



ISOLATION AND IDENTIFICATION OF FATTY ACIDS FROM THE ACETONE EXTRACT OF *Parkia biglobosa* (JACQ.) R. BR. EX G. DON FRUIT HUSK USING COLUMN CHROMATOGRAPHY AND ¹H NMR SPECTROSCOPY

¹A. D. TELTA* ²M. ADAMU ³J. BOSHA, ⁴M. CHIROMA, ⁵V. UPEV, ¹K. PARNDA

¹ Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria

² Department of Veterinary Parasitology and Entomology, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria

³ Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria

⁴ Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Maiduguri, Nigeria

⁵ Department of Veterinary Physiology and Biochemistry, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria

Correspondance Email/Tel: ayubatel@gmail.com Tel: 08062545410

ABSTRACT

*This study examined phytochemicals in the acetone extract of *Parkia biglobosa* (African locust bean) fruit husk, a traditionally used but underutilised plant part in West Africa. The fruit husks were air-dried, pulverised, and extracted by acetone maceration, producing a 16 % yield. The extract was fractionated using column chromatography and monitored by thin-layer chromatography (TLC) across different solvent systems, which showed multiple compounds based on distinct R_f values. Based on their chromatographic profiles, only three major fractions (VT14, VT22, and VT40) were selected for further characterization by proton nuclear magnetic resonance (¹H NMR) spectroscopy. The ¹H NMR spectra exhibited characteristic signals consistent with long-chain fatty acids. Structural elucidation tentatively identified the fractions VT14, VT22 and VT40 as Oleic acid, Myristic acid and Lauric acid respectively. This is the first report of Oleic, myristic and lauric acids from *P. biglobosa* fruit husk. The detection of these bioactive fatty acids supports the plant's traditional medicinal use and suggests potential for therapeutic applications and drug development.*

Keywords: Acetone extract, Column chromatography, Fruit husk, ¹H NMR spectroscopy, *Parkia biglobosa*, Thin layer chromatography.

I. INTRODUCTION

Natural products have long been a vital source of bioactive compounds with diverse pharmacological properties (Knoth and Kenar, 2004). Among these, medicinal plants continue to attract scientific interest due to their rich phytochemical

compositions and therapeutic potential (Atanasov et al. 2021). *Parkia biglobosa* (Jacq.) R. Br. ex G. Don (African locust bean) is a leguminous tree widely distributed across West Africa. This plant is traditionally recognised for its nutritional, medicinal, and economic values. Various

parts of *Parkia biglobosa*, such as its seeds, leaves, and bark, have been widely used in ethnomedicine for their antihypertensive, antidiabetic, anti-infectious, and anti-inflammatory properties (Ajaiyeoba et al 2003;Ibrahim et al. 2014). This traditional knowledge forms the basis of this research, connecting the reader to the potential impact of our findings on their field of study.

The secondary metabolites present in this plant, such as alkaloids, flavonoids, tannins, and phenolics, have demonstrated significant biological activities, including antimicrobial, antioxidant, anti-inflammatory, antidiabetic, and anticancer properties (Daglia 2012). These compounds' extraction and structural elucidation offer helpful information regarding their potential therapeutic applications (Elott, 2004). Acetone, a medium polarity solvent, has been widely employed in phytochemical extractions due to its ability to dissolve a broad spectrum of bioactive compounds efficiently (Azimi et al , 2025).

Column chromatography was a key purification technique to isolate these bioactive constituents. This method utilises a stationary phase, typically silica gel, to separate compounds based on their polarity and their interaction with the mobile phase (Ozlem, 2016). The fractions obtained were systematically analysed using thin-layer chromatography (TLC), which allowed for the rapid identification and monitoring of compound separation patterns (Wagner and Blatt, 1996). By comparing retention factors (R_f values) and observing spots under UV light, the purity and composition of each fraction were assessed for further analysis. Following isolation, purified

compounds were identified using Proton Nuclear Magnetic Resonance (^1H NMR) spectroscopy. This technique provided detailed information on the molecule's hydrogen environment, aiding in identifying functional groups and molecular frameworks (Pretsch et al. 2006). The interpretation of ^1H NMR spectra tentatively identified the compound structures (Silverstein, 2014).

This study aims to isolate, identify, and elucidate the structures of the bioactive compounds present in the acetone extract of the *Parkia biglobosa* fruit husk. The research employs chromatographic and spectroscopic techniques to tentatively identify the pharmacologically relevant compounds contributing to the plant's medicinal properties. The findings of this study provide a scientific basis for the traditional uses of *P. biglobosa* fruit husk.

MATERIALS AND METHODS

Plant Collection and Identification

A branch of the *Parkia biglobosa* tree containing the leaves, stems, and fruits was collected from Chibok town (Latitude: 10.8364, Longitude 12.7705) in Borno state, Nigeria (10° 52' 11" N 12° 50' 48"E) in July, 2022 and submitted for confirmation and authentication by a Plant taxonomist in the Department of Biological Sciences, University of Maiduguri. A voucher specimen with the name and number *P. biglobosa*, No. 16/10151/PHD, was deposited in the Department of Veterinary Pharmacology and Toxicology herbarium, Joseph Sarwuan Tarka University, Makurdi.

Extract Preparation

The fruit husks of *Parkia biglobosa* were removed from the plant, air-dried at room

temperature, and then pulverised by grinding with a pestle and mortar. Using the cold macerated method described by Umeh et. al. (2005) acetone was the solvent used for the extractions. Two hundred grams (200 g) of the pulverised plant material were weighed and mixed with one litre (1 L) of the solvent in a conical flask. The mixture was allowed to stand for four days and then filtered using Whatman No. 1 filter paper in a one-way filtration. The extract was recovered by evaporation using a water bath at 45 °C, then labelled, and stored at 4 °C until used.

Column Chromatography

Column chromatography is a fundamental technique for separating and purifying bioactive compounds from plant extracts. In this study, the acetone extract of the *Parkia biglobosa* fruit husk was subjected to column chromatography to isolate its constituent compounds based on their polarity and affinity for the stationary phase.

Setup and Column Geometry

The glass column had a 3.0 cm internal diameter (I.D), Packed Silica bed is 30.0 cm high, 1 Column Volume = $\pi r^2 l$, $\pi \times (1.5)^2 \times 30 = 212$ ml, Silica = 300 g for a 10 g crude load, Eluents = 100 % n-hexane and ethyl acetate

Chromatographic Separation

Silica gel (300 g) was packed into a 30 cm glass column as a wet slurry in hexane, ensuring uniform packing and complete removal of entrapped air bubbles to achieve a consistent bed height. The column was gently tapped during packing to eliminate voids and ensure even distribution of the stationary phase. After packing, the column was equilibrated with 100 % hexane by

passing the solvent through the silica bed until three clear column volumes (approximately 636 mL) had eluted. This equilibration step was carried out to stabilize the column and establish uniform solvent flow conditions prior to sample loading (Umeh et al. 2005).

Ten grams (10 g) of the acetone crude extract obtained from the fruit husk of *Parkia biglobosa* was accurately weighed and dissolved in 25 mL of dichloromethane to ensure complete solubilization. The resulting homogeneous solution was thoroughly mixed with approximately 15 g of silica gel to facilitate uniform adsorption of the extract onto the silica surface. The solvent was then evaporated under reduced pressure to yield a dry, free-flowing powder, herein referred to as the pre-adsorbed sample. This pre-adsorbed silica mixture was carefully and evenly loaded onto the top of the equilibrated silica gel column to maintain the integrity of the stationary phase. A protective layer of approximately 1 cm of plain silica gel was gently added above the loaded sample to prevent disturbance of the adsorbed band during elution.

The chromatographic separation was initiated using 100 % hexane as the mobile phase, resulting in the collection of fractions designated VT1–VT12. The polarity of the mobile phase was then gradually increased by introducing a mixture of hexane and ethyl acetate in a ratio of 95:5 (v/v), from which fractions VT13–VT29 were obtained. Finally, elution was continued with a hexane:ethyl acetate mixture in a ratio of 90:10 (v/v), yielding fractions VT30–VT55. Each fraction was collected sequentially at a constant flow rate and uniform volume to

ensure consistent separation, about 11.6 mL per fraction was collected.

All collected fractions were monitored by thin-layer chromatography (TLC) on silica gel 60 F₂₅₄ plates. The TLC plates were developed in solvent systems corresponding to the elution polarity and visualized under UV light at 254 nm and 365 nm. Further visualization was performed by spraying the plates with vanillin sulfuric acid reagent, followed by gentle heating to reveal distinct chromatographic spots. Fractions exhibiting similar TLC profiles based on comparable *R_f* values and spot coloration were combined to yield pooled subfractions for subsequent purification and characterization.

Upon concentration under reduced pressure and drying to constant weight, three major compounds were isolated. Fraction VT14 afforded 1.06 g (10.6 % yield relative to crude extract 10g) of a pale yellow crystalline solid, fraction VT22 gave 0.99 g (9.9 % yield relative to crude extract of 10g) of a bluish amorphous powder, and fraction VT40 provided 1.15 g (11.5 % yield relative to crude extract of 10g) of a pale yellow amorphous solid. The combined yield of these isolated compounds represented approximately 32 % of the total chromatographic recovery relative to the initial crude extract. All purified fractions were stored in airtight vials at 4 °C until further analysis.

The purified compounds (VT14, VT22, and VT40) were subsequently subjected to detailed nuclear magnetic resonance (NMR) spectroscopy using JEOL JNM-ECZ400S/L1 400 MHz NMR Spectrometer

for compound identification and structural elucidation.

Thin Layer Chromatography

Thin-layer chromatography (TLC) was carried out on the fractions using a TLC (Silica Gel F254) plate, which showed the rate of movement of compounds in the sample. The analyte solution was added in one spot, dropwise, to a pencil line drawn about 1 cm from the bottom of the TLC plate. When spotting the plate, a drop of the fraction solution was added gently so as not to destroy the silica. Once the solvent had evaporated from the analyte "spot," the TLC plate was placed in a developing chamber. A small glass jar with a lid large enough to fit the TLC plate is needed to create a TLC-developing chamber. Three (3) different eluent mixtures were used at different ratio for the TLC; Ethyl-acetate: *n*-Hexane (8:2), Ethyl-acetate: Chloroform (5:5), Ethyl acetate: *n*-Hexane:Chloroform (6:2:2). The eluent were added to a height of ~0.5 cm. The level was below the origin line drawn on the TLC plate. A piece of filter paper was then fitted around approximately half the vertical walls of the jar with the bottom edges immersed in the eluent to aid capillary action. This wetted paper ensures that the eluent's vapour is uniform throughout the developing jar once its lid is secured. When the TLC plate was placed in the jar, the eluent served as the mobile phase, moving up the silica on the plate (also by capillary action). The different compounds in the analyte mixture were carried in the eluent (up the plate) at different rates depending on polarity. The TLC plate was removed before the eluent could move beyond the top of the silica powder on the plate. The line to which the

eluent moves is called the solvent front. The solvent front was marked immediately after removing the plate from the developing jar. The TLC plate was then observed under UV light; the result was quantified by

calculating the retention factor (R_f). An R_f value is the ratio of the distance the analyte travelled versus the distance the eluent travelled.

$$R_f = \frac{\text{Distance from origin to the analyte}}{\text{Distance from the origin to solvent front}}$$

The distance an analyte travels depends on its structure and the identity of the stationary and mobile phases. Furthermore, the distance an analyte travels remains as long as the stationary and mobile phases are the same.

Structural Elucidation of Compounds

The structures of the isolated compounds were elucidated using proton nuclear magnetic resonance (^1H NMR) spectroscopy. The purified fractions were dissolved in deuterated chloroform (CDCl_3), and tetramethylsilane (TMS) was used as the internal chemical shift reference ($\delta = 0.00$ ppm) (Gottlieb et al. 1997). The ^1H NMR spectra were recorded on a Fourier transform nuclear magnetic resonance (FT-NMR) spectrometer. Nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical technique for the

structural characterization of organic compounds. It provides information on the number, type, and chemical environment of hydrogen atoms within a molecule, thereby facilitating structural elucidation (Silverstein, 2014; Claridge, 2016). The isolated compounds were identified by interpreting their chemical shifts, signal multiplicities, integration values, and coupling patterns and comparing the resulting spectral data with published literature values (Claridge, 2016; Field et al. 2013). The assignments obtained from the ^1H NMR spectra enabled the tentative identification of the isolated compounds as oleic acid, myristic acid and lauric acid.

RESULTS AND DISCUSSION

The percentage yield of the extract was calculated using the formular:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Starting plant material}} \times 100$$

Thus the extraction yield of 16 % (32 g) was obtained from the extract of *Pakia biglobosa* fruit husk.

Thin-layer chromatography:

The result of thin-layer chromatography of the acetone extract in different solvent mixtures was recorded in Table 1.0. The fractions spotted a minimum of two groups of compounds in various solvent mixtures, and their R_f values were calculated and

recorded. The spots observed were colourless in daylight but coloured under UV light (Fig 1).

Thin-layer chromatography (TLC) was used to optimize solvent systems and monitor the separation of the crude extract

and column fractions. Several developing solvent systems of varying polarity, including ethyl-acetate:n-Hexane (8:2, v/v), Ethyl-acetate:Chloroform (5:5, v/v) and ethyl-acetate:n-Hexane:Chloroform (6:2:2, v/v), were evaluated to achieve optimal resolution of the components. These solvent systems were employed exclusively for TLC analysis and were distinct from the mobile phases used for column chromatography, which consisted of n-hexane and n-hexane:ethyl acetate mixtures of increasing polarity.

Ethyl acetate: *n*-Hexane (8:2) solvent mixture gave two (2) spots with *R_f* values of 0.213 cm (Yellow) and 0.313 cm (Blue), Ethyl acetate:Chloroform (5:5) solvent mixture gave two (2) spots with *R_f* values of 0.286 cm (Yellow) and 0.120 cm (Blue) and Ethyl acetate:*n*-Hexane:Chloroform (6:2:2) also gave two (2) spots with *R_f* values of 0.120 cm (Yellow) and 0.200 cm (Blue-black).

Identification and structural elucidation of the compounds present in the acetone extract

Three fractions of the acetone extracts labeled VT14, VT22, and VT40 were subjected to proton NMR spectroscopic analysis to identify the compounds present and their structural elucidation. The results of the spectra for these fractions are represented in Figs. II, III, and IV, respectively.

¹H NMR spectroscopic analyses were conducted on fractions VT14, VT22, and VT40

¹H NMR spectroscopic analyses of fractions were carried out. Three fractions showed similar signal areas: an olefinic region (circa 5.35 ppm), an aliphatic region typical

of undisturbed methylene (1.25-1.37 ppm) and disturbed methylene (2.63-2.78 ppm), and a methylic proton (0.88 ppm) area; these signal areas are typical of long-chain fatty acids (Knothe and Kenar, 2004). Integration of the ¹H NMR signal areas for each sample, however, showed no significant differences in the undisturbed methylene area (1.25-1.37 ppm) because the area of the spectra signals is proportional to the number of hydrogen atoms of each proton type in the samples; the chain lengths of each were calculated, and signals from the olefinic region (5.32-5.35 ppm), including the attendant allylic (1.98-2.02 ppm), were taken arising from a second compound due to their inchoate integral values (Fig 1)(Knothe and Kenar, 2004). Thus, VT14 had the following data: ¹H NMR (400 MHz, Chloroform-d) 2.33 (t, *J* = 7.5 Hz, 2H), 2.00 (q, *J* = 6.5 Hz, 1H), 1.62 {(p, *J* = 7.3 Hz, 3H); Solvent}, 1.38 – 1.20 (m, 22H), 0.87 (t, *J* = 6.7 Hz, 3H) (Fig. II), VT14 displayed resonances at δ 5.34 (td, 1H), characteristic of an olefinic proton, δ 2.33 (t, 2H) attributable to methylene protons α to the carboxyl group, δ 2.00 (q, 2H) assigned to allylic methylene protons, a multiplet centred at δ 1.62 corresponding to β -methylene protons, a broad methylene envelope at δ 1.26, and a terminal methyl triplet at δ 0.87 (3H). These spectral features are consistent with those of a long-chain monounsaturated fatty acid; VT22 revealed the following: ¹H NMR (400 MHz, Chloroform-d) δ 5.42 – 5.26 (m, 0H), 2.33 (t, *J* = 7.5 Hz, 3H), 1.62 (p, *J* = 7.2 Hz, 3H), 1.26 (d, *J* = 13.6 Hz, 20H), 0.87 (t, *J* = 6.7 Hz, 3H) (Fig. III) at the same time, VT40 had the following data: ¹H NMR (400 MHz, Chloroform-d) δ 5.42 – 5.26 (m, 0H), 2.33 (t, *J* = 7.5 Hz, 2H), 1.62 (p, *J* = 7.2 Hz, 2H), 1.26 (d, *J* = 13.6 Hz, 20H), 0.87 (t, *J* =

6.7 Hz, 3H) (Fig. IV). Table 2 shows some characteristic signal areas of fatty acids.

Characterisation of VT14, VT22, and VT40 as Oleic acid, Myristic acid and Lauric acid

Because saturated fatty acids do not contain olefinic protons, the resonance observed at δ 5.30–5.40 ppm was attributed to trace unsaturated fatty acid impurities. Assuming that a monounsaturated fatty acid contributes two olefinic protons per molecule (or four protons in the case of a diunsaturated fatty acid), the relative abundance of the impurity was estimated by comparing the integrated olefinic resonance with the total aliphatic proton signals. Based on these integrations, the estimated

impurity ratios were approximately 1:5 for VT14, 1:7 for VT22, and 1:4 for VT40. The corresponding methylene proton contributions from the impurity were subtracted from the integrated methylene envelope (δ 1.20–1.40 ppm), yielding methylene integrals of 16H for VT14, 17H for VT22, and 15H for VT40. These values were subsequently used to estimate the carbon chain lengths of the isolated fatty acids. Similar approaches have been reported for the interpretation of fatty acid ^1H NMR spectra in the presence of minor unsaturated components (Claridge, 2016; Silverstein, 2014) for a straight-chain fatty acid, the carbon chain length can be estimated from the ^1H NMR integrals using the formula:

$$\text{Chain length (number of carbons)} = 1 + \left(\frac{I_{\text{CH}_3}}{3}\right) + \left(\frac{I_{\alpha\text{-CH}_2}}{2}\right) + \left(\frac{I_{\beta\text{-CH}_2}}{2}\right) + \left(\frac{I(\text{CH}_2)_n}{2}\right)$$

where:

I_{CH_3} = integral of the terminal methyl protons (3H),

$I_{\alpha\text{-CH}_2}$ = integral of the methylene adjacent to the carboxyl group (2H),

$I_{\beta\text{-CH}_2}$ = integral of the β -methylene group (2H),

$I(\text{CH}_2)_n$ = corrected integral of the remaining methylene protons,

the additional 1 accounts for the carboxyl carbon, which has no directly attached proton and is therefore not observed in the ^1H NMR spectrum.

Therefore:

Chain length (VT14) = 1 + (methyl region integral/3) + (undisturbed methylenes/2) + (H-3 acyl moiety/2) + (H-2 acyl moiety/2) + carboxylic carbon = + 18,

Chain length (VT22) = 1 + (methyl region integral/3) + (undisturbed methylenes/2) +

(H-3 acyl moiety/2) + (H-2 acyl moiety/2) + carboxylic carbon = + 14, and

Chain length (VT40) = 1 + (methyl region integral/3) + (undisturbed methylenes/2) + (H-3 acyl moiety/2) + (H-2 acyl moiety/2) + carboxylic carbon = +12.

Based on their chain lengths and literature comparisons, VT14, VT22, and VT40 were said to be consistent with oleic acid, myristic acid, and lauric acid, as represented in table 3.0

The different R_f values of the spotted compounds gave an idea of their polarity. Highly polar compounds have lower R_f values, while low-polar compounds have higher R_f values. The R_f value of a compound is constant across experiments only if the chromatographic conditions are constant, including the solvent system, adsorbent, adsorbent thickness, and number of spots identified. Since these factors are difficult to keep constant from experiment to experiment, relative R_f values are generally considered. "Relative R_f " means that the values are reported relative to a standard, or you compare the R_f values of compounds running simultaneously on the same plate. TLC profiling of the fractions in this experiment indicates an impressive result, pointing toward the presence of some bioactive compounds in the plant. Various phytochemicals demonstrate different R_f values in different solvent systems; the variation in R_f values of the phytochemicals provides an essential clue in understanding their polarity.

The TLC plates were visualized under ultraviolet light (254 and 365 nm) and after spraying with vanillin sulfuric acid reagent followed by heating. The chromatograms revealed several distinct spots with varying R_f values and colour responses, indicating the presence of multiple constituents within the extract. Although vanillin sulfuric acid is a useful general detecting reagent for a wide range of phytochemicals, the observed spot colours alone are insufficient for the definitive identification of specific classes

of compounds. Therefore, the TLC results were used primarily to monitor the separation and assess the purity of the fractions

1. Yellow TLC spots with R_f values 0.213, 0.286, and 0.120, the low R_f values indicate these constituents are relatively polar, likely due to hydroxyl groups that interact strongly with the stationary phase.

2. Blue TLC spots with R_f values 0.313, 0.185, and 0.200, their intermediate higher R_f values and variation indicate structurally diverse secondary metabolites (Benmehdi et al. 2012; Harborne, 1998; Wagner and Bladt, 1996).

The NMR spectra further tentatively identified the bioactive compounds present in this plant extract as oleic, myristic and lauric acids. This result supports the research of Olowokere *et al.*, (2019); where lauric acid was present in the seed of *Parkia biglobosa*. Also, Mathew *et al.*, (2015), revealed the presence of myristic and lauric acid in a study comparing the effects of the fatty acid composition of *Parkia biglobosa* and Mesquite bean seeds (Matthew et al 2015). The tentative occurrence of an oleic acid-like compound in the acetone extract of *Parkia biglobosa* fruit husk is consistent with previous reports demonstrating the presence of oleic acid among the fatty acid constituents of *P. biglobosa* seeds and seed oil (Aremu et al, 2006; Oluwaniyi and Dosumu, 2009). These studies identified oleic acid together with linoleic, palmitic, stearic, myristic, and lauric acids as naturally occurring fatty acids in *P. biglobosa*, although their relative abundance varied depending on the plant part and analytical method employed.

Although the spectral characteristics of the sample compounds in this plant extract resembled those of the known compounds oleic, myristic and lauric acid, definitive identification would require additional spectroscopic techniques such as ^{13}C NMR and/or mass spectrometry. Table 3.0 shows the comparison of proton nuclear magnetic resonance (^1H NMR) spectra of the unknown compound (VT14, VT22 and VT40) with the known oleic acid, myristic acid and lauric acid. Both myristic and lauric were reported to have an inhibitory effect against bacteria and helminths (Chifu et al. 2011; Matsue et al. 2019; Giancarlo et al. 2021). Lauric acid has been shown to possess antifungal activity against *Aspergillus niger* and *Candida albicans* (Akula et al. 2001; Bergsson et al. 2002). Myristic acid and lauric acid were shown to inhibit the growth of *Staphylococcus aureus* isolated from bovine mastitis in a study conducted by Kelsey et al., (2006), the presence of these compounds in the fruit husk of *Parkia biglobosa* may be responsible for the antimicrobial and anthelmintic activity by this plant. Both myristic acid and lauric acid have been shown to have anticancer activities (Nishikawa et al. 1976; Rosemaria et al. 2017) the cytotoxic activity of the fruit husk of *Parkia biglobosa* may be attributed to the presence of these bioactive compounds. Myristic acid has also been shown to reduce the risk of Type II diabetes (Takato et al. 2017) myristic acid is, therefore, a potential compound for preventing and treating Type II diabetes mellitus and its related diseases.

CONCLUSION

The present study successfully isolated three major compounds from the acetone extract of *Parkia biglobosa* fruit husk using

column chromatography. Based on the interpretation of ^1H NMR spectral data and comparison with published literature, the isolated compounds were tentatively identified as an oleic acid-like monounsaturated fatty acid (VT14), myristic acid (VT22), and lauric acid (VT40). These findings indicate that the fruit husk of *P. biglobosa* contains both saturated and unsaturated fatty acids, which may contribute to its reported biological properties.

Since the structural assignments were based primarily on ^1H NMR spectroscopy, further characterization using complementary analytical techniques such as ^{13}C NMR, GC-MS, or high-resolution mass spectrometry is recommended to confirm the identities of the isolated compounds. Future studies should also investigate the pharmacological activities of these compounds individually and explore their potential applications in the development of natural products for medicinal, veterinary, and agricultural use. In addition, comparative phytochemical studies involving *P. biglobosa* fruit husk and other plant-derived sources may provide further insight into the occurrence, abundance, and bioactive potential of these fatty acids.

V. DECLARATION OF INTEREST, FUNDING AND ACKNOWLEDGEMENTS

Declaration of interest: There is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported

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Table 1.0. Thin-layer chromatographic separation of the acetone extract of *Parkia biglobosa* fruit husk using different TLC developing solvent systems

Solvent mixture	Extract	No. of spots	R _f value (cm)	Color spot
Ethyl acetate:	Acetone	2	0.213	Yellow
n-Hexane (8:2)			0.313	Blue
Ethyl acetate:	Acetone	2	0.286	Yellow
Chloroform (5:5)			0.185	Blue
Ethyl acetate:	Acetone	2	0.120	Yellow
n-Hexane:			0.200	Blue Black
Chloroform (6:2:2)				

Table 2.0 Some Characteristic Signal Areas of Fatty Acids

Signal	Chemical shift	Functional group [29, 30]
1	0.83-0.93	-CH ₃ (saturated, oleic, and linoleic acyl chains)
2	0.93-1.03	-CH ₃ (linolenic acyl chains)
3	1.22-1.42	-(CH ₂) _n (acyl chains)
4	1.52-1.70	-OCO-CH ₂ -CH ₂ - (acyl chains)
5	1.94-2.14	-CH ₂ -CH=CH- (acyl chains)
6	2.23-2.36	-OCO-CH ₂ - (acyl chains)
7	2.70-2.84	=HC-CH ₂ -CH= (acyl chains)
8	4.10-4.32	-CH ₂ OCOR (glyceryl group)
9	5.20-5.26	>CHOCOR (glyceryl group)
10	5.26-5.40	-CH=CH- (acyl chains)

Table 3.0 Comparative ¹H NMR Spectral Data and Tentative Identification of Compounds Isolated from the Acetone Extract of *Parkia biglobosa* Fruit Husk

Fraction	Key ¹H NMR signals (δ, ppm)	Estimated carbon chain length	Literature comparison	Proposed identity
VT14	5.34, 2.33, 2.00, 1.62, 1.20–1.38, 0.87	C18	Consistent with published ¹ H NMR data for oleic acid, including the characteristic olefinic and allylic proton resonances	Tentatively assigned as Oleic acid-like monounsaturated fatty acid
VT22	2.33, 1.62, 1.20–1.40, 0.87	C14	Consistent with published ¹ H NMR data for myristic acid	Tentatively identified as Myristic acid
VT40	2.33, 1.62, 1.20–1.40, 0.87	C12	Consistent with published ¹ H NMR data for lauric acid	Tentatively identified as Lauric acid

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Figure I: TLC plate indicating two spots with black arrows under UV light and the X is the solvent front.

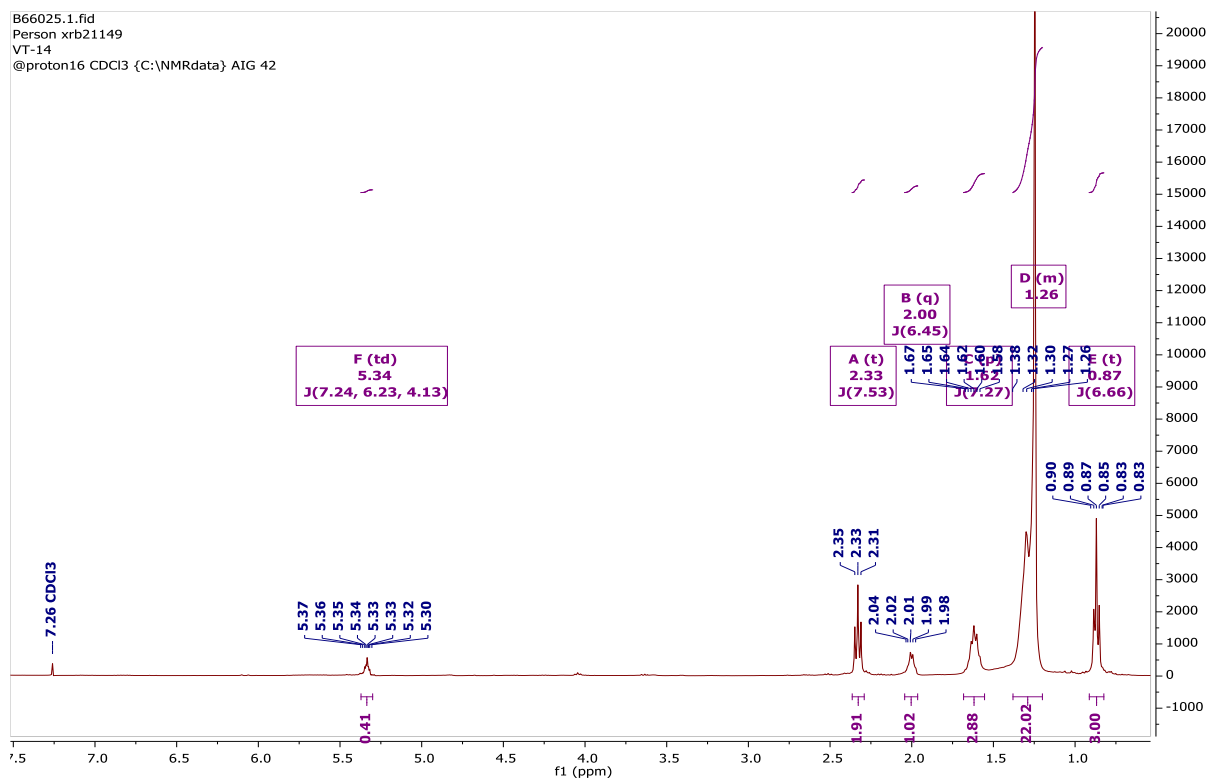


Figure II: Proton NMR Spectrum for VT14

Complete ^1H NMR spectrum of VT14 (400 MHz, CDCl_3) with expanded views of the aliphatic region (δ 0.5–3.0 ppm) and olefinic region (δ 5.0–6.0 ppm), showing the diagnostic resonances used for tentative structural assignment.

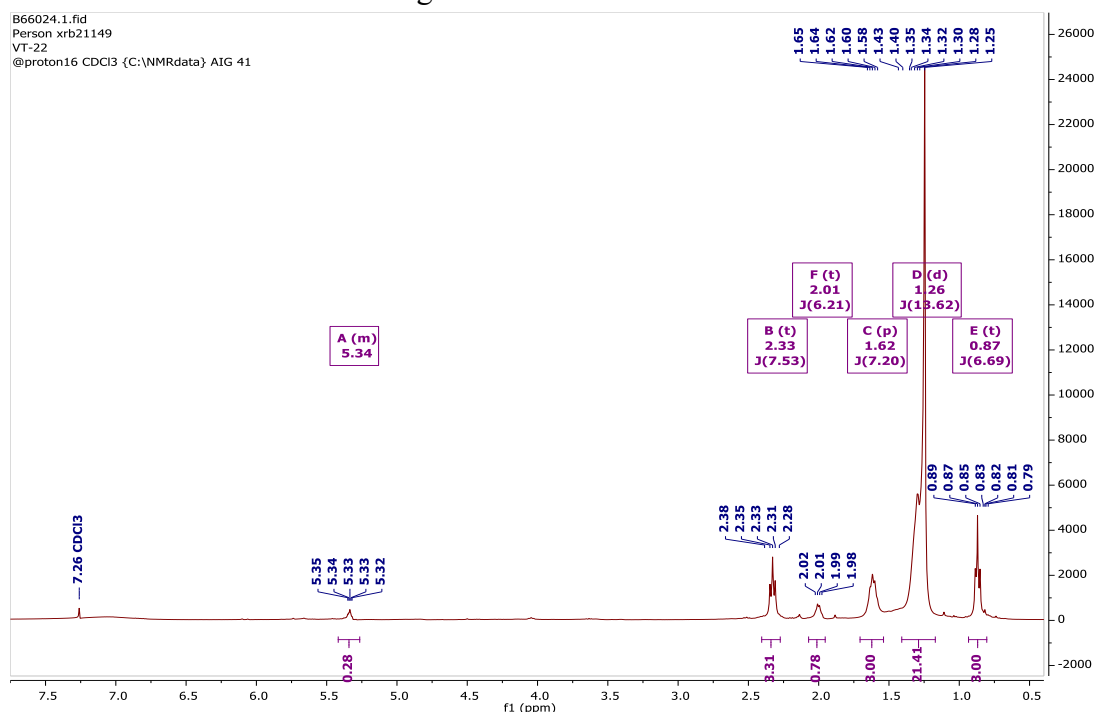


Figure III: Proton NMR Spectrum for VT22

Complete ^1H NMR spectrum of VT22 (400 MHz, CDCl_3) with expanded views of the aliphatic region (δ 0.5–3.0 ppm) and olefinic region (δ 5.0–6.0 ppm).

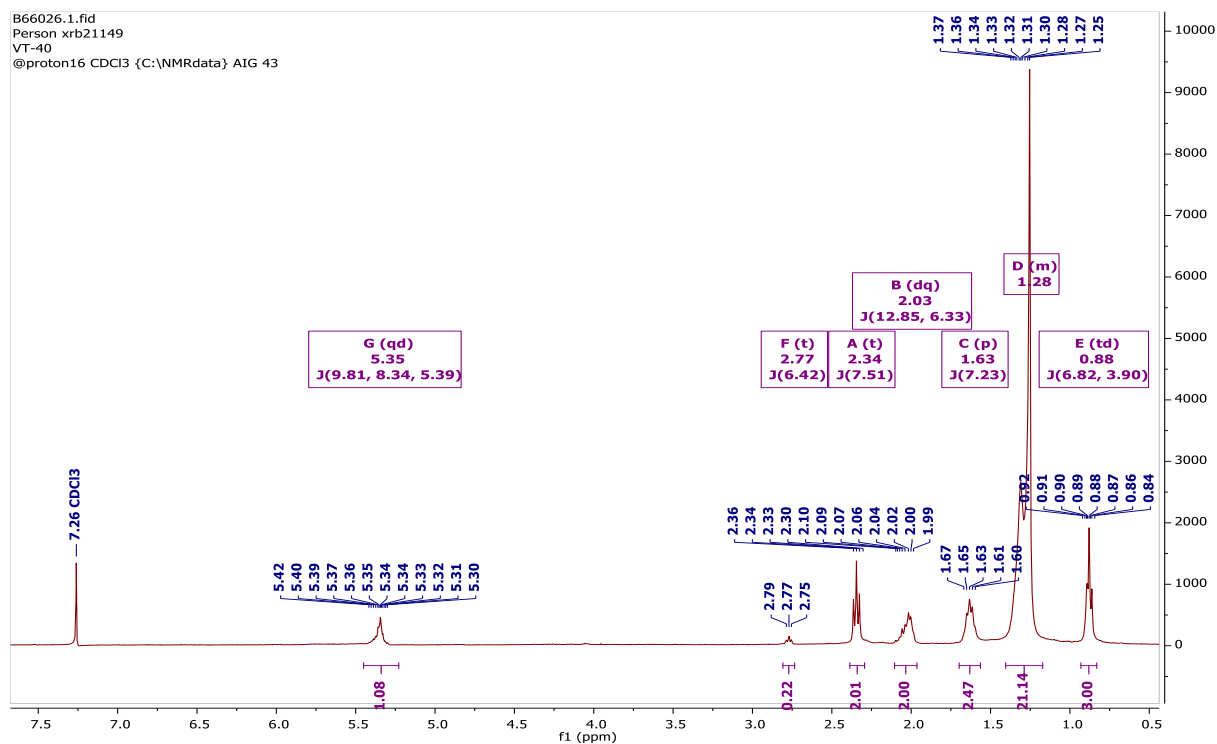


Figure IV: Proton NMR Spectrum for VT40

Complete ^1H NMR spectrum of VT40 (400 MHz, CDCl_3) with expanded views of the aliphatic region (δ 0.5–3.0)

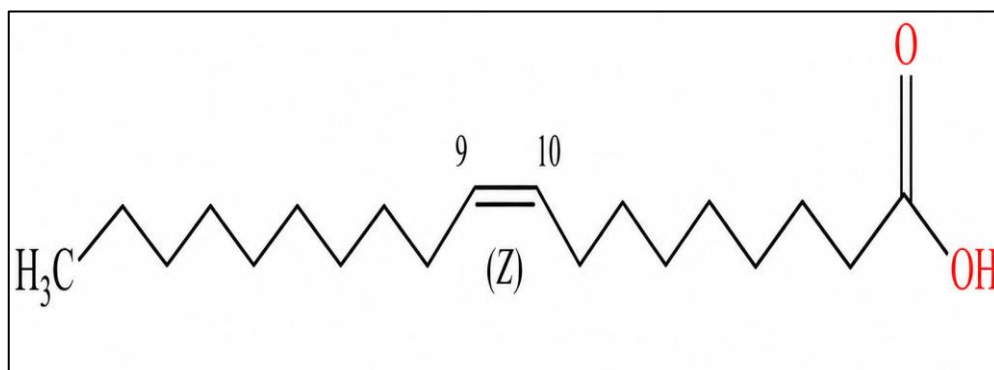


Figure VII: The structure of Oleic acid (VT14)

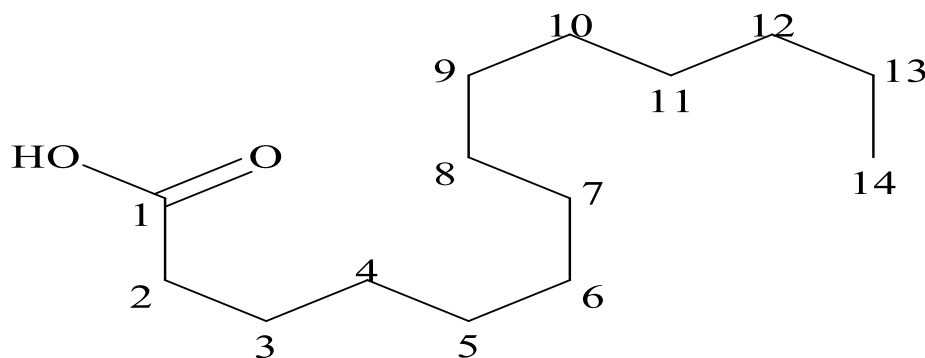


Figure VI: The structure of Myristic acid (VT22)

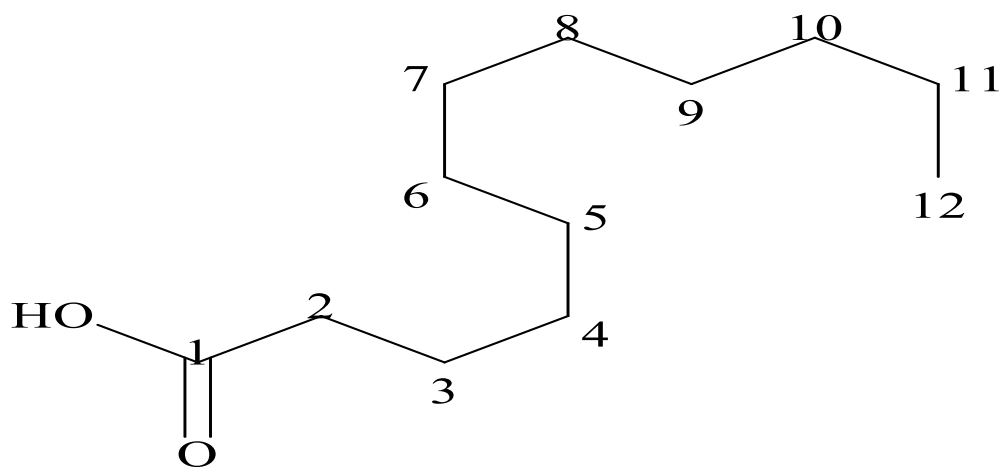


Figure V: The structure of Lauric acid (VT40)