Pentacyclic Triterpenoid from the Stem Bark of *Gmeliana arborea* and its Activities Against Some Clinical Isolates

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ABSTRACT

This investigation was conducted with the goal of determining the chemical constituent(s) and antibacterial activities of the stem bark of the Gmelina arborea plant. The stem bark of the Gmelina arborea plant was extracted using the Soxhlet technique in conjunction with hexane and ethyl acetate as the solvents. This extraction process was carried out in a sequential fashion. Using a rotary evaporator and lower pressure, these extracts were condensed to a higher concentration. All of the extracts were put through a qualitative phytochemical screening to determine whether or not they contained secondary metabolites. These secondary metabolites included alkaloids, tannins, flavonoids, cardiac glycosides, saponins, and anthraquinone. Both extracts included alkaloid, tannins, saponins, and cardiac glycosides, but only the hexane extract had flavonoid, while the ethyl acetate extract contained anthraquinone. Additionally, the hexane extract did not contain anthraquinone. The results revealed that the ethyl acetate extract did not contain anthraquinone. A quantitative phytochemical study was carried out in order to investigate the metabolite concentrations of the various compounds. Quantification and characterization were performed on eighteen (18) different bioactive substances. Ephedrine had the greatest concentration, measuring in at 45.066 g/mg, while kaempferol had the lowest concentration, measuring in at 0.386 g/mg. Antimicrobial activity against Gram negative organisms (Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi); Gram positive organisms (Staphylococcus aureus, Methicillin Resistant staphylococcus aureus, Vancomycin Resistant enterococci, Enterococcus faecalis) using disc and broth diffusion methods was also carried out on the two different crude extracts and results compared with standard drugs as control. It was established where the zone of inhibition was and how sensitive the bacteria were. The Soxhlet extraction of ethyl acetate resulted in the crude extract that shown the best antibacterial activity against all of the pathogens that were tested. After a further round of purification using column chromatography with silica gel as the packing material, the extract was then eluted with hexane, ethyl acetate, and methanol. In a solvent solution consisting of 7 parts hexane to 3 parts ethyl acetate, column fractions were analyzed using a technique called thin layer chromatography (TLC). The Rf value obtained from fraction PHE-64 was 0.53 correspondingly. After drying with vanillin sulphuric acid reagent (VSA), this fraction produced white, non-crystalline compounds with melting points ranging from 190-1910 degrees Celsius to 131-1330 degrees Celsius, respectively. For the purposes of characterization and identification, spectroscopic analyses, namely 1H-NMR and 13C-NMR, were performed on this fraction. The data from PHE-64's spectra seemed to point

toward the presence of a triterpenoid called lupeol. The findings of this research provide evidence in favor of the Ikwerre people's traditional use of the stem bark of the plant known scientifically as Gmelina arborea for medicinal purposes. Because of this, the bark from the stem of this plant has the potential to be used as a possible medication candidate for antimicrobial and for the treatment of infectious disorders caused by the pathogens employed in this current investigation.

Introduction

Man's usage of plants is commendable because to their speedy production of nourishment, medicines, oxygen for humans and other animals, and raw materials for several businesses. Additionally, plants provide the basic materials for our structures as well as the production of biofuels, dyes, fragrances, insecticides, and absorbents. Because of the chemical compounds they contain, plants have shown to be the most effective in the treatment of diseases. They also play important roles in the global pharmaceutical industry. These molecules, which are often referred to as phytochemicals or secondary metabolites, include steroids, terpenes, flavonoids, alkaloids, tannins, glycosides, phenols, anthraquinones, carotenoids, and saponins (Nwokonkwo, 2014). In contrast to macronutrients and micronutrients, phytochemicals are physiologically active, naturally occurring chemical substances that are present in plants. They protect plants from a variety of diseases and enhance the scent, flavor, and color of plants. Plants include phytochemicals that protect them from environmental dangers such pathogenic assault, all types of pollution, drought, and UV exposure as well as enhancing their effectiveness in ethnomedicine (Sofowora, 1993).

As a result, it is general knowledge that several animals, including snakes, consume herbs when they are ill. It is conceivable that early man found herbal medicines by instincts like these or through exposure, as many herbalists say. On the third day, God created plants. The unique ability that God gave to plants allows them to create a large range of organic substances that fall into almost every imaginable structural class. Plants serve as significant raw materials for the chemical industry as a consequence. God Almighty has ordered herbal healing. In Nigeria, traditional medicine practices include using herbs extensively (Okwu and Morah, 2007). The chemical components of the plants affect how well traditional medical practices work.

The exact interaction of medications with biomolecules like nucleic acids and proteins in the body enhances the physiological effects of pharmaceuticals in the body because medicinal plants serve as the scaffold for many key drugs. Because different medications are needed for various objectives, plants may serve as important precursors for the production of new pharmaceuticals (Ajibesin, 2011). Plants therefore play a significant role in homeopathy, aromatherapy, and traditional or herbal medicine. For instance, to strengthen their immune systems, women consume foods and spices made from medicinal herbs (Akinpela and Onukoya, 2006). Due to certain biological and pharmacological activities like anti-inflammatory, diuretics, laxatives, anti-plasmodics, antihypertensive, and antimicrobial potential of these plants, a variety of knowledge has been developed regarding the effectiveness of plants as food and medicine for humans (Adesokan et al., 2007).

Due to the widespread use of medications in recent years, various drug resistance in pathogens of both human and animal origin has emerged. As plant medications are biodegradable, harmless, and have fewer side effects, this emphasizes the need to screen medicinal plants for new

bioactive chemicals (Stern et al., 2003). Plants have long been used to cure human ailments because they are thought to be less expensive than synthetic medications; as a result, medicinal plants have become increasingly important as alternative sources of potent medications. Untapped chemical reserves in plants and natural products that might be used as therapeutic candidates and distinctive templates for the synthesis of innovative medications are still present. Because of this, this work aims to use spectroscopic techniques to extract, analyze, and discover bioactive chemicals in the stem bark of Gmeliana arborea and their antibacterial properties against various clinical infections.

Materials and Methods

Sample Collection, Identification and Preparation

The Community Secondary School, Rumuodomaya in the Obio Akpor Local Government Area of Rivers State, Nigeria, would be where the stem bark of the Gmelina arborea is harvested. Dr. W.S. David, a botanist at Rivers State University in Port Harcourt, recognized and verified the plant. Samples were placed in their Herbarium and given a voucher specimen number. Three weeks of air drying the sample was followed by a mortar and pestle powdering. It was then delivered to the Department of Chemistry at Ignatius Ajuru University of Education for extraction and further analysis after being kept in a glass container.

General Procedure

The study was conducted in 2022 between July and September. In order to accomplish column chromatography, silica gel and sephadex were used, respectively. To get rid of greasy and other contaminants, they were cleaned many times with hexane and acetone separately. Separately, the sephadex and silica gel were dried in an oven set at 40°C for 30 minutes before being stored. On plates that had already been coated with silica gel (Merck, TLC grade), thin layer chromatography (TLC) was performed. Before being exposed to iodine vapour in a tank, TLC bands were viewed under ultraviolet light.On a Bruker Avance 3 spectrometer, 1H and 13C NMR studies of CDCl3, deuterated acetone, and DMSO were captured. An SMP1 Stuart Scientific melting point equipment was used to calculate melting points (m.p). Agilent Technologies' ATR-FT 5500 series FTIR device measured infrared (IR) with 32 scans and reported frequencies in cm-1.

Extraction of Sample

Gmelina arborea powdered stem bark weighing about 500g was put in a Soxhlet apparatus and consecutively extracted over the course of three days using hexane and ethyl acetate. In a rotary evaporator set at 400C, the extracts were dried out. For both ethyl acetate and hexane extract, all dried extracts were gathered, stored in various sample bottles, and labeled PHE/EAO1, PHE/EX/01, PHE/EX-01, and PHE/HEX-01. A preliminary phytochemical screening of around 10g of each crude extract was conducted, and the remaining grams were adsorbed on silica gel and left to dry in preparation for column chromatography.

Qualitative Phytochemical Screening

On the raw plant extracts, phytochemical assays were run to determine if certain secondary metabolites (phytochemicals) were present or absent. Using the methods given by Nna (2020), these phytochemicals may be identified as alkaloids, tannins, saponins, flavonoids, cardiac glycosides, steroids, and triterpenoids.

Chromatographic methods

A crude extract's reasonably refined fractions, which may be mixed or handled independently, are obtained using column chromatography. It is the quickest and most trustworthy approach for isolating and purifying natural chemicals. The research used the Nande and Igoli (2017) published column chromatographic technique.

Dichloromethane was used to dissolve around 6.3g of dried Gmelina arborea extract, which was then put into a small beaker. The extract was mixed with 10g of silica gel, added, and left to dry in a fume closet. Silica gel was added to help the extract adsorb. A slurry was created by continually stirring 100g of silica gel with 500 mL of hexane until it was produced. To prevent contamination, the column was clamped to a retort stand and washed three times with hexane. A little amount of hexane was added to the column, and two minutes later, the slurry was added. The column tap was then gently opened, allowing the solvent to drain as it settled. Adsorbed extract slurry was transferred to the column surface with the tap closed until there was approximately 10 cm of solvent above the silica packing. The tap was then opened to enable any remaining solvent to drain out of the column and dry only on the silica. Hexane and 5% ethyl acetate were added to the column, and the tap was left open to drip roughly 10-15 drops each minute. A total of 25 mL of fractions were consistently collected using vials with serial numbers. The eluates were stored in a dust-free fume hood and allowed to dry.The column chromatography fractions were examined by TLC, and fractions with comparable characteristics were mixed. The column chromatography fractions were examined on pre-coated (MERCK) F254 TLC plates. On TLC plates, a little amount of material was spotted using a capillary tube and left to dry for three minutes. On the TLC plates, the spot's location was noted. The plates were then put in a TLC tank that had been prepared (3:7, ethyl acetate and hexane), and the solvent distance traveled was tracked over a period of time. The TLC plates were then taken out of the tank and dried using a pistol heater. After that, plates were put under a UV light to check the compounds' (samples') UV activity and fluorescence. To identify the greatest number of components in each fraction and the distance traveled, the plates were checked for densed coloration, sprayed with general spraying reagent (5% H_2SO_4 in anialdehyde), and then allowed to dry in a fume cabinet for 2–5 minutes. Each sample's retention factor (Rf), which measures how much a chemical is delayed relative to the solvent front, was computed. In contrast, fractions containing several components based on the number of spots seen were further purified using sephadex column chromatography. The fractions with identical Rf values were gathered and prepared for NMR analysis.

Sephadex column chromatography

Methanol was poured into a glass column in an amount of around 15 mL. In 100 mL of methanol, 50 g of sephadex powder was dissolved before being added to the column and allowed to settle. In order to purify an impure fraction produced through column chromatography, sephadex was then added to the column. Following that, the column tap was left to run while 4-5 mL of fractions were collected into vials with serial labels. For dryness, the eluates were stored in a cabinet free of dust. Fractions' Rf values were calculated, and fractions with comparable Rf values were mixed and put through spectroscopic analysis. To get the pure fraction PHE 64, samples PHE-16–18 (Hexane extract) were purified using a sephadex column.

Melting Points Determination of Isolated Sample

SMPI Stuart Scientific melting point equipment was used to measure the melting point of each separated chemical (solid).

Infrared Spectroscopy

Frequencies were given in cm-1 for the IR studies, which were conducted using a thermos Scientific Nicolet Is10.

Mass Spectrometry (MS)

The MS of each isolated compound was performed on an Agilent technologies 1220 series LC column, Poroshell 120 EC-C18 (4.6 75mm, particle size 2.7 m), in a gradient of MeCN + 5mM ammonium acetate using an Agilent 6100 series quadrupole mass spectrometer in positive and/or negative electrospray ionization (ESI) mode.

Nuclear Magnetic Resonance (NMR) Spectroscopic Analysis

Using a Pasteur pipette, the cleaned samples were diluted in chloroform (CDCl3) and then transferred to clean NMR tubes where they underwent NMR investigations to determine the structures of the chemicals present. On a Bruker Avance 3 spectrometer, the NMR studies were captured at 400 MHz (1H) and 376 MHz (13C). All spectra were measured in CDCl3 (Deuterochloroform), and chemical shifts were corrected at 7.26 parts per million (ppm) to account for this. The splitting patterns were identified as singlets (s), doublets (d), doublet of doublets (dd), triplets (t), and multiplets (m). Coupling constants (J) were measured in Hertz (Hz).

Antimicrobial Screening

Using a few animal and plant infections, the isolated chemicals from the plants under inquiry were tested for their antibacterial effects. The UPTH Department of Medical Microbiology provided the animal pathogens. Methicillin was one of the animal pathogens (bacteria) utilized in the antimicrobial test. Plant pathogens (fungi) used were Aspergillus flavus, Aspergillus fumigatus, Aspargillus nigre, Coniophora puteana, Fibrophoria vaillentii, Fomitopis pinicola, Fusarium oxysporum, Fusarium proliforatum, Rhizopus sp, Sclerotium rofsii, and Serpula lacrymans. All microorganisms underwent purity testing and were kept on agar slants (Tor-Anyiin et al., 2016).

RESULTS AND DISCUSSION

Using accepted techniques, the qualitative phytochemical screening of Gmelina arborea stem bark extracts in hexane and ethyl acetate was performed. The findings are shown in table 1.

Table 1: Qualitative Phytochemical Screening of *Gmelina Arborea* Stem Bark Extract

Class of Compound	Test	Ethyl acetate	Hexane Extract
Alkaloid	Mayer	+	+
Tannins	$FeCl_3$	+	+
Flavonoids	Shinoda	-	+
Cardiac glycoside	Keller-killiani	+	+
Saponins	Frothing	+	+
Triterpenoids	Liebermann-Burchard	+	+
Anthraquinones	Borntrager	+	-

Table 4.1 lists the phytochemicals that are present, including anthraquinones, alkaloids, flavonoids, cardiac glycosides, saponins, and triterpenoids. According to the findings, only flavonoids were missing from the sample's ethylacetate extract, while anthraquinones were missing from the hexane extract. Both the crude extracts (ethylacetate and hexane) of the research sample included alkaloids, tannins, cardiae glycoside, saponins, and triterpenoids.

Table 2: Quantitative Phytochemical Analysis of ethyl acetate extract of the stem bark of *Gmelina arborea*

Components	Concentrations (μg/mg)
Kaempferol	0.386
Catechin	0.873
Steroid	2.093
Aphylidine	5.483
Anthocyanin	7.873
Narigenin	9.350
Dihydrocytisine	14.036
Ammodendrine	19.973
Tannin	20.616
Cyanogenic glycoside	23.363
Flavonones	25.960
Flavone	29.326
Ribalinidine	31.653
Spartine	34.150
Phytate	36.773
Oxalate	38.640
Sapogenin	43.536
Ephedrine	45.066

The stem bark of Gmelina arborea contains eighteen (18) bioactive chemicals, according to the quantitative phytochemical screening. According to the findings, ephedrine has the greatest concentration (45.066 mg/kg), whereas kaempferol has the lowest concentration (0.386 mg/kg). Table 4.1 displays a phytochemical qualitative analysis of the extracts. Alkaloid, tannins, flavonoids, cardiac glycoside, saponins, and triterpenoids were present in the hexane extract's product, however anthraquinone was not present. According to Nna and Okwelle (2021), this was accurate. According to the results of the ethylacetate phytochemical screening, flavonoids were not found, but alkaloids, tannins, cardiac glycosides, saponins, triterpenes, and authraquinones were. This was consistent with the findings published by Koshkoneba et al. (2017). Alkaloids, tannins, saponins, flavonoids, cardiac glycosides, and phytosterols were found in previous phytochemical screening of aqueous and ethanolic leaf extracts of Gmelina arborea (Diso et al., 2017). reducing sugars, tannins, anthraquinones, triterpenoids, saponins, flavonoids, alkaloids, and cardiac glycosides were all found in the root of Gmelina arborea in a different research (Sha et al., 2010). This research concurs with that written up by Udeme et al (2018). This demonstrated the large variety of physiologically significant phytochemicals present in the plant under study. For many years, alkaloids like quinine and artheminisin have been used to treat malaria (Fagbohum et al., 2012; Nna and Ejiofor, 2023). According to Nna et al. (2019), certain alkaloids have been shown to be effective against AIDS-related intestinal infections, COVID-19 infection, and HIV infection.

A class of plant compounds known as flavonoids is thought to have antioxidant characteristics, block key viral enzymes, and kill pathogenic protozoa in people (Ogoko, 2018). In addition to their

roles as antioxidants and free radical scavengers, flavonoids found in plants also protect cells from oxidative stress, exhibit strong anti-cancer properties, and combat carcinogens (Tene et al., 2016). Additionally, it reduces the incidence of coronary artery disease, intestinal inflammation, allergy illnesses, and heart disease (Omodamiro et al., 2016). The capacity of flavonoids to interact with extracellular and soluble proteins of bacterial cells has been revealed to be a key factor in their antibacterial action against microbes (Ogoko, 2018). The existence of these phytochemicals attests to the plant's efficacy in various ethnomedical practices over its whole body.

5.2 Quantitative Phytochemical Analysis

Gmeline arborea's ethyl acetate extract was subjected to a quantitative study, which identified a total of 18 bioactive components with concentrations ranging from 0.386 to 45.066 g/mg. the effects of kaemferol's (0.386 g/ml) presence. In terms of concentration, epihedrine had the greatest value (45.066 g/mg), followed by sapogin, oxalate, phytate, spartein, and ribalinidine (43.536, 38.640, 36.773, 34.150, and 31.653 g/mg), in that order (Table 2). Aphylidine, narigbriun, dihydrocytisine, ammodendrine, tannin, cyanogenic glycoside, flavonones, and flavones with concentrations of 5.483, 7.873, 9.350, 14.036, 19.973, 20.616, 23.263, 25.960, and 29.326 g/mg, respectively, are other bioactive substances that have been measured and discovered (Table 2). Kaempferol's antioxidant defense against the body's free radicals, anti-inflammatory, antibacterial, cardiovascular, and neurological capabilities have all been noted. Kaempferol may be utilized to treat hormone-regulated tumors such ovarian, breast, cervical, hepatocellular carcinoma, and leukemia since it resembles estrogen hormone (Nna and Okwelle, 2022). Studies have shown that kaempferol's anticancer action extends beyond just causing cell death, since it also has antiangiogenic and antimetastatic capabilities. Human chronic disorders including inflammatory bowel disease (IBD) have been claimed to be prevented and treated with catechin, which was measured in the research sample. Cancer, particularly breast and prostate cancer, is also prevented by catechin. Plants contain the antinutrient oxalate. Complexes (calcium oxalate crystals) are created when it binds to calcium. These oxalate crystals result in diseases like rickets and osteomalacia by preventing the body from absorbing and utilizing calcium. According to reports, steroids have been used to treat rheumatologic conditions such lupus, vasculitis, and rheumatoid arthritis (Ogoko, 2018). Ephedrine is a central nervous system stimulant that is used to treat myasthenia gravis, breathing difficulties (as a bronchodilator), nasal congestion, and orthostatic hypotension issues.

According to Wang et al. (2015), the alkaloidal compound sparteine has been shown to have anti-arrhythmic, anti-convulsant, and activity to decrease locomotor effects. Triterpene sapogenin has potential to inhibit methanogenesis and regulate rumen fermentation (Nna, 2019b). Guinoline alkaloid ribalinidine is known for its capacity to scavenge free radicals (Amise et al., 2016). A flavonoid known as anthocyanin has been shown to prevent cataract development in rats and enhance eyesight (Nna et al., 2019b). A teratogenic substance called ammodendrine is an alkaloid of the piperidine family. In other words, it may impede the embryo or fetus' growth. This may be related to the herb under investigation's ethnomedical usage in ending undesired pregnancies. According to Wang et al. (2015), naringenin has a wide range of biological effects on human health, including a reduction in lipid peroxidation, protein carbonylation, promotion of carbohydrate metabolism, increased antioxidant defenses, scavenging of reactive oxygen species, modulation of immune system activity, and anti-atherogenic and anti-inflammatory effects.

Table 3: Zone of inhibition of the crude hexane extract against some test organism

Test Organism	K	y	Z	Mean	Control Drug Ciprofloxacin x	Y	Z	Mean
Salmonella typhi	13	12	14	13.00	27	28	28	27.67
Staphylococcus aureus	0	0	0	0	43	43	43	43.00
Pseudomonas aeruginesa	18	20	21.5	19.8	40	43	41	41.33
Enterococcus faecalis	15.5	16	17	16.17	45	47	47	46.33
Escherichia Coli	19.5	21	19	19.83	45	45	46	45.33
Vancomycin resist enterococci	29	28	30	29.00	32	29	30	30.3
Methicillin resist staphylococcus aureus	28	29	33	30.00	34	33	32	33.3

The minimal inhibition of the crude extract against certain clinical pathogens is shown in Table 3. In this investigation, ciprofloxacin was utilized as the control (standard) antibiotic and seven (7) clinical pathogens were evaluated. Salmonella typhi had the lowest zone of inhibition, while methicillin-resistant Staph aureus had the largest, with a mean zone of inhibition ranging from 13.00 to 30.00. Staphylococcus aureus doesn't exhibit an inhibitory zone.

Table 4: Antimicrobial activity of the crude hexane extract against some clinical pathogens

Test Organism	Extract	Standard drug (Ciprofloxacin)
Salmonella typhi	S	S
Staphylococcus aureus	R	S
Pseudomonas aeruginesa	S	S
Enterococcus faecalis	S	S
Escherichia Coli	S	S
Vancomycin resist enterococci	S	S
Methicillin resist staphylococcus aureus	S	S

Table 4 shows the microbial response to both the extract and the common (control) medications. The table shows that every test organism, with the exception of staphylococcus aureus, was susceptible to the extract.

Table 5: Zone of inhibition of the crude ethyl acetate extract against some clinical pathogens (mm)

Test Organisms	B	C	D	Mean	Control Drug Ciprofloxacin

Salmonella typhi	22	24	23	23	32
Staphylococcus aureus	0	0	0	0	28
Pseudomonas aeruginesa	28	19	18	21.7	26
Enterococcus faecalis	18	17	15	16.7	27
Escherichia Coli	23	24	26	24.3	25
Vancomycin resist enterococci	28	29	28	28.3	38
Methicillin resist staphylococcus aureus	31	28	26	28.3	36

Table 6: Antimicrobial activity of the crude ethyl acetate extract against some clinical pathonges (mm)

Test Organism	Extract	Ciprofloxacin
Salmonella typhi	S	S
Staphylococcus aureus	R	S
Pseudomonas aeruginesa	S	S
Enterococcus faecalis	S	S
Escherichia Coli	S	S
Vancomycin resist enterococci	S	S
Methicillin resist staphylococcus aureus	S	S

The zone of inhibition of the crude ethyl acetate extract against a few clinical pathogens is shown in Table 6. The results indicated that the test organisms had an average zone of inhibition between 15 and 28 millimeters, while the typical medication inhibition zone was 25 to 38 millimeters. However, Table 6 demonstrated that all test organisms, with the exception of Staphylococcus aureus, were responsive to the extract and resistant to the control medication (ciprofloxacin).

Antibacterial Studies of the Crude extracts

Salmonella typhi, staphylococcus aureus, pseudomonas aeruginosa, enterococcus faecalis, Escherichia coli, enterococci resistant to vancomycin, and staph aureus resistant to methicillin were the microorganisms employed in the research. The outcome (Table 6) showed that all bacteria, with the exception of staphylococcus aureus, were susceptible to crude hexane extract. The results also demonstrate that vancomycin-resistant enterococci recorded a mean zone of inhibition of 29.00mm, whereas methicillin-resistant staph aureus had the largest zone of inhibition (30.00mm). The zone of inhibition for salmonella typhi was reported to be the smallest (13.00mm), followed by the zone of inhibition for enterococcus faecalis, which was 16.17mm on average. The mean zones of inhibition for Escherichia coli and pseudomonas aeruginosa were 19.8 and 19.83 mm, respectively. The extract's distinct zone of inhibition may indicate that it inhibits a number of cellular enzymes that are essential to the metabolism of the tested bacteria (Amise et al., 2016). A gram-negative bacterium called Pseudomonas aeruginosa is responsible for various human diseases such bacteremia, pneumonia, eye and ear infections, among others. Gram-negative Escherichia coli bacteria are often discovered in the digestive tracts of warm-blooded animals. Although certain E. coli strains are safe for people and other animals to consume, they have been implicated in the development of pneumonia, urinary tract infections, and diarrhea in humans

(Allocate et al., 2013). The bioactive components that were identified and described from the plant may be the source of the plant extract under investigation's antibacterial properties (Alsiddig et al., 2017). As a result, Gmelina arborea extract may successfully replace ciprofloxacin. According to Ahmed et al. (2012), the mode of action of antimicrobials may involve a number of targets in the microorganisms, including interference with the synthesis of cellular walls, damage that can result in altered cell permeability characteristics or disorganized lipoprotein arrangements, ultimately leading to cell death, deactivation of various cellular enzymes that are crucial for these microorganisms' metabolic pathways, and denaturation of one or more proteins.

Characterization of PHE-64 as a pentacyclic triterpenoid

Table 7: HMNR and ^{13}CNMR Chemical Shifts for PHE-64

Spectroscopic Technique	Data
1R (Cm^{-1})	3000, 2900, 150
MP	190-191^{0}C
RF	0.53
^{1}HNMR ($CDCl_3$)	δ 0.67, 0.94, 0.75, 0.69, 0.82, 0.97, 0.98, 0.68, 3.21, 3.20, 3.18, 3.17, 4.60, 4.61, 4.73, 4.74 ppm
^{13}C NMR ($CDCl_3$)	δ 38.9, 27.5, 79.0, 39.8, 55.3, 18.3, 34.3, 40.9, 50.5, 37.7, 21.2, 25.2, 38.3, 43.0, 27.5, 34.8, 43.0, 48.7, 48.3, 151.0, 31.1, 40.0, 29.8, 15.6, 16.8, 16.7, 14.7, 18.4, 109.3, 19.3 ppm

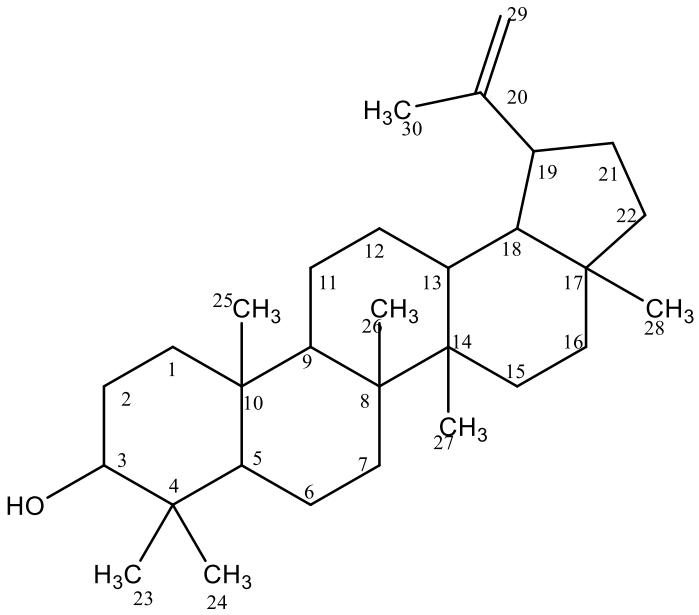
After drying, the fraction PHE-64 from Gmelina arborea produced a white needle with a melting point of 190-1910C (lit 190-1920C; Nna et al., 2018). The TLC advertisement did not display under UN but did on spraying and heating as a pink spot. The resultant Rf value was 0.53. The IR spectra showed the hydroxyl group absorption at 3000 cm-1, significant intramolecular hydrogen bonding off that resulted in the absorption at 3000 cm-1 (broad), C-H stretching at 2900 cm-1, and the carbon double bond (C=C) absorption at 1500 cm-1. The methyl group's stretching and bending vibrations were seen as a strong band at 2940 cm-1 and a medium-intensity band at 1456 cm-1. At 2869 cm-1, the methylenic group was detectable. At 1038 cm-1, the associated C-C stretching vibration was seen as a band.

According to a biogenetic comparison, the proton multiplet at 3.2 in the 1H-NMR spectrum was ascribed to H-3 (Upendra et al., 2018). The seven methyl groups that resonate at ppm values of H0.67, 0.75, 0.69, 0.94, 0.82, 0.68, and 0.97. The methylene group at C-20 was thought to be the cause of two doublets that were present at 4.73 and 4.74 and each integrated for one proton. The chemical is a pentacyclic triterpenoid, as shown by the H-NMR spectrum, which also showed the presence of methyl singlets and two olefinic protons. The existence of 20 carbon atoms, including six quaternary carbons, six methane carbons, eleven methylene carbons, and seven methyl groups—characteristics of triterpenoids—was verified by the 13C-NMR spectra (Appendices a and b). One of the carbons showed up at c 79.0 ppm and was oxygenated. The existence of a C=C group, of which one carbon was a quaternary carbon and the other was a carbon of a vinyl methylene group, was revealed by the olefinic carbon resonance at c 151.0 and 109.3, respectively. The spectra also revealed peaks for methylene carbons at c 18.3, 21.1, 25.2, 34.3, 34.8, 38.3, 37.7,

27.5, and 40.0 ppm. At concentrations of 14.7, 15.6, 16.8, 16.7, 18.4, and 29.8 ppm, the methyl carbon atoms were detected. The percentage PHE-64 was identified as 20(29) - lupen-2-ol (Lupeol) and has been isolated from numerous plant species, including Dacryodes edulis and Zanthoxylum Zantholoides (Nna et al., 2019), based on the spectroscopic data and comparison with literature reports as given below (Table 8).

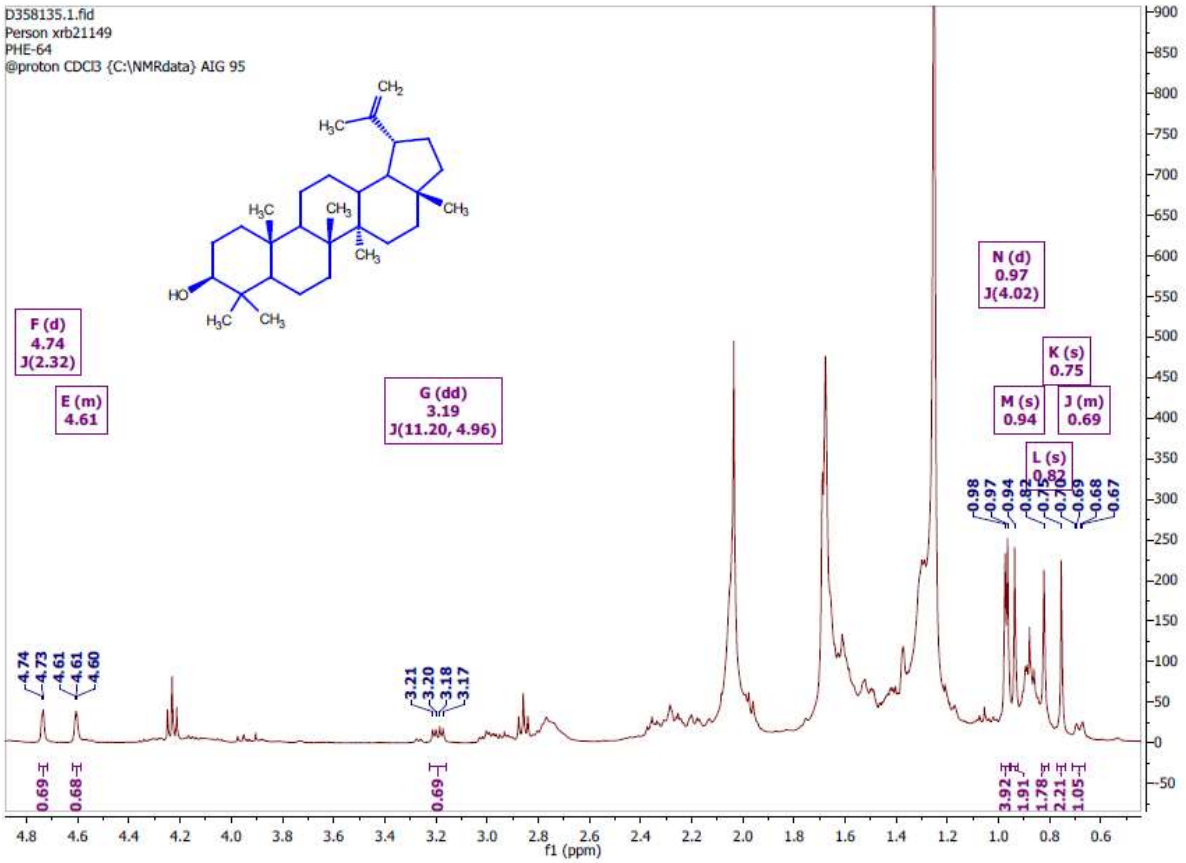
Table 8: NMR data for PHE-64

Position	Experimental		literature data (Tor-Anyiin and Akpuaka, 2011)	
	$^{1}H(\delta)$	$^{13}C(\delta)$	$^{1}H(\delta)$	$^{13}C(\delta)$
1	0.82	38.9	1.66, 0.83	38.78
2	1.56	27.5	1.37	27.51
3	3.21	79.0	3.19	79.09
4		39.8	-	38.94
5	0.75	55.3	0.75	55.36
6	1.34	18.3	1.34, 1.24	18.40
7	1.37	34.3	1.37	34.35
8		40.9	-	40.90
9	1.28	50.5	1.24	50.50
10	-	37.7	-	38.11
11	1.16	21.1	1.05, 1.34	21.00
12	1.36	25.2	1.01, 1.37	25.20
13	1.65	34.8	1.66	38.11
14		43.0	-	42.90
15	1.05	27.5	1.87, 1.05	27.50
16	1.99	34.8	1.99, 1.23	35.65
17		43.0	-	43.09
18	1.37	48.7	1.37	48.36
19	2.39	48.3	2.38	48.07
20	-	151.0	-	151.05
21	1.92	21.1	2.01, 1.37	29.90
22	0.98	40.0	1.59, 1.05	40.07
23	0.97	29.8	0.96	28.07
24	0.68	15.6	0.78	15.47
25	0.67	16.7	0.81	16.21
26	1.00	16.8	1.01	16.05
27		14.7	0.95	14.63
28	0.81	18.4	0.82	18.08
29	4.73, 4.61	109.3	4.67, 4.56	109.44
30	1.66	19.3	1.67	19.39

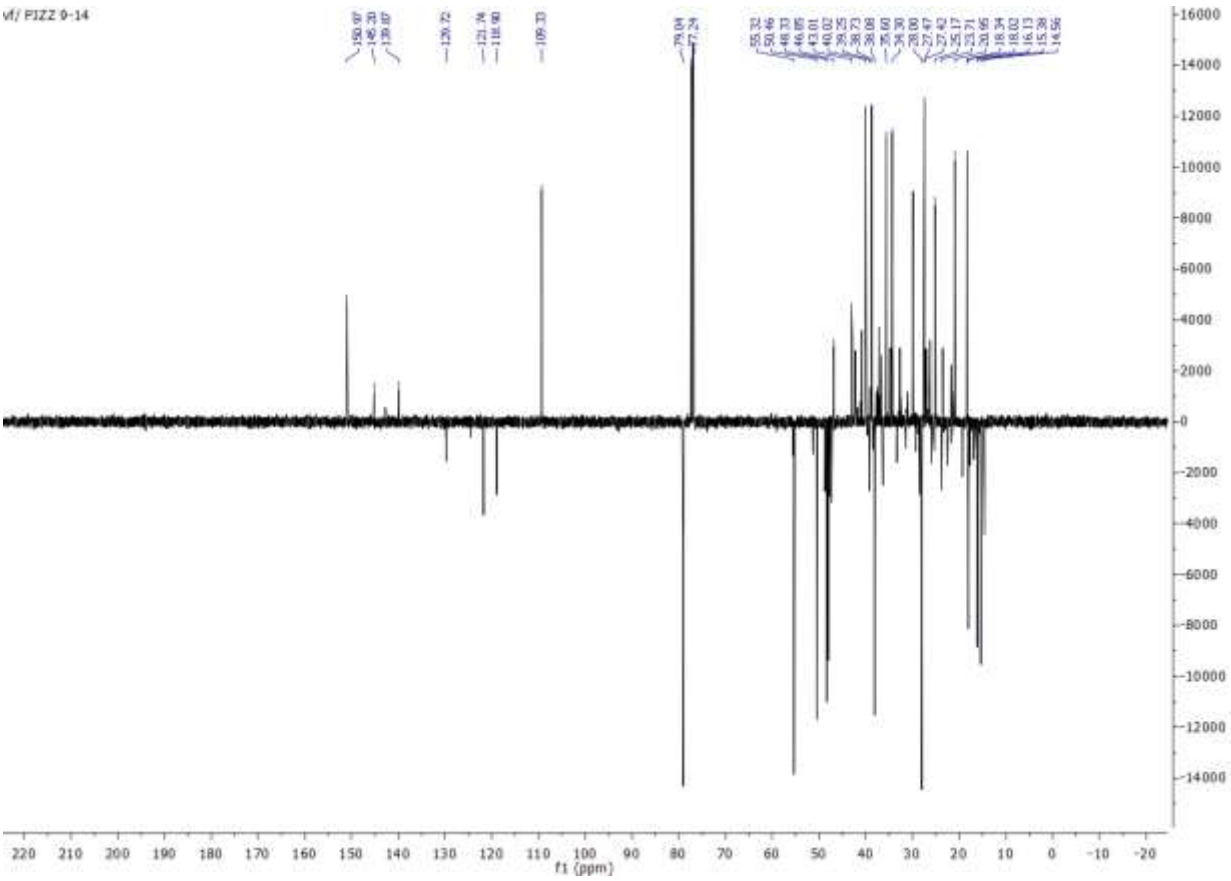


Structure of PHE-64: Lupeol

APPENDIX A: H-NMR SPECTRUM OF PHE-64 (Lupeol)



APPENDIX B: ^{13}C-NMR SPECTRUM OF PHE-64 (Lupeol)



Conclusion

With support from the findings of the antimicrobial assays performed on the clinical isolates used in this investigation, this study demonstrates the presence of some secondary metabolites in the plant that have been confirmed to be biologically active and account for the high medicinal values of the plant under investigation in traditional medicine. Based on spectroscopic analysis and comparison with previously published literature, the structure of the main chemical that was isolated, described, and identified in the research was determined. Therefore, this research advises that the separated compounds and plant extracts might be employed as medication candidates in the treatment of any illness brought on by the isolates used in this investigation that exhibits high sensitivity.

REFERENCES

Adesokan, A.A., Akanji, M.A. and Yakubu, M.I. (2007). Antibacterial potentials of aqueous extracts of *Enantia chlorantha* stem bark. *Africa Journal of Biotechnology*, 6(22): 2502-2505.

Ahmed, A.A., Yesmiem, K.A., Heba, H.I and Ali, A.A. (2012). Antioxidant, antimicrobial and theoretical studies of the thiosemicarbazone derivative Schiff base 2-(2-imino-1-methylimidazolidin-4-ylidene) hydrazinecarbothioamide (IMHC). *Organic Medicinal Chemistry Letter*, 2:4.

Ajibesin, K.K. (2011). *Dacryodes edulis*: A review of its medicinal, phytochemical and economic properties, *Journal of Traditional 51*: 32-41.

Akinpelu, D.A. and Onukoya, Z.T.M (2006). Antimicrobial activities of medicinal plants used in folklore remedies in South-Western Nigeria. *African Journal of Biotechnology,* 7(5): 1078-1081.

Alsiddig, O., Sufyan, A., Mai, O., Saga, Y., Yousra, B., Thoyba, E. and Afnan, A. (2017). Antimicrobial activity and elemental composition of *Sarcolephalus latifolius* fruits: An ethnopharmacological based evaluation. *Journal of Advances in Microbiology*, 3(2): 1-5.

Amise, A.F., Lennox, J.A. and Agbo, B.E. (2016). Antimicrobial Potential and Phytochemical Analysis of *Dacryodes edulis* Against selected clinical Bacterial isolates. *International Journal of Pharmacognosy and Phytochemical Research*, 8(11):1795-1800.

Diso, S.U., Ali, M., Mukhtar, S.I. and Garbs, M. (2017). Antibacterial activity and phytochemical screening of *Mangifera indica* stem and leaf-extracts on clinical isolates of methicillin resistant, staphylococcus aureus. *Journal of Advance in Medical and Pharmaceutical Sciences*, 1391): 1-6.

Ejiofor, N. and Nna, P.J. (2022): Analysis of the Phytoconstituents of Chromolaena odorata leaves and its Bioactivities against some clinical and plant pathogens. FNAS-Journal of Scientific Innovations, 3(2): 1-9.

Fagbohum, E.D., Asare, R.R. and Egbebi, A.O.(2012). Chemical composition and antimicrobial activities of *Urena lobate L*.(Malvaceae). *Journal of Medicinal Plants Research*, 6(12): 2256-2260.

Koshkoneba, E., Touckia, I., Worowounga, X., Toumuou, A.L., Mololi, A., Mukeina, G., Sanbella, S., Batauilla, K. and Akoagana, K. (2017). Case study and phytochemical investigation of *Zanthoxylum xanthoxyloids* and *Zanthoxylum macrophylum* (Rutaceae) in the central Africa Republic of Togo: A comparative approach. *American Journal of Phytomedicine and Clinical Therapeutics*, 6(11): 1-9.

Nande, W.U and Igoli, J.O (2017). Isolation and Characterization of Bioactive compounds from plant materials: The Nigerian situation. *Nigerian Journal of Pure and Applied Sciences*, 9(1): 132-143.

Nna, P.J, Tor-Anyin, T.A & Igoli, J.O (2019b). Fagaramide and Pellitorine from the Stem Bark of *Zanthoxylum zanthoxyloides* and their Antimicrobial Activities. *South Asian Research Journal of Natural Products, 2*(3), 1-8

Nna, P.J. (2020): Isolation and characterization of Tyrosol from the stem bark of *Gmelina arborea* and its antimicrobial activities. *Direct Research Journal of Chemistry*

and material Science.

Nna, P.J. and Ejiofor, N. (2023): Extraction and Characterisation of Steroidal Compounds from Gmelina arborea stem bark for Pharmaceutical Applications. International Journal of Chemistry and Chemical Processes, 9(1):36-43.

Nna, P.J. and Okwelle, A.A. (2021): Spectroscopic Characterization and identification of Friedelan-3-ol from Mangifera indica root and its anti-microbiological activities against some clinical pathogens. FNAS-Journal of Scientific Innovations, 3(1):60-69.

Nna, P.J. and Okwelle, A.A. (2022): Quantitative phytochemical screening of four Nigerians medicinal plants and their biological activities against some clinical pathogens. International Journal of Research in Pharmacy and Pharmaceutical sciences. 7(4):8-13.

Nna, P.J.(2022): Spectroscopic Characterization of D-Glucitol from Mangifera indica root. FNAS-Journal of Scientific Innovations,3(2): 28-34.

Nna, P.J., Tor-Anyiin, T.A. and Khan, E.M. (2019a): Stigmasterol and β-Sitosterol from the Root of *Mangifera indica* and their Biological Activities against some Pathogens. Journal of Complementary and Alternative Medical Research, 7(4): 1-10.

Nwokonkwo, D.C. (2014). The phytochemical study and antibacterial activities of the seed extract of *Dacryode edulis* (African native pea). *African Journal of Science Industrial Research*,.5:7-12.

Ogoko, E.C. (2018). Phytochemcial and heavy metal analysis of *Gongronema latifolium*, *Talinum Triangulane* and *Amaranthus Hybridus*. *Fuw Trends in Science and Technology Journal*, 3(1): 195-200.

Okwu, D.E. and Morah, F.I (2007). Isolation and characterization of flavanone glycoside 4,6,5,7 trihydroxy flavanone Rhamnoglucose from *Garcinia kola* seed. *Journal of Applied Science*, 7(2).

Omodamiro, O.D., Jimoh, M.A., and Ewa, I.C. (2016): Hepatoprotective and haemopoeitic activity of ethanol extract of *Persea Americana* seed in paracetamol induced toxicity in wista ralbino rat. *International Journal of Parasitology*, 5(3): 149-165.

Sha, K.A, Petal, R.J. and Parma, P.K (2010). *Mangifera indica* (mango): *Phamacognosy Review*, 4:42-48.

Sofowora, E.A. (1993). Medicinal plants and traditional medicine in Africa. 2nd edition. Spectrum books Ltd, Ibadan, Nigeria Pp. 289.

Stern, R., Kingsley, S.J. and James, E.B. (2003). Introduction to plant biology, 9th edition, New York, McGraw Hill Company Inc. Pp. 557.

Tene, T.O., Nyamen, L.D., Ngouafong, T.F., Seukep, A.J. (2016): Ethnobotanical uses, Phytochemical and Pharmacological Profiles, and Toxicity of Persea Americana mill: An Overview. *Pharmacology Online*. 3:213-221.

Tor-Anyiin, T.A. and Akpuaka, M.U. (2011): A Triterpenoid from stem Bark of *Sterculia* setigera Del. *Journal of Chemical Society of Nigeria*, 36 (2).

Tor-Anyiin, T.A., Igoli, J.O. and Anyam, J.N. (2016). Studies on *Dacryodes edulis* III: Isolation and characterization of Gallic acid from methanolic extract of raw (Untreated) seed of

Dacryodes edulis and its Antimicrobial properties. *Journal of chemical society of Nigeria*. 40(1): 6-9.

Udeme, G.C., Dahiru, D. and Etteh, C.C. (2018). Invitro antioxidant activities of aqueous and ethanol extracts of *Manifera indica* leaf, stem bark and root bark. *Pharmacognosy Communications,* 8(3): 119-124.

Uprendra, B.G., Duraiswamy, B., Mayur, R.B and Sanjayi, A.A. (2018). Isolation and Characterisation of Ipeola triterperiod from Stem bark of *Ziziphus Xylopny* (rets) wild ineative rapid pharmacecutical Analysis and Quality Assurance, 4(4):1-8.

Wang, F., Li, Z., Cui, H. and Liang, M. (2015). Two new triterpenoids from the resin of *Boswellia canterii*. *Journal of Asian Natural Products Research*, 13(3): 193-197.