchased from Aldrich Chemical Company (Milwaukee, WI) and used as received. Solvent was removed under reduced pressure on rotary evaporator. The crude oil was refined by agitating with 18M NaOH (1: 30 g/g of alkali: powder) for 15 minutes. The resultant was heated to 75-80° C to break the soap stock and neutral oil separated by centrifugation.

— Oil characterization

The TPO fatty acid composition was determined by chromatography analyzer. The fatty acid methyl esters were separated using HP 6890 Gas Chromatography analyzer powered by a Hp ChemStation Rev A 09.11 (1206) software and equipped with flame Ionization Detector (FID). The carrier gas was Nitrogen and the oven initial temperature was at 60 °C. The first ramping was at 10 °C/minute for 20 minutes and maintained for 4 minutes. The second ramping was at 15 °C/minute for 4 minutes and maintained for 10 minutes. The detector temperature was 320°C while hydrogen and compressed air pressures were 7 KPa and 11 KPa, respectively.

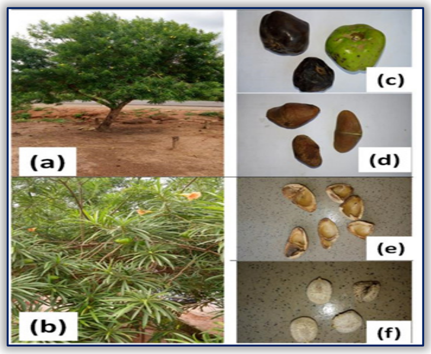

Figure 1: (a) *Thevetia peruviana* Plant, (b) Leaves and Flowers, (c) Fruits, (d) Nuts, (e) Peels, and (f) Meats

Proton nuclear magnetic resonance ('H NMR) spectrum was recorded in $CDCl_3$ (Deuterated Chloroform) on a broker Avance DPX spectrometer operating at 300 MHz and 400 MHz for ^{13}C while Fourier Transform Infrared spectroscopy (FTIR) spectrum was determined from Tensor 27FTIR-H1026302 (Bruker Optik, GmbH, Germany).

3. RESULTS AND DISCUSSION

— Gas Chromatography Analysis

The fatty acid composition and profile of TPO were as shown in Table 1 and Figure 2, respectively. The results indicated that TPO was highly unsaturated (78.64 %) with the major fatty acids as Oleic acid (52.4 %), Linoleic acid (25.03 %), Linolenic acid (0.6 %), Arachidonic acid (0.6 %) and Palmitoleic acid (0.01 %). This is an indication of the presence of multiple C═C bonds as a result of the presence of highly unsaturated fatty acid [7].

Table 1: Fatty acid constituents of *Thevetia Peruviana* oil

Name	Acronym	Volume percentage
Myristic Acid	14:0	0.35
Palmitic Acid	16:0	14.10
Palmitoleic Acid	16:1	0.01
Stearic Acid	18:0	6.50
Oleic Acid	18:1	52.40
Linoleic Acid	18:2	25.03
Linolenic Acid	18:3	0.60
Arachidic Acid	20:0	0.20
Arachidonic Acid	20:4	0.60
Behenic Acid	14:0	0.11
Erucic Acid	16:0	0.10

— Determination of percentage of oil content

Table 2: Determination of percentage of oil extract

Quantity of seeds used (g)	Quantity of oil extracted (g)
40.01	25.74

From Table 2, the oil yield was calculated to be 64.33 %, using equation (1) by Olisakwe *et al.*,[10].

$$\text{Oil yield \%} = \frac{\text{Qty of oil extracted}}{\text{Qty of seeds used}} \quad (1)$$

This value falls within the acceptable range of the percentage oil content (52-65 %) of *Thevetia peruviana* seed reported in literature [7 - 9].

Based on the fatty acid composition in Table 3, the carbon – carbon double bonds per triglyceride of *Thevetia peruviana* oil was calaculated using equation (2) by Akintayo *et al.*[11].

$$\text{Mole of double bonds/Mole of Triglyceride} = \frac{\sum \text{mole of double bond/100g}}{\sum \text{mole of fatty acid/100g}} \times 3 \quad (2)$$

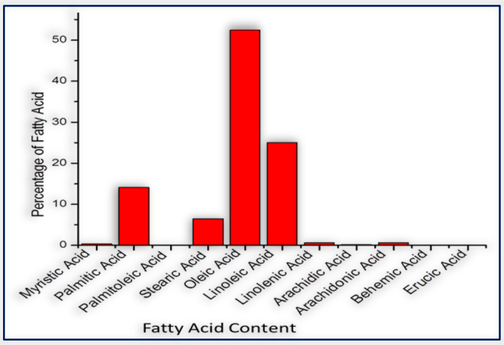

Figure 2: Profile of Fatty acid Content of *Thevetia peruviana* oil

Table 3: Fatty acid composition, moles of double bond and fatty acid per 100g of TPO

Fatty acid	% Fatty acid	Molar Mass	No of double bounds	Moles of double bounds/100g	Moles of Fatty acid/100g
Myristic	0.35	228.3709	0	0	0.0015
Palmitic	14.1	256.4241	0	0	0.0550
Stearic	6.5	284.4772	0	0	0.0228
Oleic	52.4	282.4614	1	0.1855	0.1855
Linoleic	25.03	280.4455	2	0.1786	0.0893
Linolenic	0.6	278.4500	3	0.0066	0.0022
Arachidic	0.2	312.5300	0	0	0.0006
Arachidonic	0.6	304.4669	4	0.0080	0.0020
Behenic	0.11	340.5836	0	0	0.0003
Erucic	0.1	338.5700	0	0	0.0003
∑				0.3787	0.3595

The TPO was calculated to have 3.16 carbon – carbon double bonds per triglyceride as presented in Table 3. This is an indication that methyl esters of TPO will have better reactivity and this is in agreement with the findings of Dejan *et al*.,[12] about peanut oil; which had been used successfully to prepare thermosetting polymers.

— 'H NMR spectrometry analysis

Figure 3 shows TPO proton NMR spectrum, the peaks at 4.1 - 4.4 ppm and 5.2 - 5.5 ppm originated from the methylene and vinylinic protons of the triglyceride of TPO. The protons of CH_2 groups sandwiched between two carbon-carbon double bonds appeared at 2.7-2.8 ppm, and this is in agreement with the findings of Akintayo *et al*.,[11].

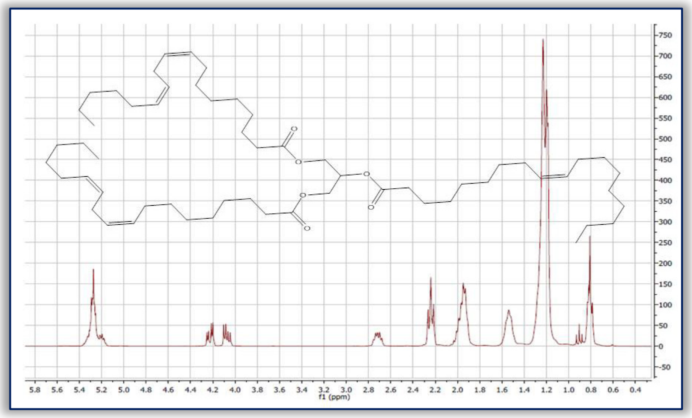

Figure 3: 'H NMR Spectrum and Structure of TPO

— FTIR spectrometry analysis

Figure 4 shows the IR spectrum of TPO. The peaks at 2851.8 cm^{-1} and 2921.0 cm^{-1} indicate symmetric and asymmetric stretching vibrations of C – H alkane groups respectively; which is in agreement with the findings of Akintayo *et al*.,[11]. Also, the peak at 3005.9 cm^{-1} shows the presence of stretching vibration of =C-H alkene groups. They are detected above wavenumber 3000 cm^{-1} in the spectrum compared to corresponding alkane C – H stretching groups detected below 3000 cm^{-1} (around 2851.8 cm^{-1} and 2921.0 cm^{-1}) as reported by some authors [13-14]. Also in the spectrum, the characteristics peaks found which was the strongest at 1742.4 cm^{-1} – 2000 cm^{-1} indicate C=O stretching vibration which is attributed to the presence of methyl esters and this is in agreement with the findings of Shenjun *et al*.,[15], peak 1376.1 cm^{-1} can be ascribed to the bending vibrations of $-CH_3$ groups, and the band at 1458.0 cm^{-1} can be ascribed to the aromatic C=C bending vibration [13]. The characteristics peaks found at peaks 1095.0 – 1158.4 cm^{-1} show C – O stretching vibration as reported by Ivanoiu *et al*.,[16] and Ndama *et al*.,[16], while peak 721.4 cm^{-1} shows the bending and overlapping rocking of C—C functional group [11]. All these confirmed the highly unsaturated and triglyceride structure of TPO established earlier.

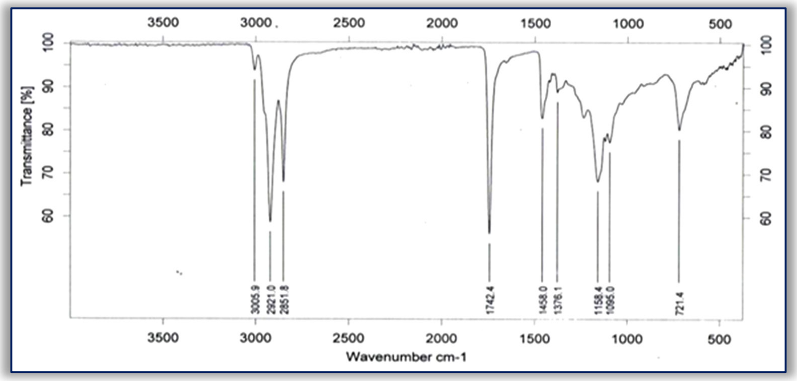

Figure 4: FTIR spectrum of TPO

4. CONCLUSION

Based on the fatty acid composition, 'H NMR and FTIR analysis, it has been established that *Thevetia peruviana* oil (TPO) has a triglyceride structure with 3.16 C═C bonds per molecule on average in the fatty acid chain. The polymer production can be achieved through either fatty acid C═C bond functionalization and subsequent copolymerization or through direct copolymerization of the fatty acid C═C bonds with a variety of alkene comonomers. The seed of the plant has high oil yield percentage (64.35%) and high sustainability which made the TPO suitable natural building block for the preparation of useful bio-polymer materials.

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