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Fatty acids from choerospondias axillaris stem bark and its activities against some clinical isolates: spectroscopic characterization and identification

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ABSTRACT: Since the use of plants in traditional medicine forms forms the foundation for natural therapeutic agents, the quest for ways to treat current illnesses in the absence of synthetic medications opened up new avenues for research into the therapeutic potential of medicinal plants. A growing number of researchers are looking for novel treatments to treat infectious illnesses that are either reemerging or emerging because of antimicrobial resistance and the apparent adverse effects of synthetic medications. By separating, describing, and identifying potential medicinal compounds from Choerospondias axillaris stem bark and assessing the compounds' efficacy against a few clinical disease-causing pathogens, this study aimed to further this quest. With the help of n-hexane and ethyl acetate, the plant sample under inquiry was crushed into a powder and then extracted in stages using a soxhlet apparatus. When phytochemicals were measured using GC-FID, the results revealed that sapogenin had the highest concentration (44.17 ppm), followed by epihedrine (42.28 ppm), and kaempferol (0.36) had the lowest concentration. The extracts were separated using a silica gel column and eluted with increasing amounts of methanol in ethyl acetate. The acquired fractions were spectroscopically investigated using TLC, and based on their profiles, 50 fractions were obtained and pooled to form 10 combined fractions, which were then subjected to 1D and 2D NMR analysis to determine the components' structures. According to the results, PE-20, 21, 22, and 29 were fatty acids, namely linoleic, stearic, eicosenoic, and docosadienoic acids. High bacterial sensitivity to the crude extract was shown by the antimicrobial result, suggesting that the plant may be utilized as a prophylactic for illnesses brought on by the pathogens used in the investigation. The plant portion is a natural supply of fatty acids, as the research has shown.

Keywords: Choerospondias axillaris, fatty acids, clinical isolates, phytochemicals
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INTRODUCTION

For a long time, the native inhabitants of Nigeria have used medicinal plants as their primary source of treatment. This is particularly true considering that research has shown that over 85% of the world's population still heavily relies on medicinal plants for healthcare (Pesic, 2015). They also provide 80% of all synthetic pharmaceuticals, according to Bauer and Bronstrup (2014), providing them with a source for the creation of novel medications. Humanity has always used medicinal plants to heal illnesses. Because of their many health advantages, plants have long been used as a source for newly developed pharmaceutical chemicals and drugs. There are two types of support offered for the creation of innovative medications. They might serve as the foundation for creating a medication, a model for creating a natural medication, or a phytomedicine to cure illnesses Iwu (1993). The active compounds found in medicinal plants are a rich source of chemicals that are

used in the food, cosmetic, and pharmaceutical sectors, and more recently, in agriculture to control pests (Rice, 1995). Herbal medicines derived from medicinal plants are chosen because they are more effective, less toxic, well-tolerated by culture, and need less testing. The chemical components found in herbal goods are thought to be better suited to the human body since they are a part of the physiological processes of living organisms. Lapsi (Choerospondias axillaris), a fruit tree that was among the earliest of its type in Nepal, is a member of the Anacardiaceae family. The fruit tree may reach heights of 20 to 40 feet and diameters of 30 to 40 centimetres.

The plum-like fruit is sometimes known as "hog plum" and has a greenish-yellow colour. It has an extremely sour flavour when mature. The pulp and seed were tightly bound together before to cooking, but they can now be readily separated.

Warm-weather fruit trees are nonetheless commonly planted as an annual income crop despite this (Tang and Eisenbrand, 1992; Manandhar, 2002). Researchers have identified the nutritional components of Choerospondias axillaris (Lapsi) fruit: amino acid, vitamin C, reductic and non-reductic saccharides, organic acid, pectin, tan fat, and trace element (Chang et al., 1996). Lapsi's blooming season lasts three months, from April to June, while its fruiting period spans from July to September.

The rapidly growing deciduous tree is significant for both medicine and the economy. At a height of around 600 metres, it thrives in hill and mountain woods. East Asian nations including China, Nepal, India, Bhutan, Japan, Cambodia, and Vietnam are the main cultivators of it. It is significant because of its hard seeds, fruit, bark, and wood.

Fruits may be eaten, and the wood is used to make wine, pickles, jellies, ice cream, spicy candies, and sweets. Fibre from the bark is used to make rope and fuel (Poudel, 2003). Pericarp, or the wall of the fruit, has medicinal uses. It is a low-sodium, healthful fruit. Brick kilns utilize the hard seed shells as fuel. The agricultural community in Nepal has traditionally grown the tree as a means of making money. International markets get the fruit that is utilized locally as well as exported (Tang and Eisenbrand, 1992; Chang et al., 1996; Manandhar, 2002; Poudel, 2003; Hoang et al., 2004). On the other hand, not much is known about Lapsi's availability or sales in Nepal's different marketplaces.

Even though certain plant kinds are important for ethnomedicine, not enough study has been done on them in the South-South. This is especially true for the ones that the people of Rivers State have historically utilized. Thus, in order to discover its use in traditional medicine and provide scientific evidence for it, it is important to isolate, characterize, and identify the bioactive components from Choerospondias axillaris stem bark extract and its biological activity against clinical infections. Choerospondias axillaris is a succulent wild fruit that grows in numerous provinces in south China. It wisely selects an elevation range of 300–800 m (He et al., 2003).

The fruit may be eaten immediately and is sweet, tart, and excellent. According to Doanh et al. (1996) and Joshi et al. (2015), the leaves may be used to make green manure, the bark can be used as a raw material for tanning and tannin extracts, and the nucleus can be used as a raw material for activated carbon. It may also be used to process sour jujube cake and wine. Therefore, Choerospondias axillaris is frequently processed for south SuanZaoGao, wild jujube juice, zizyphus jujube wine, and other products.

In particular, south SuanZaoGao has always been the most popular food in the market. However, due to its flavour, acidity, and perishable fermentation after harvest, it loses a lot of nutrients and is not conducive to fresh jujube fresh-keeping and circulation (Mi et al., 2019).

Customers both domestically and internationally have grown to strongly favour Choerospondias axillaris goods because of their unique flavour, owing to worries about food safety (Qing et al., 2005; Wang et al., 2014). However, the growth of jujube has been hampered by the absence of well-known processing businesses, breeding, and efficient auxiliary technologies (Li et al., 2016; Mi et al., 2015).

Therefore, we should aggressively pursue the development of the green sector, completely use the potential of the Choerospondias axillaris resource, and work towards ensuring the sustainable, all-encompassing use of resources. We should also effectively utilize innovation to transform resource advantages into economic benefits.

This may be achieved by considering methods for industrializing ecological building, changing the way forest products are processed, and expanding the industrial supply chain. To optimize the utilization of Choerospondias axillaris, the molecular active components, cracking processes, and cleavage products of the bacterium are examined in this experiment.

The lapsi tree is often used for private planting on slopes as part of a community forestry programme (CBS 1998).

Lapsi trees are scarce in the natural forest. Over 40,000 fruit-bearing trees and over 450,000 additional trees have been planted throughout the nation. In Nepal, there is a great potential to generate income and jobs via the proper maintenance and use of the lapsi tree (Poudel, 2003).

In order to establish the antibacterial effectiveness of the crude extract and to elucidate the structure of the detected component, this work examined the phytochemical screening of the Choerospondias axillaris stem bark crude extract, which was followed by its isolation and characterization using NMR.

MATRIALS AMD METHODS

Sample collection and preparation

In April of 2022, Choerospondias axillaris was taken from Bara-kpete Bunu in the Tai Local Government Area of Rivers State, Nigeria. A botanist from the biology department of Ignatius Ajuru University of Education in Port Harcourt recognized it and verified its identity. After being stored in the herbarium, the sample was air dried for two weeks and then crushed using a mortar and pestle to a powder. It was then put in a glass container for extraction and further analysis.

Extraction of sample

A thimble containing 10g of the powdered sample was sealed and put into the soxhlet apparatus's thimble chamber after it had been weighed appropriately. After adding 250 millilitres of n-hexane to the round-bottom flask, which has a boiling point between 75 and 800 degrees Celsius, the soxhlet apparatus was put together, heated with a heating mantle, and left to reflux for six hours. The round bottom flask's n-hexane vaporizes in the condenser due to heat, and then it drips back to the sample thimble. The liquid is dumped into the circular bottom flask once again when it reaches the Syphon arm. After the procedure was complete, the soxhlet apparatus was disassembled, and the mixture was recycled to create a concentrated sample that was then put into a vial for examination.

Preparation of sample for GC analysis

After dissolving 1 ml of the filtered residue in 20 ml of chloroform, the mixture was poured into a 100 ml volumetric flask and diluted to the appropriate level. After the majority of the chloroform had evaporated at ambient temperature, 1 ml of the reagent (10 vol% benzene and 20 vol% methanol) was added. The mixture was then sealed and heated for 30 minutes in a water bath at 400 c. After that, the organic sample was extracted using hexane and water, resulting in a final reagent combination of hexane and water that was added to the reaction mixture in a 1:1:1 ratio (one millilitre of each hexane and water). For two minutes, the liquid was violently shook by hand to create a stable emulsion. The organic layer was eliminated and half of the top hexane phase was transferred to a tiny test tube for injection (Nna and Ejiofor, 2023).

Chromatographic separation

The filtrate was run via column chromatography using a silica gel stationary phase. The solvent system had an ethyl acetate and n-hexane combination. About 25 mL of fractions were extracted using gradient elution, and the elutes were seen using thin layer chromatography using a solvent ratio of 3:7 for ethyl acetate and hexane. The fractions exhibiting the matching Rf value were combined after being visualised by a UV laser. Anisaldehyde-sulphuric acid was used to precipitate obtained fractions in thin layer chromatography in order to verify them. The retention factor for each sample was determined. Fractions with similar Rf values were combined for NMR analysis, and those with a lot of spots underwent sephadex column chromatography for further purification (Nna, 2023)

Spectroscopic characterization

The purified coding fraction was also submitted for IR, spectroscopic analysis (Proton and Carbon NMR), and melting point determination of the chemical. Using chloroform (CDCl3) as the solvent, the University of Strathclyde in Glasgow, Scotland, the United Kingdom, verified the 1H and 13C NMR spectra using a Bruker Avance 3 spectrophotometer.

Antimicrobial activity of extracts against some selected test bacteria

The organisms were acquired from the University of Port Harcourt's Microbiology Laboratory's Reference Laboratory Section. For a whole day, the organisms were kept on nutrient broth. Three to five pure cultures of the test microorganism were selected using a sterile wire loop to standardize the test germs, which were then emulsified in three to four millilitres of sterile physiological saline. Using physiological saline, the turbidities of the test organisms were adjusted to match the absorbance of the 0.5 McFarland standards at the same wavelength. The turbidity reading of the standards was recorded as absorbance in a Spectrophotometer at 540 nm.

Antimicrobial susceptibility test

Using modified disc diffusion techniques, the extracts' antibacterial properties against the test microorganisms were assessed (Agu et al., 2013; Adindu et al., 2016). Mueller-Hinton plates were cultivated with exactly 25 µl of 0.5 McFarland standardized suspension of test bacteria (1.5x108 cfu ml-1) using the pour plate technique. The 6mm filter paper discs were impregnated with exactly 50 µl of the extracts and then put on two sections of the agar plate. The sizes of the inhibition zones on the different plates were measured and documented in millimetres. Every experiment was carried out three times. Sterile physiological saline was used to set up negative controls, whereas 50 µg/ml was used to set up positive controls. Ciprofloxacine.

Determination of minimum inhibitory concentration (MIC)

For this investigation, the Pallota et al. (2007) approach was modified by Agu et al. (2013). The broth dilution technique was used to establish the Minimum Inhibitory Concentration (MIC). Twenty percent, forty percent, sixty percent, and eighty percent of the extracts were diluted with a dilution factor of 10-2 for fungi and 0.5 McFarland adjusted cultures for bacteria and yeast, respectively, and then inoculated with known quantities of bacterial and fungal inocula in Nutrient Broth and Sabouraud Dextrose Broth. Positive and negative controls were also put up. The tubes were placed on a metabolic rotary shaker (220 rev/min) and incubated at room temperature for 24 hours for bacteria and 48 hours for fungus. After that, the test tubes were incubated for a total of 48 hours for fungus and 24 hours for bacteria before being sub-cultured onto sterile, newly prepared plates. The plates were tallied and the total microbial count was reported in CFU/ml at the conclusion of the incubation time. The minimum indicator plate (MIC) was identified by its lowest count. The minimum inhibitory concentration (MIC) is the concentration at which, after 24 or 48 hours of incubation, the bacteria stop growing.

RESULTS AND DISCUSSION

GC-MS was used to identify and measure the presence of several plant metabolites. Alkaloids, tannins, saponins, flavonoids, and steroids were the chemical classes that were measured (Table 1 and Figure 1). The phytochemical content (measured in parts per million) reveals that saponin (44.17 ppm) and epihedrine (42.28 ppm), both of which are alkaloids and saponins, have the greatest composition of phytochemicals. Kaempferol was the least amount of phytochemical that was measured (0.36 ppm). Ribalinidine (32.99 ppm), spartein (34.60 ppm), aphyllidine (17.96 ppm), and ammodendrine (20.31 ppm) were the individual alkaloids found. The plant has a wide range of potential medical uses due to its high concentration of heterocyclic chemicals such alkaloids (Kurek, 2019).

Kaempferol (0.36ppm), naringenin (10.37ppm), catechin (6.02ppm), proanthocyanidin (4.12ppm), anthocyanin (7.47ppm), flavonones (25.65ppm), flavones (29.86ppm), and tannins (22.73ppm) were among the phytochemicals that belonged to polyphenolics, flavonoids, or tannins. These groups of substances are strong scavengers of free radicals. The present study has verified the use of extracts as antioxidants in pharmacology and medicine. Yadav et al. demonstrated in 2022 that the fruits of Choerospondias axillaris may scavenge free radicals. According to Labh et al. (2015), plant polyphenols seem to have a major impact on reducing the risk of developing a number of illnesses, including inflammation, cancer, arthritis, immune system disorders, and heart disease.

For example, alkaloids have antibacterial, anti-asthmatic, anti-cancer, and anti-malarial pharmacological effects in humans. According to Akinpelu et al. (2014), nutraceuticals and dietary supplements containing saponins are becoming more and more well-liked. Additionally, saponins are utilised to decrease blood cholesterol and as an anticancer agent. Additionally, it facilitates protein translocation across cell membranes due to its amphipathic properties. The fact that glycosides have antibacterial properties is well-established. The phytoconstituents found in this analysis may be effectively compared with studies from previous literature (Owolabi et al., 2010; Yasir et al., 2010). Due to their antifungal and antibacterial properties, alkaloids such aphyllidine, ammodendrine, ribalinidine, spartein, and epihedrine shield plants against pests including insects and herbivores as well as bacteria and fungi, which is essential for plant survival (Molineux et al., 1996). Alkaloid-based plant chemicals have been used as poisons, dyes, and medicinal agents since the beginning of human civilization. The prevention of cancer, hypertension, malaria, and arrhythmia is only one of the many health advantages of indole alkaloids. There are just not enough examples to show how important and highly lucrative this group of plant components is. Alkaloids are used as analgesics and anti-malarial drugs because they exhibit intoxicating qualities comparable to those of nicotine, morphine, caffeine, and quinine (Wink et al., 1998).

Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis, and Escherichia coli were the microorganisms that were isolated and identified in this investigation (Table 2). It is well known that these bacteria are resistant to a wide range of antibiotics (Magili et al., 2016, Nna et al., 2018). The results of this experiment were shown as mean ± standard deviation of measurements made in triplicates (Table 2).

The stem bark plant extract of Choerospondias axillaris exhibited antimicrobial activity against all microorganisms, as shown by the antimicrobial investigation (Table 2), with mean zones of inhibition of microbial growth ranging from 14.67 to 20 mm. These actions were contrasted with those of the control, ciprofloxacin, whose mean zones of inhibition for microorganisms varied from 27.67 to 46.33 mm. Enterococcus faecalis had the lowest mean zone of inhibition (14.67 mm), whereas Salmonella typhi had the largest (20 mm). The plant's antibacterial properties are consistent with a study by Meghashree and Latha (2020), which found that Choerospondias axillaris stem bark ethyl actetate extract inhibited the growth of S. aureus and E. coli. The ethyl acetate extract's zones show that the stem bark of Choerospondias axillaris contains potential

Table 1: Quantitative Phytochemical screening *Choerospondias axillaris* stem bark.

Phytochemical	Composition ppm
Kaempferol	0.36
Steroids	2.39
Proanthocyanidin	4.12
Catechin	6.02
Anthocyanin	7.47
Naringenin	10.37
Aphyllidine	12.97
Cyanogenic glycosides	15.46
Aphyllidine	17.97
Ammodendrine	20.31
Tannins	22.73
Flavonones	25.65
Cardiac glycosides	27.54
Flavones	29.86
Ribalinidine	32.99
Spartein	34.6
Phytate	36.88
Oxalate	39.20
Epihedrine	42.28
Sapogenin	44.17

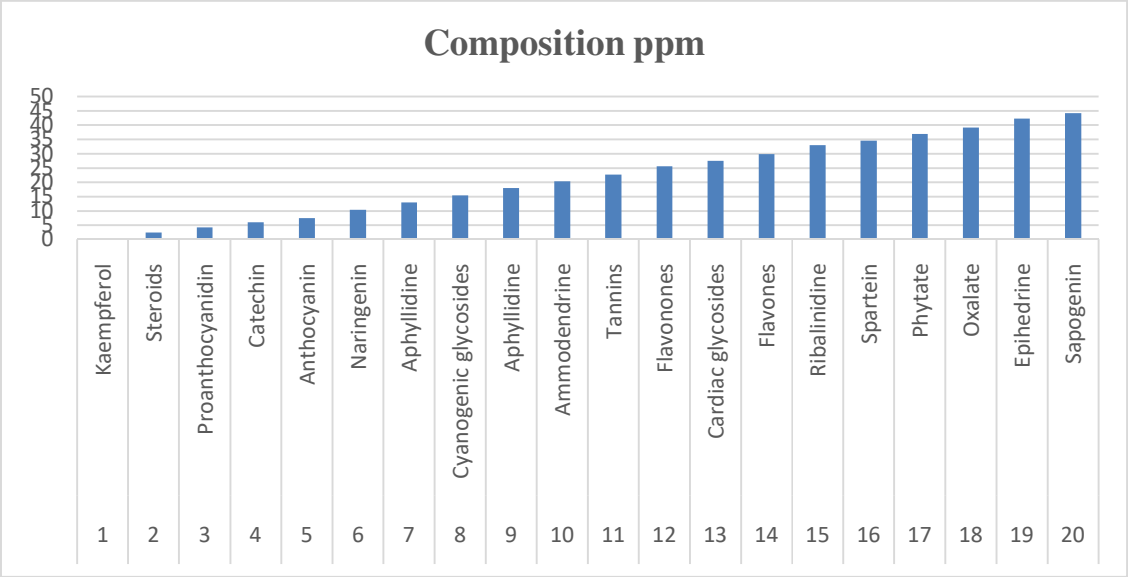


Figure 1: Comparison of Composition in ppm of Phytochemicals

antibacterial agents. Choerospondias axillaris stem bark has been shown in antibacterial trials to reduce growth even on resistant bacteria such as Staphyloccocus aureus, suggesting that it may be a viable choice for treating bacterial disorders. The findings validate the conventional study on the stem bark of Choerospondias axillaris plant and show that secondary metabolites are the cause of the antimicrobial action.

[1]HNMR Spectroscopic Analyses of Fractions PE20, PE21, PE22 and PE29

[1]HNMR spectroscopy was used to examine the portions PE20, PE21, PE22, and PE29. An olefinic region (about 5.35 ppm), an aliphatic region typical of both disturbed and undisturbed methylenes (2.63-2.78 ppm) and 1.25-1.37 ppm), and an area with a methylic proton (0.88 ppm)

Table 2: Preliminary Antimicrobial Susceptibility Screening of *Choerospondias axillaris* stem bark extract.

	Plant Extract				+ve ctrl				-ve ctrl			
Test Bacteria	**x**	**y**	**z**	**Mean±SD**	**X**	**y**	**z**	**Mean±SD**	**x**	**y**	**z**	**Mean ±SD**
Salmonella typhi	20	22	18	20.00±2.00	27	28	28	27.67±0.58	-	-	-	-
Staphylococcus aureus	18	16	18	17.33±1.15	43	43	43	43.00±0.00	-	-	-	-
Pseudomonas aeruginosa	17	14	16	15.67±1.53	40	43	41	41.33±1.53	-	-	-	-
Enterococcus faecalis	15	16	13	14.67±1.53	45	47	47	46.33±1.15	-	-	-	-
Escherichia coli	16	14	17	15.67±1.53	45	45	46	45.33±0.58	-	-	-	-

Table 3: Some characteristic signal areas of fatty acids.

Signal	**Chemical shift**	**Functional group (**Guillén & Ruiz, 2003)
1	0.83-0.93	$-CH_3$ (saturated, oleic and linoleic acyl chains)
2	0.9 3-1.03	$-CH_3$ (linolenic acyl chains)
3	1.2 2-1.42	$-(CH_2)_n$ (acyl chains)
4	1.5 2-1.70	$-OCO-CH_2-CH_2-$ (acyl chains)
5	1.9 4-2.14	$-CH_2-CH=CH-$ (acyl chains)
6	2.2 3-2.36	$-OCO-CH_2-$ (acyl chains)
7	2.7 0-2.84	$=HC-CH_2-CH=$ (acyl chains)
8	4.1 0-4.32	$-CH_2OCOR$ (glyceryl group)
9	5.2 0-5.26	>CHOCOR (glyceryl group)
10	5.2 6-5.40	-CH=CH- (acyl chains)

were all similar across the four samples. These signal areas are typical of long chain fatty acids (Guillén and Ruiz, 2003; Knothe and Kenar, 2004). However, the integration of the 1HNMR signal areas for each sample revealed significant differences in the undisturbed methylene area (1.25-1.37 ppm); the chain lengths of each were calculated including signals from the olefinic region (5.32-5.35 ppm), including the attendant allylic (1.98-2.02 ppm) and bisallylic (2.78 ppm) signals because the area of the proton NMR spectral signals is proportional to the number of hydrogen atoms of each proton type in the samples. According to Guillén and Ruiz (2003) and Knothe and Kenar (2004), the inchoate integral values of PE20 signals from the olefinic area (5.32-5.35 ppm), together with the allylic (1.98-2.02 ppm) and bisallylic (2.78 ppm) attendant signals, were assumed to originate from a second, minor molecule (Table 1). PE20 therefore contained the following information: 1.65 – 1.53 (m, 2H), 1.26 (d, J = 4.4 Hz, 29H), 0.87 (t, 3H), and 1.34 (t, J = 7.5 Hz, 2H) were the results of 1H NMR (400 MHz, Chloroform-d). (Appendix 1); PE21 made the following discoveries: 1H NMR (chloroform-d, 400 MHz) 1.71–1.51 (m, 2H), 1.43–1.17 (m, 24H), 2.44–2.25 (m, 2H), 2.14–1.96 (m, 2H), and 0.95–0.84 (t, 3H) are the values obtained from 1H NMR (400 MHz, Chloroform-d). A 1H NMR (400 MHz, Chloroform-d) δ 5.41 – 5.26 (m, 4H), 2.81 – 2.72 (m, 2H), 2.34 (t, J = 7.5 Hz, 2H), 2.14 – 1.98 (m, 4H), 1.71 – 1.50 (m, 2H), 1.40 – 1.17 (m, 22H), 0.94 – 0.83 (t, 3H) was shown by PE22 (Appendix 2). (See Appendix 3) PE29, however, had the accompanying data:1.41–5.26 (m, 4H), 2.81–2.72 (m, 2H), 2.34 (t, J = 7.5 Hz, 2H), 2.14–1.98 (m, 4H), 1.71–1.50 (m, 2H), 1.40–1.17 (m, 22H), and 0.94–0.83 (t, 3H) are the values obtained from 1H NMR (400 MHz, Chloroform-d) (Tables 3 and 4 and Appendix 1).

Characterization of PE-20 as stearic acid

White solids, or PE-20, were separated, prominent signals characteristic of a saturated molecule were seen in its proton NMR spectra at δH 2.34 (2H, t, J=7.5 Hz), 1.59 (2H, m), 1.26 (28H, d, J= 4.38 Hz), and 0.87 (3H, t). The methylene-(CH2)14 protons of a fatty acid are characterized by integration owing to 28 hydrogen at δH 1.26. The α-CH2 and β-CH2 groups exhibit typical signals at δH 2.34 and 1.59, respectively. Additionally, a signal characteristic of the terminal methyl group (ω1-CH3) was seen at δH 0.87. PE-20 is identified as stearic acid, a saturated fatty acid of the C18 type, based on an analysis of signals from several groups. The current NMR findings for stearic acid are consistent with those reported by Di-Pietro et al. (2020) (Table 5).

Characterization of PE-21 as gondoic acid (cis-11-Eicosenoic acid)

The fact that the integration of methylene signals at δH 1.26 results in 24 hydrogens suggests that PE-21 is a fatty acid. Additional evidence that the chemical is a fatty acid (FA) may be found at δH 2.33 (α-CH2), 1.63 (β-CH2), and 0.88 (ω1-CH3), which are signals attributed to saturated groups. The observation of an absorption at δH 5.34 attributed to vinylic protons indicates that PE-21 is an unsaturated fatty acid. PE-21 is a mono unsaturated

Table 4: Proton NMR data for Isolated Samples.

PE-20		PE-21		PE-22		PE-29	
Proton chemical sifts/δ, (no., multi., J(Hz))	Group	Proton chemical sifts/δ, (no., multi., J(Hz))	Group	Proton chemical sifts/δ, (no., multi., J(Hz))	Group	Proton chemical sifts/δ, (no., multi., J(Hz))	Group
2.34 (2H, t, 7.5)	α-CH_2-	2.33 (2H, m)	α-CH_2	2.34 (2H, t, 7.5)	α-CH_2-	2.34 (2H, t, 7.5)	α-CH_2-
1.59 (2H, m)	β-CH_2-	1.63 (m)	β-CH_2-	1.62 (2H, m)	β-CH_2-	1.63 (2H, m)	β-CH_2-
1.26 (28H, d, 4.38)	-$(CH_2)_{14}$-	1.26 (24H, m)	-$(CH_2)_{12}$-	1.28 (22H, m)	-$(CH_2)_9$-	1.29 (18H, m)	-$(CH_2)_9$-
-	-	2.03 (4H, m)	-CH_2-CH=CH--CH_2- Allylic	2.04 (4H, m)	-CH_2-CH=CH-CH_2-CH=CH-CH_2- Allylic	2.06 (4H, m)	-CH_2-CH=CH-CH_2-CH=CH-CH_2- Allylic
-	-	-	-	2.77 (2H, m)	-CH=CH-CH_2-CH=CH-, Bis-allylic	2.78 (2H, m)	-CH=CH-CH_2-CH=CH-, Bis-allylic
-	-	5.34 (2H, m)	-CH=CH-, Vinylic	5.35 (2H, m)	-CH=CH-CH2-CH=CH-, Vinylic	5.35 (4H, m)	-CH=CH-CH_2-CH=CH-, Vinylic
0.87 (3H, t)	ω_1-CH_3	0.88 (3H, m)	ω_1-CH_3	0.88 (3H, m)	ω_1-CH_3	0.87 (3H, m)	ω_1-CH_3

Table 5: Comparison of PE-20 NMR Data with Literature Report.

Experimental (ppm)	Di Pietro *etal.* (2020)	Group
2.34	2.35	α-CH_2-
1.59 (2H, m)	1.64	β-CH_2-
1.26 (28H, d)	1.26	-$(CH_2)_{14}$-
0.87 (3H, t)	0.88	ω_1-CH_3

Table 6: Comparison of PE-21 NMR Data with Literature Report.

Experimental (ppm)	Di Pietro *et al.* (2020); Ivanova *et al.* (2022)	Group
2.33 (2H, m)	2.33	α-CH_2-
1.63 (m)	1.63	β-CH_2-
1.26 (24H, m)	1.25	-$(CH_2)_{12}$-
2.03 (4H, m)	2.03	-CH_2-CH=CH-CH_2-CH=CH-CH_2- Allylic
5.34 (2H, m)	5.34	-CH=CH-CH_2-CH=CH-, Vinylic
0.88 (3H, m)	0.88	ω_1-CH_3

fatty acid, as shown by the integration of the vinylic signal to two protons. A significant signal was also seen at δH 2.03, attributed to allylic protons. Based on the study of 1H-NMR data and comparison with published data Di Pietro et al. (2020); Ivanova et al. (2022), it was consequently deduced that PE-21 is gondonic acid (Table 6).

Characterization of PE-22 as cis-13, 16-docosadienoic acid

The 1H-NMR spectra of PE-22 revealed the following signals, which are indicative of fatty acid signals: δH 5.35, 2.77, 2.34, 2.04, 1.62, 1.28, and 0.88. Given that these signals resemble those seen for PE-21, PE-29 is also an unsaturated FA. But there's also another signal at δH 2.77 that is unique to protons that are bis-allylic. Following a comparison of the current NMR findings with studies from the literature (Alexandri et al., 2017; Ivanova et al., 2022), PE-22 was determined to be cis-13,16-docosadienoic acid (Table 7).

Characterization of PE-29 as linoleic acid

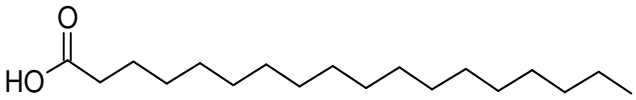
The 1H-NMR spectra of PE-29 revealed signals that are indicative of fatty acids: δH 5.35, 2.78, 2.34, 2.06, 1.68, 1.29, and 0.88. Since these signals resemble those of PE-22, it may be concluded that PE-29 is also an unsaturated FA with an isolated diene group. The 1H-NMR findings for oleic acid are consistent with those reported by Knothe and Kenar (2004) and Ivanova et al. (2022). PE-29 was thus determined to be oleic acid (Table 8).

Table 7: Comparison of PE-22 NMR Data with Literature Report.

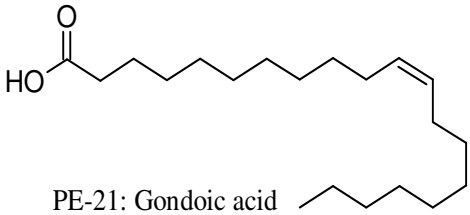
Experimental (ppm)	Alexandri *et al.* (2017); Ivanova *et al.* (2022)	Group
2.34 (2H, t, 7.5)	2.34	α-CH_2-
1.62 (2H, m)	1.62	β-CH_2-
1.28 (22H, m)	1.27	-$(CH_2)_9$-
2.04 (4H, m)	2.05	-CH_2-CH=CH-CH_2-CH=CH-CH_2- Allylic
2.77 (2H, m)	2.77	-CH=CH-CH_2-CH=CH-, Bis-allylic
5.35 (2H, m)	5.35	-CH=CH-CH_2-CH=CH-, Vinylic
0.88 (3H, m)	0.88	ω_1-CH_3

Table 8: Comparison of PE-29 NMR Data with literature report.

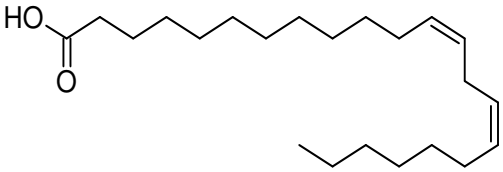
Experimental (ppm)	Knothe and Kenar (2004); Ivanova *et al.* (2022)	Group
2.34 (2H, t, 7.5)	2.34	α-CH_2-
1.63 (2H, m)	1.62	β-CH_2-
1.29 (18H, m)	1.27	-$(CH_2)_9$-
2.06 (4H, m)	2.05	-CH_2-CH=CH-CH_2-CH=CH-CH_2- Allylic
2.78 (2H, m)	2.79	-CH=CH-CH_2-CH=CH-, Bis-allylic
5.35 (4H, m)	5.36	-CH=CH-CH_2-CH=CH-, Vinylic
0.87 (3H, m)	0.87	ω_1-CH_3



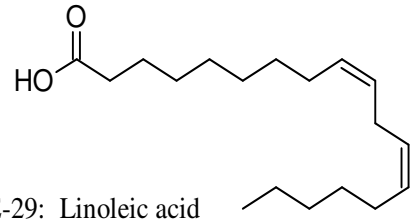
PE-20: Stearic acid



PE-21: Gondoic acid



PE-22: cis-13,16-docosadienoic acid



PE-29: Linoleic acid

Figure 2: Fatty acids Isolated from *Choerospondias axillaris* Stem Bark

Characterization of PE20, PE21, PE22 and PE29as Stearic acid, Eicosenoic acid, Docosadienoic acid,and Eicosadienoic acid

The following formula was used to calculate the compounds' chain lengths.

Chain length *(Sample)* = (methyl region integral/3) + (undisturbed methylenes/2) + (H-3 acyl moiety/2) + (H-2 acyl moiety/2) +(allylic proton signal/2) + (bisallylic proton signal/2) + carboxylic carbon (Johnson and Schoolery, 1962;Knothe and Kenar, 2004).:

Therefore Chain length $_{(PE20)}$ = (methyl region integral/3) + (undisturbed methylenes/2) + (H-3 acyl moiety/2) + (H-2 acyl moiety/2) +

carboxylic carbon = $\frac{3}{3}+\frac{28}{2}+\frac{3}{2}+\frac{2}{2}+1 \approx 18$,

Chain length $_{(PE21)}$ = (methyl region integral/3) + (undisturbed methylenes/2) + (H-3 acyl moiety/2) + (allylic protons/4) + (H-2 acyl moiety/2) + (olefinic protons/1) + carboxylic carbon= $\frac{3}{3}+\frac{24}{2}+\frac{2}{2}+\frac{2}{2}+\frac{4}{2}+\frac{2}{2}+\frac{2}{1}+1 \approx 20$,

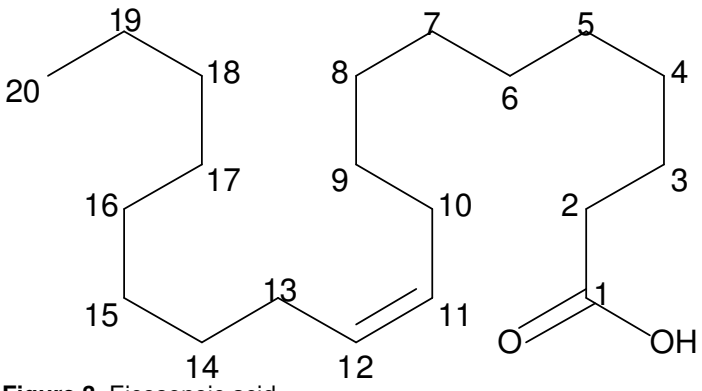


Figure 3. Eicosenoic acid

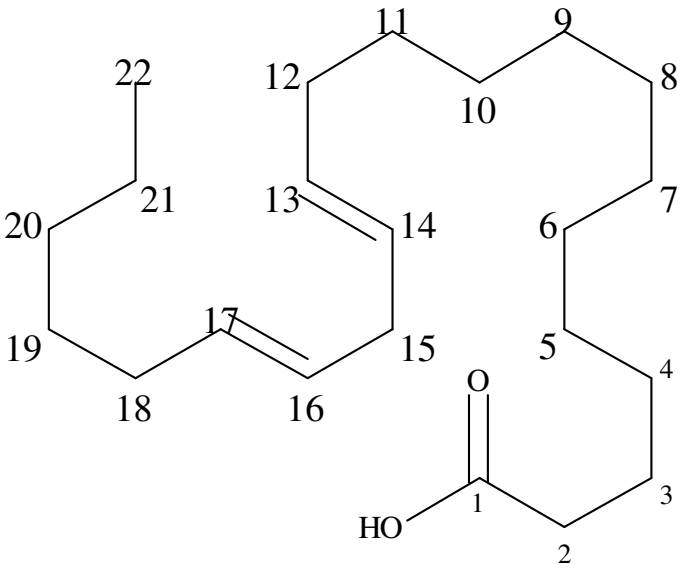


Figure 4. Docosadienoic acid

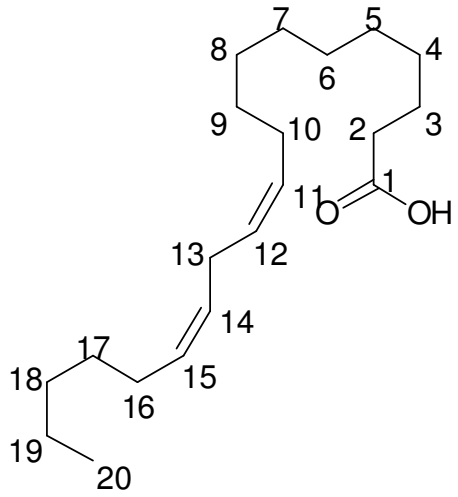


Figure 5. Eicosadienoic acid

Chain length *(PE22)* = (methyl region integral/3) + (undisturbed methylenes/2) + (H-3 acyl moiety/2) + (allylic protons/4) + (H-2 acyl moiety/2) + (bisallylic protons/2) + (olefinic protons/2) + carboxylic carbon =

$$\frac{3}{3}+\frac{22}{2}+\frac{2}{2}+\frac{4}{2}+\frac{2}{2}+\frac{2}{2}+\frac{4}{1}+1 \approx 22$$

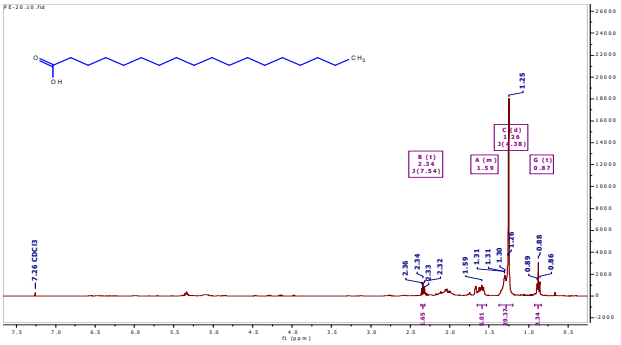
,

and

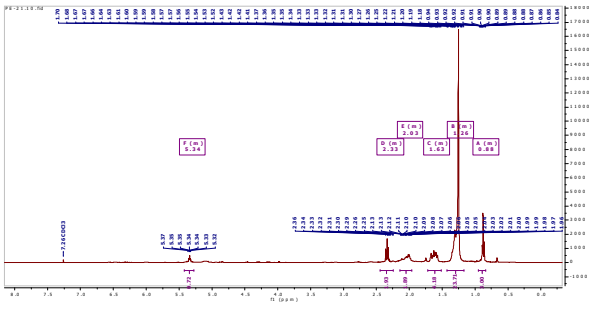
Chain length *(PE29)* = (methyl region integral/3) + (undisturbed methylenes/2) + (H-3 acyl moiety/2) + (allylic protons/4) + (H-2 acyl moiety/2) + (bisallylic protons/2) + (olefinic protons/2) + carboxylic carbon

$$=\frac{3}{3}+\frac{18}{2}+\frac{2}{2}+\frac{4}{2}+\frac{2}{2}+\frac{2}{2}+\frac{4}{1}+1 \approx 20$$

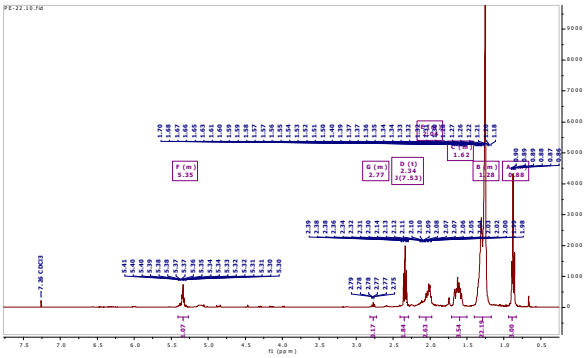
Furthermore, based on their chain lengths and literature comparisons, PE20, PE21, PE22 and PE29 were determined to be Stearic acid, Eicosenoic acid, Docosadienoic acid, and linoleic acid (Figures 2-5), respectively (Johnson and Schoolery, 1962; Vlahov, 1999; Guillén and Ruiz, 2003a; Guillén and Ruiz, 2003b; Knothe and Kenar, 2004;Gunstone and Herslöf, 2012; Sopelana et al., 2013; Ibargoitia et al., 2014; Nieva-Echevarría et al., 2015; Smith et al., 2015).



Appendix I: Proton NMR Spectrum for PE20



Appendix II: Proton NMR Spectrum for PE21.



Appendix III: Proton NMR Spectrum for PE22.

Conclusion

Choerospondias axillaris is used by traditional healers to treat a wide range of ailments, such as myocardial ischemia, external burns, sleepiness, tongue infections, chest discomfort, and improved blood circulation. The stem bark of Choerospondias axillaris has a number of pharmacological properties, including as detoxifying, anti-tumor, anti-inflammatory, antidiabetic, and enhanced heart function. These characteristics are caused by the bioactive compounds found in the plant, which include linoleic acid, cis-13, gondoic acid, stearic acid, and 16-docosadienoic acid. This is because they have several biological and medicinal properties, including the ability to be anti-inflammatory, anti-diabetic, anti-cancer, immunomodulatory, antifungal, antibacterial, antioxidant, anti-parasitic, and neuroprotective. Choerospondias axillaris stem bark has been shown in antibacterial investigations to be a viable choice for treating bacterial illnesses.

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