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Original Research Article

Isolation and Characterisation of Bioactive Principles from Sapwood of *Pterocarpus santalinoides*

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ABSTRACT

Pterocarpus santalinoides is a medicinal plant widely employed in traditional Nigerian medicine for managing diverse health conditions. The present study focuses on the isolation and characterisation of biologically active compounds from the sapwood of Pterocarpus santalinoides. . Sequential extraction was carried out using n-hexane, ethyl acetate, and methanol, followed by purification of the ethyl acetate extract through column chromatography. Thin Layer Chromatography (TLC) was employed for fraction monitoring, while the structures of isolated compounds were elucidated using ^{1}H, ^{13}C, and 2D NMR techniques, including HMBC, HSQC, and COSY, in comparison with existing literature. The identified compounds included lupeol, a mixture of *β*-sitosterol and stigmasterol, a 1,2-diacylglycerolipid containing polyunsaturated ω-6 and ω-3 acyl chains, a diacylglycerol with one polyunsaturated and two saturated/monounsaturated chains, and oleanolic acid. Crude extracts of *Pterocarpus santalinoides*, including ethyl acetate, methanol, and hexane, exhibited significant antimicrobial activity against both Gram-positive and Gram-negative microorganisms. Notably, fraction EPs14 demonstrated superior efficacy, with inhibition zones of 23 mm to 27 mm against various fungi and bacteria. EPs26 and EPs36 showed the highest inhibition zones of 29 mm and 30 mm, respectively, against *Phaeolus schweinitzii* and *Coniophora puteana*. Minimum inhibitory concentrations (MIC) for these fractions were as low as 6.25 µg/mL. These findings support the traditional use of *P. santalinoides* in the treatment of diarrhoea, placentitis, skin infections, and gastrointestinal disorders, marking the first report of these bioactive compounds from the plant's sapwood.

Keywords: *Pterocarpus santalinoides,* Lupeol, Glycerolipids, Phytosterols, Oleanolic

Introduction

Plants have long served as the foundation for treating ailments in both traditional and orthodox medicinal practices, with evidence dating back millennia. [1] Traditional medicine encompasses a broad spectrum of health practices, methodologies, beliefs, and knowledge systems, incorporating remedies derived from plants, animals, and minerals, alongside spiritual, hands-on, and physical therapies. These approaches are utilised either independently or in combination to diagnose, treat, prevent, or maintain health and wellness. [2,3] This knowledge, often passed down through generations, remains relevant today, particularly in regions where access to modern healthcare is limited[1]. In developing countries like Nigeria, the high cost of conventional medicines further exacerbates healthcare challenges, particularly due to low living standards. [4] Nigeria, however, is endowed with a wealth of plant species whose roots, barks, stems, and leaves are extensively employed by traditional healers to treat various ailments. Consequently, there is a growing focus on traditional medicine practices and the screening of plant-derived bioactive compounds to identify those with genuine therapeutic potential. These bioactive compounds, or phytochemicals, are natural, non-nutritive substances found in plants that exhibit significant health benefits and possess disease-preventive properties. [5]

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Pterocarpus santalinoides (Family: Leguminosae Papilionoideae) is an evergreen tree with a dense crown, typically growing to a height of 9–12 metres, and is commonly found in lowland forests across Africa and tropical South America. The plant's distribution spans countries such as Brazil, Cameroon, Ghana, Nigeria, and Senegal. [6] In traditional African medicine, various parts of P. santalinoides are utilised for the treatment of a wide range of human ailments. [7] In Nigeria, the plant is known by various local names, including Ikyarkya (Tiv), Uturukpa (Igede), Nturukpa (Igbo), Gbengbe (Yoruba), Gunduru or Gyadarkurmi (Hausa), Okumeze (Edo), Maganchi (Nupe), and Nja (Efik). [8] Among the Tiv, Igede, and Igbo communities, the fresh leaves are often consumed in soups and are believed to be effective in managing gastrointestinal disorders, including diarrhoea. [4] Additionally, the leaves are used in veterinary medicine to alleviate abdominal pain in goats and regulate menstruation in females. [9] Fresh leaf extracts, when combined with Solanum macrocarpon leaves, are traditionally used to manage high blood pressure. [9] In Ivory Coast, the bark and leaves are employed in the treatment of sleeping sickness, while tannins extracted from the plant are used in dyeing processes. [8]Furthermore, P. santalinoides has been traditionally used to treat conditions such as rheumatism, diarrhoea, dysentery, cough, asthma, diabetes, malaria, elephantiasis, colds, and more. [3] Its efficacy in treating skin conditions, including eczema and candidiasis, has also been documented. [2]

The rising resistance of microorganisms, particularly those responsible for diarrhoea and gastrointestinal diseases, has spurred research into the effectiveness of traditional treatments[4]. Despite global advancements in antimicrobial research, antibiotic resistance remains a critical public health issue, with a growing concern over resistant strains of bacteria, viruses, and parasites. This issue disproportionately affects rural populations with limited access to modern healthcare. [6] To date, there has been no comprehensive investigation into the sapwood of P. santalinoides, which presents a significant gap in the current body of

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knowledge. This study seeks to address this gap by conducting a sequential extraction, isolation, antimicrobial activity and characterisation of bioactive compounds from the sapwood of P. santalinoides using column chromatography and Nuclear Magnetic Resonance (NMR) spectroscopy. To the best of our knowledge, this is the first report on the isolation and characterisation of bioactive compounds from P. santalinoides sapwood.

Materials and Methods

Plant Collection

Leaves and bark of *P. santalinoides* were collected in July, 2018 from Federal Housing Estate North-Bank, Makurdi Benue state, Nigeria (latitude 7° 45' 42.20"N; longitude 8° 34' 21.86"E). The fresh sapwood of the plant was rendered into sawdust using a power saw.

The sawdust of *Pterocarpus santalinoides* sapwood was collected, air-dried under ambient laboratory conditions for two weeks, and labelled as EPs for easy identification. The dried sapwood (1 kg) was sequentially extracted using hexane, ethyl acetate, and methanol. Each solvent extraction was conducted by soaking the plant material in the solvent for 48 hours. After extraction, the solvent was decanted, filtered through Whatman filter paper, and allowed to evaporate at room temperature. The dried extracts were adsorbed onto silica gel (230–400 mesh, Merck KGaA, Darmstadt, Germany) to form a free-flowing powder before being subjected to column chromatography. The isolation protocol used here is as per methods described by Hostettmann, Hostettmann and Marston with slight modifications. [21]

Column chromatography was performed using a silica gel slurry (100 g) as the stationary phase, packed into a glass column. Cotton wool was placed at the bottom of the column to prevent loss of material, and the dried extract (1.3 g) was introduced using a funnel onto the gel bed. Prior to extract loading, the gel bed was gently tapped to remove trapped air bubbles. Gradient elution was carried out using a hexane/ethyl acetate mixture, starting from 190:10 (v/v) and progressing in decreasing hexane concentrations (180:20, 170:30, etc.) up to 100:100. Fractions (EPs1 -EPs120) were collected and pooled based on their TLC profiles. EPs26 (**1) (**27 mg**)** was obtained at a solvent ratio of 180:20 (hexane: ethyl acetate) without further purification. EPs36 (**2** & **3**) (40 mg) was isolated at a solvent ratio of 180:20 (hexane: ethyl acetate) without further purification, EPs42 (**4**) (36 mg) was obtained at a solvent ratio of 170:30 (hexane: ethyl acetate) without further purification. EPs61 (**5**) (40 mg) was obtained at a solvent ratio of 170:30 (hexane: ethyl acetate) without further purification. EPs14 (**6)** (20 mg) was obtained at a solvent ratio of 190:10 (hexane: ethyl acetate) without further purification.

The antimicrobial activity of the isolated compounds (EPs14, EPs26, EPs36) and crude extracts of *P. santalinoides* was evaluated against a panel of pathogenic microorganisms using the agar well diffusion method. Microbial strains were obtained from the Institute for Agricultural Research, Ahmadu Bello University (Zaria, Nigeria). The crude extracts (5 mg/mL) and fractions (100 µg/mL) were prepared by dissolving in dimethyl sulfoxide (DMSO). Mueller-Hinton agar was used as the growth medium for bacteria, while Sabouraud dextrose agar was used for fungi. The media were sterilised at 121°C for 15 minutes and poured into sterile Petri dishes.

Bacterial inocula (0.1 mL) were seeded onto the surface of Mueller-Hinton agar plates, while fungal inocula were spread evenly on Sabouraud dextrose agar using sterile swabs. Wells (6 mm in diameter) were cut into the inoculated agar, and 0.1 mL of the prepared fraction or extract solution was introduced into each well. Plates were incubated at 37°C for 24 hours for bacterial strains and at 30°C for 7 days for fungal strains. Zones of inhibition were measured and recorded in millimetres.

The MIC of the fractions and extracts was determined using the broth microdilution method, as described by Odeh and Tor-Anyiin and Rodríguez-Melcón *et al*. [3,10] Fractions were serially diluted two-fold in Mueller-Hinton broth to achieve concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL, while extracts were diluted to concentrations of 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.63 mg/mL, 0.31 mg/mL, and 0.16 mg/mL. A 0.1 mL aliquot of each dilution was inoculated with 0.1 mL of microbial suspension (1.5 x 10^8 CFU/mL). Tubes were incubated at 37°C for 24 hours and observed for turbidity, with the lowest concentration showing no visible growth being recorded as the MIC.

MBC/MFC was determined by transferring 0.1 mL from tubes showing no growth (from the MIC assay) onto antibiotic-free Mueller-Hinton agar plates. Plates were incubated at 37°C for 24 hours, and the absence of colonies was recorded as the MBC/MFC. The lowest concentration of extract or fraction showing no microbial growth was considered the MBC or MFC. [3,10]

The bioactive components of the fractions were analysed using ^{1}H, ^{13}C, and 2D NMR spectroscopy on a 500 MHz Bruker BioSpin spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany). Mnova 14 software was employed to process the spectral data and confirm the structural identities of the isolated compounds.

Results and Discussion

The crude ethyl acetate, methanol, and hexane extracts of *Pterocarpus santalinoides*, along with isolated compounds (EPs14, EPs26, EPs36), exhibited significant antimicrobial activity against both Gram-positive and Gram-negative microorganisms. Crude extracts effectively inhibited the growth of *Aspergillus niger*, *Coniophora puteana*, *Gloeophyllum sepiarium*, and *Rhizopus sporangia* with zones of inhibition ranging from 18 mm to 25 mm (Table 1).

Table 1: Fungal Activities and Zone of Inhibition (ZOI) of the extracts

Test organism	Ethylacetate Sensitivity (ZOI)	Methanol Sensitivity (ZOI)	Hexane Sensitivity (ZOI)
Aspergillus flavus	R (00)	R (00)	R (00)
Aspergillus fumigatus	R (00)	R (00)	R (00)
Aspergillus niger	S (21)	S (20)	S (18)
Coniophora puteana	S (25)	S (21)	S (20)
Fibroporia vaillantii	S (20)	S (20)	R (00)
Fomitopsis pinicola	R (00)	R (00)	R (00)
Gloeophyllum sepiarium	S (20)	S (21)	S (20)
Phaeolus schweinitzii	R (00)	R (00)	R (00)
Rhizopus sporangia	S (23)	S (22)	S (21)
Sclerotinia sclerotiorum	R (00)	R (00)	R (00)
Serpula lacrymans	R (00)	R (00)	R (00)

Key: S=Sensitive, R=Resistant ZOI=Zone of Inhibition in mm

In comparison, fraction EPs14 demonstrated superior antimicrobial activity, inhibiting *C. puteana, Fomitopsis pinicola, G. sepiarium, Phaeolus schweinitzii, Sclerotium rolfsii,* and *Serpula lacrymans* with inhibition zones between 23 mm and 27 mm, surpassing the efficacy of the crude extracts. EPs26 exhibited the highest zone of inhibition (29 mm) against *P. schweinitzii*, while the lowest was 25 mm against *C. puteana.* EPs36 showed the strongest inhibition (30 mm) against *C. puteana*. (Table 2). All extracts of *P. santalinoides* inhibited the growth of *Acidobacteria* with zones ranging from 18 mm to 26 mm, and they also effectively inhibited *Alphaproteobacteria, Betaproteobacteria, Enterococcus faecium, Bacillus subtilis*, and *Proteus mirabilis*, with inhibition zones between 16 mm and 23 mm. The highest activity was observed in the ethyl acetate extract against *B. subtilis* (Table 3).

The isolated compounds demonstrated potent antibacterial properties as well. EPs14 showed high sensitivity against *Acidobacteria, Alphaproteobacteria, Betaproteobacteria*, *E. faecium*, and *P. mirabilis*, with a maximum inhibition zone of 30 mm, similar to the inhibition observed for *Actinobacteria* and *E. faecium* (Table 4). EPs26 exhibited

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the highest inhibition zone (31 mm) against *Alphaproteobacteria*, while EPs36 recorded the same highest inhibition (31 mm) against *Pseudomonas aeruginosa*. The minimum inhibitory concentration (MIC) of EPs14 was 25 µg/mL against *C. puteana*, *G. sepiarium*, and *S. lacrymans*, while EPs26 showed an MIC of 12.5 µg/mL against *Aspergillus fumigatus*, *C. puteana*, *G. sepiarium*, and *S. lacrymans*. EPs36 recorded the lowest MIC of 6.25 µg/mL against *C. puteana*. EPs14 had an MIC of 25 µg/mL against *Betaproteobacteria* and 12.5 µg/mL against *Acidobacteria*, *Alphaproteobacteria*, and *P. mirabilis*. These antimicrobial tendencies lend credence to the plant's use in treating gastro-intestinal infection, placentitis, mucormycosis and abortion in traditional medicine.[3, 7]

Table 2: Antifungal activities and Zones of Inhibitions (mm) for EPS14, EPS26 and EPS36

Test organisms	EPS14 Sensitivity (ZOI)	EPS26 Sensitivity (ZOI)	EPS36 Sensitivity (ZOI)
Aspergillus flavus	R (00)	R (00)	S (28)
Aspergillus fumigatus	R (00)	S (27)	R (00)
Aspergillus niger	R (00)	R (00)	S (27)
Coniophora puteana	S (26)	S (25)	S (30)
Fibroporia vaillantii	R (00)	R (00)	S (29)
Fomitopsis pinicola	S (24)	R (00)	R (00)
Gloeophyllum sepiarium	S (23)	S (27)	R (00)
Phaeolus schweinitzii	S (27)	S (24)	S (28)
Rhizopus sporangia	R (00)	S (28)	R (00)
Sclerotinia sclerotiorum	S (26)	R (00)	S (29)
Serpula lacrymans	S (25)	R (00)	S (27)

Key: S=Sensitive, R=Resistant ZOI= Zone of Inhibition in mm

Table 3: Antibacterial Activities and Zone of Inhibition for *Pterocarpus santalinoides* extracts

Test organism	Ethylacetate Sensitivity (ZOI)	Methanol Sensitivity (ZOI)	Hexane Sensitivity (ZOI)
Acidobacteria	S (26)	S (22)	S (18)
Actinobacteria	R (00)	R (00)	R (00)
Alphaproteobacteria	S (22)	S (21)	S (19)
Enterococcus faecium	R (00)	R (00)	R (00)
Betaproteobacteria	S (20)	S (20)	S (16)
Bacillus subtilis	S (23)	S (20)	S (18)
Gammaproteobacteria	S (20)	S (18)	S (18)
Proteus mirabilis	R (00)	R (00)	R (00)
Pseudomonas aeruginosa	R (00)	R (00)	R (00)

Key: S=Sensitive, R=Resistant ZOI=Zone of Inhibition in mm

Table 4: Bacteria Activities and Zone of Inhibition (mm) for EPS14, EPS26 and EPS36 fractions

Test microbes	EPS14 Sensitivity (ZOI)	EPS26 Sensitivity (ZOI)	EPS36 Sensitivity (ZOI)
Acidobacteria	S (28)	S (26)	S (30)
Actinobacteria	R (00)	S (30)	R (00)
Alpha proteobacteria	S (27)	S (31)	S (26)
Enterococcus faecium	S (30)	S (30)	S (27)
Betaproteobacteria	S (26)	R (00)	R (00)
Bacillus subtilis	R (00)	R (00)	R (00)
Gammaproteobacteria	R (00)	R (00)	R (00)
Proteus mirabilis	S (27)	S (26)	S (26)
Pseudomonas aeruginosa	R (00)	S (28)	S (31)

NMR Spectroscopic Characterisation of Isolated Fractions

Lupeol (**1**): off-white powder, melting point 213–217°C, Rf value = 0.96. ^{1}H NMR data: δ_H: 0.91 (1H, *m*, H-1α), 1.67 (1H, *m*, H-1β), 1.6 (1H, *m*, H-2α), 1.56 (1H, *m*, H-2β), 3.19 (1H, *dd*, *J* = 11.1, 5.7 Hz, H-3), 0.68 (1H, *d*, H-5), 1.54 (1H, *m*, H-6α), 1.39 (1H, *m*, H-6β), 1.39 (2H, *m*, H-7), 1.25 (1H, *m*, H-9), 1.42 (1H, *m*, H-11α), 1.23 (1H, *m*, H-11β), 1.08 (1H, *m*, H-12α), 1.62 (1H, *m*, H-12β), 1.66 (1H, *m*, H-13), 1.03 (1H, *m*, H-15α), 1.68 (1H, *m*, H-15β), 1.35 (1H, *m*, H-16α), 1.47 (1H, *m*, H-16β), 1.36 (1H, *m*, H-18), 2.38 (1H, *dd*, *J* = 11.0, 6.0 Hz, H-19), 1.32 (1H, *m*, H-21α), 1.89 (1H, *m*, H-21β), 1.18 (1H, *m*, H-22α), 1.39 (1H, *m*, H-22β), 0.95 (3H, *s*, H-23), 0.76 (3H, *s*, H-24), 0.89 (3H, *s*, H-25), 1.02 (3H, *s*, H-26), 0.94 (3H, *s*, H-27), 0.79 (3H, *s*, H-28), 4.56 (1H, *t*, *J* = 2.1 Hz, H-29α), 4.68 (1H, *t*, *J* = 2.5 Hz, H-29β), 1.68 (3H, *s*, H-30). ^{13}C NMR (100 MHz, $CDCl_3$) δ_C: 38.9 (C-1), 27.5 (C-2), 79.2 (C-3), 39.0 (C-4), 55.4 (C-5), 18.4 (C-6), 34.4 (C-7), 41.0 (C-8), 50.6 (C-9), 37.3 (C-10), 21.0 (C-11), 25.3 (C-12), 38.2 (C-13), 43.0 (C-14), 27.6 (C-15), 35.7 (C-16), 43.2 (C-17), 48.4 (C-18), 48.2 (C-19), 151.1 (C-20), 29.9 (C-21), 40.2 (C-22), 28.2 (C-23), 15.5 (C-24), 16.3 (C-25), 16.1 (C-26), 14.7 (C-27), 18.2 (C-28), 109.5 (C-29), 19.5 (C-30). The data aligns closely with literature reports on lupeol, [11, 12] a well-known triterpenoid found in various natural sources, including lupin seed skin, figs, and edible fruits. Lupeol (**1**) **(Figure 1)** is recognised for its therapeutic properties in managing multiple health conditions.

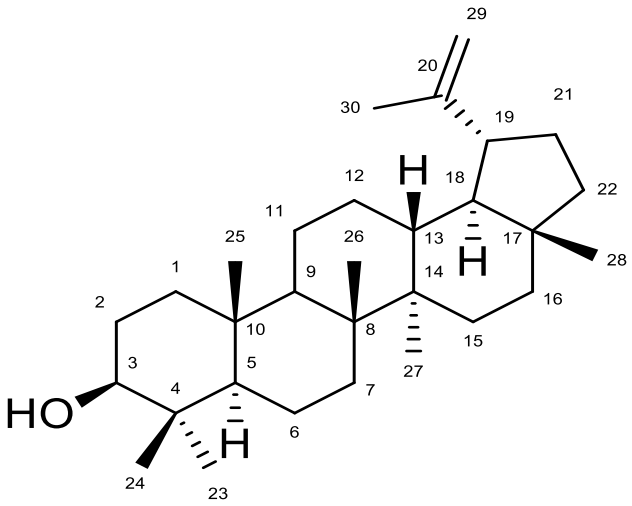

Figure 1: Structure of Lupeol (1)

Stigmasterol (2) and *β*-Sitosterol (3): white crystalline powder, Rf 0.69 (hexane: ethyl acetate, 9:1), melting point = 133-136°C, NMR data: δ_H 5.35 (1H, *dd*, *J* = 4.8, 2.4 Hz, H-22), 5.15 (0.33H, *dd*, *J* = 15.2, 8.6 Hz, H-23a), 5.02 (0.31H, *dd*, *J* = 15.2, 8.6 Hz, H-23b), 3.52 (1H, *tt*, *J* = 11.1, 4.6 Hz, H-3), 2.38–2.18 (3H, *m*, H-7, H-8), 1.25 (4H, *s*, H-2, H-12), 1.01 (4H, *d*, J = 3.2 Hz, H-21), 0.93 (3H, *s*, H-24), 0.84 (3H, *d*, *J* = 1.9 Hz, H-26), 0.80 (3H, *d*, *J* = 2.0 Hz, H-27), 0.69 (3H, *d*, J = 9.1 Hz, H-18.The spectral data closely aligned with literature reports for Stigmasterol (2) and *β*-Sitosterol (3) (Figure 2), which are frequently co-isolated as mixtures. [13,14] The key distinguishing feature between these two compounds is the presence of a C22=C23 double bond in stigmasterol and a C22-C23 single bond in *β*-sitosterol. The NMR data strongly suggest that EPs36 is a mixture of these well-known sterols, further supported by comparisons with existing literature. [13,14]

1,2-diacylglycerolipid (4): white powdery solid, δ_H 5.35 (3H, *qt*, *J* = 11.2, 3.5 Hz, olefinic), 5.28 (1H, *t*, *J* = 3.7 Hz, *SN*-2 glyceryl proton), 5.08 (0H, *p*, *J* = 5.0 Hz), 4.49 (0H, *dd*, *J* = 9.5, 6.5 Hz), 4.34–4.03 (4H, *m*, *SN*-1 and *SN*-2 glyceryl protons), 3.73 (1H, *dd*, *J* = 5.0, 1.8 Hz, glyceryl group in 1,2-diglycerides), 2.84–2.73 (2H, *m*, bisallylic protons), 2.33 (4H, *dt*, *J* = 12.1, 7.5 Hz, H-2 acyl moiety), 2.03 (5H, *h*, *J* = 6.4, 5.9 Hz), 1.61 (5H, *dq*, *J* = 13.6, 6.6 Hz), 1.39–1.21 (37H, m, methylene protons), 0.95–0.82 (12H, *m*, terminal methyl signals). The spectrum is consistent with a 1,2-diacylglycerolipid (4) (Figure 2) containing polyunsaturated ω-6 and ω-3 acyl chains. [20]

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Oleanolic acid (5): whitish crystals, melting point, 306 - 313°C. Rf value = 0.50: hexane: ethyl acetate: 9:1, 1H NMR (400 MHz, $CDCl_3$) δH: 1.02, 1.57 (2H, *m*, H-1), 1.82, 1.82 (2H, *m*, H-2), 3.44 (1H, *dd*, *J* = 12.0, 4.2 Hz, H-3), 0.88 (1H, *d*, *J* = 11.0 Hz, H-5), 1.58, 1.39 (2H, *m*, H-6), 1.53, 1.36 (2H, *m*, H-7), 1.71 (1H, *t*, *J* = 6.7 Hz, H-9), 1.96, 1.96 (2H, *m*, H-11), 5.49 (1H, *s*, H-12), 1.22, 2.19 (2H, *m*, H-15), 2.12, 1.96 (2H, *t*, *J* = 6.9 Hz, H-16), 3.30 (1H, *dd*, *J* = 11.4, 4.3 Hz, H-18), 1.83, 1.32 (2H, *m*, H-19), 1.46, 1.23 (2H, *m*, H-21), 1.82, 2.04 (2H, *m*, H-22), 1.24 (3H, *s*, H-23), 1.02 (3H, *s*, H-24), 0.93 (3H, *s*, H-25), 1.04 (3H, *s*, H-26), 1.30 (3H, *s*, H-27), 0.97 (3H, *s*, H-29), 1.02 (3H, *s*, H-30). ^{13}C NMR (100 MHz, $CDCl_3$) δC: 33.77 (C-1), 27.31 (C-2), 79.17 (C-3), 38.54 (C-4), 55.33 (C-5), 18.45 (C-6), 32.78 (C-7), 38.54 (C-8), 47.75 (C-9), 40.83 (C-10), 22.85 (C-11), 122.78 (C-12), 143.67 (C-13), 41.73 (C-14), 27.80 (C-15), 24.85 (C-16), 46.64 (C-17), 41.20 (C-18), 46.00 (C-19), 29.85 (C-20), 33.21 (C-21), 32.53 (C-22), 29.21 (C-23), 15.70 (C-24), 62.23 (C-25), 17.07 (C-26), 26.06 (C-27), 177.61 (C-28), 32.78 (C-29), 23.53 (C-30), 51.20 (C-28—OH).

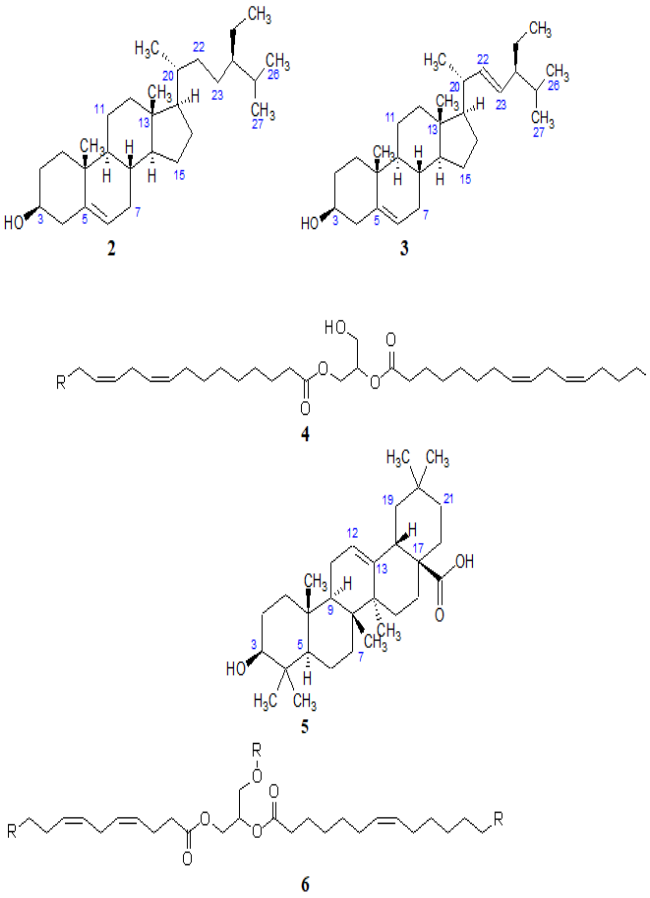

Figure 2: Structure of isolated compounds: *β*-sitosterol (2), Stigmasterol (3) Polyunsaturated glycerolipid (4) , Oleanolic acid (5) and Glycerolipid with one polyunsaturated chain (6).

A combination of 1D and 2D NMR methods, along with comparison with the reported literature data, let to characterisation of compound **5** as Oleanolic Acid [18, 19]

Oleanolic acid (**5)** (Figure 2) is a widely occurring pentacyclic triterpene found in various edible and medicinal plants. It is known for its broad range of pharmacological activities, including anticancer, antidiabetic, antimicrobial, antioxidant, antihypertensive, and anti-inflammatory properties. Clinical trials have also explored its safety, dosage, and pharmacokinetic profiles, further validating its therapeutic potential. [19]

Diacylglycerol (6): white, waxy crystals. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.35 (*qt*, *J* = 11.4, 6.3 Hz, 12H), 5.27 (*td*, *J* = 10.3, 9.1, 3.8 Hz, 1H), 4.29 (*dd*, *J* = 11.9, 4.3 Hz, 2H), 4.14 (*dd*, *J* = 11.9, 6.0 Hz, 2H), 2.79 (*dt*, *J* = 18.8, 6.5 Hz, 8H), 2.31 (*td*, *J* = 7.6, 3.0 Hz, 6H), 2.05 (*dhept*, *J* = 19.5, 6.8, 6.1 Hz, 11H), 1.66 – 1.54 (*m*, 8H), 1.40 – 1.23 (*m*, 36H), 0.97 (*t*, *J* = 7.6 Hz, 4H), 0.89 (*t*, *J* = 6.8 Hz, 5H). The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) displayed characteristic signals, including an olefinic proton resonance at δ_H 5.35 (qt, *J* = 11.4, 6.3 Hz, 12H) and an SN-2 glyceryl proton at δ_H 5.27 (td, *J* = 10.3, 9.1, 3.8 Hz). Glyceryl protons with ABX coupling appeared at δ_H 4.29 (dd, *J* = 11.9, 4.3 Hz, 2H) and δ_H 4.14 (dd, *J* = 11.9, 6.0 Hz, 2H). Bisallylic protons were observed at δ_H 2.79 (*dt*, *J* = 18.8, 6.5 Hz, 8H), while the H-2 protons of the acyl moiety appeared at δ_H 2.31 (*td*, *J* = 7.6, 3.0 Hz, 6H). Allylic protons were noted at δ_H 2.05 (*dhept*, *J* = 19.5, 6.8, 6.1 Hz, 11H), with methylene protons spanning δ_H 1.66–1.54 (*m*, 8H) and δ_H 1.40–1.23 (*m*, 36H). [20] The terminal methyl groups of the linolenoyl and saturated/monounsaturated acyl chains resonated at δ_H 0.97 (*t*, *J* = 7.6 Hz, 3H) and δ_H 0.89 (*t*, *J* = 6.8 Hz, 3H), respectively. [20] The NMR data strongly suggest that EPs14 is a diacylglycerol containing one polyunsaturated acyl chain and two additional saturated or monounsaturated chains (6). However, due to the limited spectral data and complexities related to the position of unsaturation in the acyl chains, further structural identification was not possible. [20]

Conclusion

The ethnomedicinal significance of *Pterocarpus santalinoides* has led to the successful isolation and characterisation of bioactive compounds from its sapwood, including the pentacyclic lupane triterpenoid lupeol, a mixture of stigmasterol and *β*-sitosterol, a 1,2-diacylglycerolipid containing polyunsaturated ω-6 and ω-3 acyl chains, a diacylglycerol containing one polyunsaturated acyl chain and two additional saturated or monounsaturated chains and oleanolic acid. The antimicrobial evaluation revealed that both the crude extracts and isolated compounds exhibited potent activity against a range of pathogenic microbes, with the isolated compounds demonstrating superior efficacy compared to the crude extracts. This research presents the first report of these compounds being isolated from the sapwood of *Pterocarpus santalinoides*, reinforcing the plant's traditional use in treating microbial infections and laying the foundation for further exploration of its pharmacological potential.

Conflict of Interest

Authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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