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Antimicrobial Activity of Lupeol and *β*-Amyrin (Triterpenoids) Isolated from the Rhizome of *Dolichos Pachyrhizus* Harm

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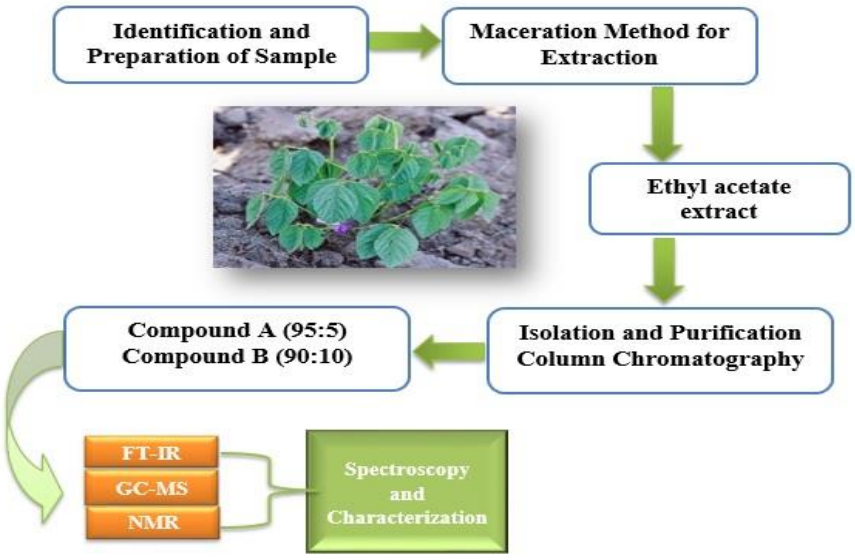
ABSTRACT

Dolichos pachyrhizus (Annonaceae) has been traditionally used in Africa to treat syphilis and many other microbial infections. However, no phytochemical study and antimicrobial investigations have been conducted on this species. Thus there is need for the discovery and development of new antibiotics and anifungal. The triterpenoids [Lupeol (1) and *β*-amyrin (2)] isolated from rhizome of Dolichos pachyrhizus (Harm) were evaluated for their antimicrobial activity against some selected Gram positive bacterial, Gram negative bacterial and Fungal isolates. The antimicrobial activity of the crude extract and compounds isolated was determined using agar well diffusion method. The compounds exhibited antimicrobial activity against most of the tested bacteria with minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) values ranging from 2.5 to 20 mg/mL and 5 to 40 mg/mL, respectively. The triterpenoids could be a potentially effective antimicrobial agent to combat infectious diseases. The structures of the compounds were elucidated using modern spectroscopic and spectrometric techniques such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) to be Lupeol and *β*-amyrin.

GRAPHICAL ABSTRACT



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Introduction

Pathogenic microorganism resistance strains are becoming more prevalent and emerging on a daily basis [1]. In addition, antimicrobial drugs in one way or another are connected to side effects or toxicity [2]. Therefore, great improvements are needed to address these problems associated to the current chemotherapeutic agents [3].

Because they contain many components with therapeutic significance, plant extracts have been utilized for a wide range of medicinal purposes for many years. Based on the occurrence of specific health issues, these features differ from region to region. As they are known to contain potent pharmacological substances, such as those with antibacterial and antifungal action, medicinal plants have therefore received considerable attention [4-5]. Phytochemicals are known for protecting plants and humans against parasitic and microbial diseases [6].

Biological properties of phytochemicals such as antibacterial [2], antioxidant [7], anticancer [8], and anti-inflammatory activities [9-10] have been reported. *Dolichos pachyrhizus* Harms (*D. pachyrhizus*) Papilionaceae (Fabaceae) a branching, sub-erect and downing herb dispersed in both hemispheres tropics and a member of fabaceae family [3].

D. pachyrhizus Harms is a common curling climber, sprawling perennial with small trifoliate leaves, bearing narrow flat curving pods when mature that are 1.5-2.0 inches long and have a persistent style at the peak. It grows in rocky soil and is a leguminous plant. The pods have 5-6 ellipsoid, flattened seeds that are 1/8 to 1/4 inch long [11].

It originates from Mexico and Central America and is a tropical plant. Due to its delicious underground tubercles, *D. pachyrhizus*, sometimes known as "jicama," has been widely cultivated in Mexico since the pre-Columbian era [12]. It was first cultivated under the name "yam bean" throughout India, China, and the USA. Since ancient times, Nigeria has used more yam beans as culinary ingredients than any other nation [13-15].

These plants are used by local citizens as fish poison [16] and insecticide [17], while in customary medicine they are used against syphilis [18].

Several phytochemical and pharmacological studies have been reported on Dolichos species such as *Dolichos mitis* [19-22], *Dolichos biflorus* [23], *Dolichos erosus* [24-25], and *Dolichos lablab* [26] among others with potentially therapeutic and prophylactic importance. On the other hand, based on the literature, there is no any phytochemical and or pharmacological activity reported on the *D. pachyrhizus* specie. Therefore, the current study aimed to isolate, characterize, and assess the antimicrobial efficacy of the bioactive component(s) and crude ethyl acetate extract of *D. pachyrhizus* rhizomes.

Experimental

Materials and methods

Reagents and solvents

The following chemicals were used: diluted hydrochloric acid (HCl), potassium iodide (KI), concentrated sulphuric acid (H_2SO_4), sodium hydroxide (NaOH), 1 % ferric chloride ($FeCl_2$), ethyl acetate ($CH_3COOC_2H_5$), and methanol (CH_3OH). The analytical-grade reagents and solvents used in this study were all procured from Sigma Aldrich in Germany.

Plant collection and identification

In February 2019, the *Dolichos pachyrhizus* rhizome was collected from Ajja bush in the Batsari Local Government Katsina State, Nigeria. The rhizome was identified at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria, with a specimen number (ABU09001) lodged.

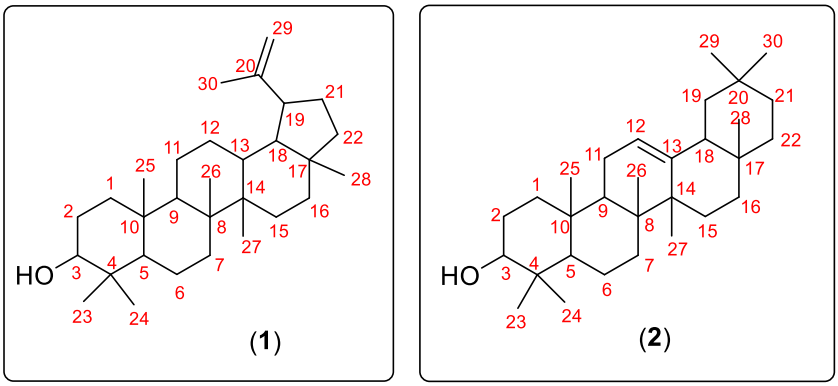
General analysis

The extraction procedure used was the cold maceration approach as described by [27-28]. For the extraction, the following solvents in increasing polarity were employed to obtain crude extracts of *D. pachyrhizus* that were saved for further use:

n-Hexane, dichloromethane, ethyl acetate, and methanol. The compounds were separated from the ethyl acetate extract (12.5 g) using column chromatography (24 700 mm; Silica gel 60; 0.063 to 0.2 mm; 70 to 230 mesh ASTM; Merck, Darmstadt, Germany).

The mobile phase was a hexane-ethyl acetate solvent gradient system (90:10 to 30:70) and fractions of 10 mL were collected. Four mixed fractions were obtained from the collected fractions after thin layer chromatography (TLC) analysis using silica gel 60 TLC aluminum sheets (20 cm 20 cm, F254). Compounds 1 (0.72 g) and 2 (1.14 g) were obtained after additional separate purification with hexane and ethyl acetate of the second and seventh mixed fractions. The melting point was determined using melting point equipment (Shalom Instruments Supplies, Durban, R.S.A.).

Nuclear magnetic resonance (NMR) techniques (^{1}H, ^{13}C, and DEPT) (in $CDCl_3$, Bruker 600 MHz) and infrared (FTIR) (KBr, Perkin-Elmer 100 FTIR) techniques were used to establish and confirmed the structures of the triterpenoids where chemical shifts (δ) were expressed in (ppm).



Scheme 1. Structure of *Lupeol* (1) and *β*-amyrin (2).

Phytochemical Screening

To identify the existence of bioactive components, a phytochemical study of the ethyl acetate extract of *D. pachyrhizus* was carried out [29-30].

Column chromatographic purification

Crude ethyl acetate extract (2 g) was weighed and transferred into a packed column carefully on top of silica gel; about 5 g of dried silica gel was applied to serve as a protective layer. A gradient solvent system of 100 % n-hexane (100 cm^3) was initially used to elute the column, followed by n-hexane-ethyl acetate (100 cm^3) in this order; 95:5; 90:10; 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45; 50:50; 45:55; 40:60; 35:65; 30:70; 25:75; 20:80; 15:85, and 10:90; 5:95.

Finally, the column was cleansed with 100% ethyl acetate (100 cm^3). Different fractions were collected in a 10 cm^3 beakers labeled (1-114) at intervals. At room temperature, each fraction was allowed to evaporate before examined through thin layer chromatography (TLC). Similar fractions were merged together based to

their TLC pattern, grouped into (K, L, M, and N), and then purified further by a short column [23], using n-Hexane: Ethyl acetate (95:5 and 90:10) as solvent systems. This led to isolation of two compounds from fractions A and B which were thoroughly washed with pentane to obtain optimal purity for spectroscopic analyses labeled isolates **1** and **2**.

Antimicrobial activity

Experimental organisms

The following species of bacteria were used to determine the antibacterial activity; four Gram-positive *Staphylococcus aureus (S. aureus),* (*Bacillus cereus (B. cereus), Streptococcus pneumonia (S. pneumonia)*, and *Staphylococcus saprophyticum (S. saprophyticum)*) and four Gram-negative (*Escherichia coli (E. coli), Klebsiella pneumonia (K. pneumonia), Selmonella typhi (S. typhi)*, *Pseudomonas aeruginosa (P. aeruginosa)*), *Candida albicans* (*C. albicans*), *Chrysosporium merdarium* (*C. merdarium*), and *Trichophyton rubrum.* (*T. rubrum*).

Determination of Zone of Inhibition

According to [31], the agar well diffusion method was employed. The ethyl acetate extract and isolated components from the Dolichos pachyrhizus rhizome were tested for their antibacterial activity. With the help of a sterile stick, the standardized innocula of the extract and isolated chemicals were uniformly smeared onto freshly prepared Mueller Hinton agar plates.

Each agar plate had five properly labeled wells drilled through it using a sterile cork borer (8 mm in diameter).

Each well received 0.2 mL of the proper concentration of extract and isolated compound, which was then given time to disperse into the agar. The inocula isolate was streaked onto a second plate, which was then covered with ciprofloxacin standard (10 ug/disc). The plates were incubated for 24 hours at 37 °C for the bacteria, and 48 hours at 25 °C for the fungi using Sabouraud Dextrose Broth. The plant extract and isolated compounds' antibacterial properties were measured in terms of the diameter of the inhibitory zones they formed.

Determination of Minimum Inhibitory Concentration (MIC)

The broth dilution procedure described by [32] was used to determine the lowest inhibitory concentration of the extract and isolated compounds. Mueller Hinton broth (MHB) was placed in test tubes along with various amounts of the extract and isolated compounds that shown antibacterial action against the test organisms. Each tube containing the diluted extracts received an inoculation of the organisms. For bacteria, the tubes were incubated at 37 °C for 24 hours, and for fungus, at 25 °C for 72 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration in the series at which the test organisms showed no discernible growth.

Minimum bactericidal/fungicidal concentration (MBC/MFC)

By analyzing the test tube contents in the MIC estimations, the minimum bactericidal/fungicidal concentration was established. A loopful of each tube's contents was streaked onto a solidified nutrient agar plate as an inoculum, after which it was incubated at 37 °C for 24 hours to grow bacteria and at 25 °C for 48 hours to grow fungus. The minimum bactericidal/fungicidal concentration was determined to be the subculture's lowest concentration when there was no growth [32].

Results

Preliminary phytochemical test

Table 1. Preliminary phytochemical constituents of *D. pachyrhizus* extract

Test Compounds	Ethyl Acetate Extract
Saponins	-
Glycosides	-
Alkaloids	-
Anthraquinones	+
Steroids	+
Terpenoids	+
Flavonoids	+
Tannins	-
Phenols	+

Key: (+) = present and (-) = absent

Electrical properties of SrSe/ZrSe deposited at a different time interval

Spectroscopic analyses

Isolated compound **1** was eluted with 95 % n-hexane: ethyl acetate and obtained as white sparkles (12 mg) which was examined for the chemical formula $C_{30}H_{50}O$ by EIMS m/z 426.40 [M^+]. Melting point ranges 210 - 212 °C.

In the IR scale, a very intensely wide peak was observed at 3325.04 cm^{-1} (OH Stretch), 2941.90 cm^{-1} (CH_2, CH_3 Stretch), 1638.02 cm^{-1} (C=C Asymmetric stretch), 1035.49 cm^{-1} (C-O Stretch of 2° alcohol), and 887.81 cm^{-1} (=C-H bend external CH_2). Analysis of ^{1}H-NMR (400 MHz, DMSO, δ, ppm): 4.65 (2H, s, H-29b), 4.57 (2H, s, H-29a), 3.07-3.10 (1H, m, H-3), 2.95-3.00 (1H, m, H-19), 2.35-2.38 (2H, t, *J* = 4.08 Hz, H-15), 1.83–1.91 (2H, m, H-21), 1.67 (3H, s, H-30), 1.50–1.62 (8H, m, H-1,2,6,13,18), 1.23–1.37 (7H, m, H-7,9,16,22), 1.06–1.17 (4H, m, H-11,12), 1.02 (3H, s, H-27), 0.95 (3H, s, H-26), 0.92 (3H, s, H-25), 0.81 (3H, s, H-24), 0.77 (3H, s, H-23), 0.75 (3H, s, H-28), and 0.61-0.62 (1H, t, *J* = 3.52 Hz, H-5). ^{13}C-NMR (400 MHz, $CDCl_3$) δ (ppm): 150.9, 108.5, 78.3, 55.5, 50.6, 49.2, 48.0, 43.0, 42.2, 40.5, 40.0, 38.7, 38.5, 38.2, 37.0, 36.9, 34.2, 30.4, 29.5, 29.3, 27.2, 25.5, 22.8, 20.7, 18.2, 18.0, 16.0, 15.3, 14.7 and 13.7 for carbon-20, 29, 3, 5, 9, 18, 19, 17, 14, 8, 22, 4, 1, 13, 10, 16, 7, 21, 23, 2, 15, 12, 11, 30, 6, 28, 25, 26, 24, and 27, respectively.

Isolated compound **2** was eluted with 90 % n-hexane and obtained as white shapeless solid (10 mg) which was examined for a chemical formula $C_{30}H_{50}O$ *via* EIMS m/z 426 [M^+]. Melting point was determined to be 212 - 214 °C. In the IR spectrum, an extremely prominent peak at 3488.30 cm^{-1} for hydrogen bonded, 2933.50 cm^{-1} for carbon-hydrogen stretch in CH_2 and CH_3, 1643.40 cm^{-1} for C = C asymmetric stretch and 1035.50 cm^{-1} for C-O stretch for 2° alcohols were observed. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.15 (H-3), 0.67 (H-5), 5.22 (H-12), 0.77 (s, H3-23), 0.90 (s, H3-24), 0.74 (s, H3-25), 0.94 (s, H3-26), 1.16 (s, H3-27), 1.06 (s, H3-28), 0.86 (s, H3-29), 0.79 (s, H3-30) 5.22 (1H, *br s*, H-12), 3.61 (1H, *dd*, *J* = 8, 4.4 Hz, H-3), 0.80 (3H, *s*), 0.84 (3H, *s*), 0.88 (3H, *s*), 0.95 (3H, *s*), 1.12 (3H, *s*), 1.34 (3H, *s*) , 1.01 (3H, *s*), and 1.11 (3H, *s*). ^{13}C-NMR (400 MHZ, $CDCl_3$): δ (ppm); 158.1, 116.9, 79.1, 55.5, 49.3, 48.8, 41.3, 39.0, 38.8, 38.0, 37.7, 37.7, 37.6, 36.7, 35.8, 35.1, 33.7, 33.4, 33.1, 29.9, 29.8, 28.8, 28.0, 27.2, 25.9, 21.3, 18.8, 17.5, 15.5, and 15.4 for Carbon 13, 12, 3, 5, 9, 18, 19, 14, 8, 4, 1, 10, 22, 21, 7, 17, 20, 16, 2, 29, 30, 28, 15, 27, 11, 23, 24, 6, 26, and 25, respectively.

Antimicrobial screening

Table 2. Zone of inhibition of crude ethyl acetate extract and isolated compounds from the rhizome of *D. pachyrhizus* against tested pathogens in millimeter (mm)

Test pathogens	Crude ethyl acetate extract (mg/mL)		*Lupeol*	*β-amyrin*	Control (mg/mL) Cipro./Fluc.
	40	20	10	10	10
B. cereus	24	22	25	25	26
S. aureus	26	23	30	31	32
S. pneumonia	30	26	28	27	25
S. saprophyticus	30	26	28	30	29
E. coli	25	22	29	28	26
K. Pneumonia	29	21	27	28	29
S. typhi	31	29	32	27	30
P. aeruginosa	29	27	27	29	28
C. albicans	25	22	24	28	26
C. merdarium	24	21	25	23	24
T. rubrum	26	23	29	26	27

B. cereus= Bacillus cereus, S. aureus= Staphylococcus aureus, S. pneumonia= Streptococcus pneumonia, S. saprophyticus= Staphylococcus saprophyticus, E. coli= Escherichia coli, K. pneumonia= Klebsiella pneumonia, S. typhi= Selmonella typhi, P. aeruginosa= Pseudomonas aeruginosa, C. albicans= Candida albicans, C. merdarium= Chrysosporium merdarium, T. rubrum= Trichophyton rubrum, Cipro.= Ciprofloxacin, and Fluc.**=** Fluconazole

Table 3. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) for crude extract and isolated compounds against tested microorganisms in mg/mL

Test Pathogens	Crude extract		*Lupeol*		*β-amyrin*	
	MIC	MBC/MFC	MIC	MBC/MFC	MIC	MBC/MFC
B. cereus	5	10	10	20	10	20
S. aureus	2.5	5	5	10	2.5	5
S. pneumonia	5	10	5	10	5	10
S. saprophyticus	5	10	5	10	2.5	5
E. coli	5	10	2.5	5	10	20
K. pneumonia	5	10	20	40	20	40
S. typhi	5	10	2.5	5	5	10
P. aeruginosa	5	10	10	20	2.5	5
C. albicans	40	ND	20	40	40	ND
C. merdarium	40	ND	40	ND	40	ND
T. rubrum	20	40	20	40	20	40

B. cereus= Bacillus cereus, S. aureus= Staphylococcus aureus, S. pneumonia= Streptococcus pneumonia, S. saprophyticus= Staphylococcus saprophyticus, E. coli= Escherichia coli, K. pneumonia= Klebsiella pneumonia, S. typhi= Selmonella typhi, P. aeruginosa= Pseudomonas aeruginosa, C. albicans= Candida albicans, C. merdarium= Chrysosporium merdarium, and *T. rubrum= Trichophyton rubrum.* ND= not determined

Discussion

The rhizome of *Dolichos pachyrhizus* indicates that anthraquinones, flavonoids, steroids, and terpenoids from ethyl acetate extract are present (Table 1). Plants with biological activity are frequently discovered to contain phytochemicals, which are compounds with biological action [33].

The findings suggest that the rhizome contains a substantial amount of secondary metabolites, including terpenoids with extreme medical significance that has been shown to support anti-diuretic activity [34]. In addition, they comprise the majority of the most beneficial drugs for treating human physiology, including those with anti-plasmodial, analgesic, antispasmodic, anti-diabetic, and anti-inflammatory properties [35,36].

The most diverse classes of phenolic compounds found in plants are flavonoids, which have antibacterial, anti-inflammatory, anti-allergic, and tumor-fighting effects [37-39]. Free radical scavengers, super antioxidants, and potentially water soluble flavonoids protect cells from oxidative damage. The presence of flavonoids, terpenoids, and steroids were confirmed via phytochemical tests of plant's specie (*Dolichos lablab* seed extracts) [40-41]. From methanolic extract of the rhizome of the plant, flavonoids, terpenoids, and steroids were also confirmed [3]. Steroids have been shown to have potent anti-cancer, anti-allergic, antithrombotic, vasoprotective, tumor inhibitory, and antiviral properties [42-44].

Triterpenoid glycosides, also known as steroids, have been reported to have anti-inflammatory, cardiac depressant, and hypocholesterolemic effects [45]. The presence of steroids was confirmed; steroidal chemicals are significant and fascinating in pharmacy because of their interactions with other drugs, such as sex hormones, for which the steroidal structure may act as a potent precursor [43]. *Dolichos lablab* also exhibited antidiabetic, anti-inflammatory, analgesic, cytotoxic, antioxidant, hypolipidemic, insecticidal, antimicrobial, antilithiatic, hepatoprotective, and antispasmodic properties, according to the preliminary pharmacological tests [46].

To maintain their hormonal stability, nursing women or expectant mothers may find a lot of benefit from the decoction of the *Dolichos pachyrhizus* rhizome [47].

The findings of the phytochemical screening are consistent with what [23] reported from the methanol extract of *Dolichos biflorus* Linn seeds, which suggests that the plant's rhizome may be used as an antibacterial agent.

A pentacyclic triterpene, which has a color and melting point similar to isolated compound 1, was produced as a white, colorless powder. It displayed a mass peak at m/z 426 [M]+ in the MS spectrum, which matched the chemical formula $C_{30}H_{50}O$. Isolated compound 1's positive Liebermann-Buchard test result indicates that the substance is a terpernoid. An extremely strong broad peak at 3325.03 cm^{-1}, which is indicative of the O-H bond stretch, could be seen in the isolated compound 1's IR spectrum. There was also a noticeable band at 2941.86 cm^{-1} that is associated with an aliphatic C-H stretch. The unsaturated section of the C-H vibrations and a peak for the C-O stretch for secondary alcohol were observed at 1035.52 cm^{-1} and 1638.03 cm^{-1}, respectively. The methylenic part's rocking motion was thought to be the cause of the fairly intense band at 632.

^{1}H-NMR spectrum of isolated compound **1** showed seven CH_3 singlets protons, two double bonded protons of methylene part and one oxygenated C-H proton at δH 1.68 (3H, br s, Me-30), 1.03 (3H, s, C-26), 0.97 (3H, s, C-27), 0.94 (3H, s, C-24), 0.83 (3H, s, C-25), 0.79 (3H, s, C-23), and 0.76 (3H, s, C-28); 4.57 (1H, dd, J = 1.2, 2.4 Hz, H-29a), 4.69 (1H, br d, J = 2.4 Hz, H-29b), and 3.19 (1H, d, J = 5.1, 10.6 Hz, H-3), respectively. The double bond between methylene carbon (C-29) and quaternary carbon (C-20) was supported by the methylene protons of H-29 while H-3 proton appears as triplet at 3.16 ppm.

On the other hand, ^{13}C-NMR spectrum of the isolated compound **1** shown the presence of thirty (30) carbon, further classified into seven (7) CH_3, eleven (11) CH_2, six (6) methines, and six (6) quaternary carbon atoms at C-23, C-24, C-25,

C-26, C-27, C-28, and C-30; C-1, C-2, C-6, C-7, C-11, C-12, C-15, C-16, C-21, C-22, and C-29; C-3, C-5, C-9, C-13, C-18, C-19, and C-4, C-8, C-10, C-14, C-17, and C-20 together with one external methylene and oxygenated carbons at δC 108.52 and 78.27, respectively. The chemical shift values (in ppm) of ^{13}C-NMR spectrum were obtained and compared with available data from literature (Table 4).

Table 4. ^{13}C Spectroscopic data values of isolated compound 1 compared with literature values (400 MHz, $CDCl_3$)

Carbon position	^{13}C-NMR δ_{ppm} Isolated compound 1	^{13}C NMR δ_{ppm} Lupeol, literature [30]
1.	38.5	38.85
2.	29.3	27.44
3.	78.3	78.99
4.	38.7	38.70
5.	55.5	55.29
6.	18.2	18.31
7.	34.2	34.27
8.	40.5	40.83
9.	50.6	50.43
10.	37.0	37.16
11.	22.8	20.92
12.	25.5	25.13
13.	38.2	38.05
14.	42.2	42.82
15.	27.2	27.41
16.	36.9	35.58
17.	43.0	42.99
18.	49.2	48.30
19.	48.0	47.98
20.	150.9	150.97
21.	30.4	29.84
22.	40.0	39.99
23.	29.5	27.98
24.	14.7	15.36
25.	16.0	16.11
26.	15.3	15.97
27.	13.7	14.60
28.	18.0	17.99
29.	108.5	109.31
30.	20.7	19.30

The bonding structure relationship of the compound **1** was confirmed via the ^{1}H and ^{13}C spectra of long range correlation. The correlation of proton at δ 1.68 (H-30) with quaternary carbon at δ 150.96 (C-20), CH_2 carbon at δ 108.52 (C-29), and CH carbon at δ 47.99 (C-19) showed that the CH_3 carbon atom (C-30) binds to the quaternary carbon (C-20). The oxygenated carbon with proton H-3 and the de-shielded signal at 78.27 are connected by methylene proton H-2 at 1.65 ppm [8,48].

Comparing the data from the literature with the one from ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra revealed that compound 1's structure is a lupeol

(Scheme 1). Isolated compound **2** was also obtained as a white residue and its mass spectroscopic information projected that it had the same chemical formula as isolated compound **1** ($C_{30}H_{50}O$), that was confirmed via ^{13}C-NMR spectrum. Isolated compound **2** displayed a positive test for terpenoids, just like isolated compound **1** above. The isolated compound **2**'s IR spectrum had an extremely broad peak at 3488.29 cm^{-1}, which is usual of the O-H bond stretch. An aliphatic C-H stretch can be assigned by a reasonably strong band at 2933.48 cm^{-1}. An intense band of C=C vibrations was seen at 1643.43 cm^{-1}, and the comparable C-H vibrations of the unsaturated region were seen at 1013.8 cm^{-1}.

A proton signifying the H-3 of a terpene moiety that emerged as a triplet at 3.26 and a different proton at 5.18 that suggested the presence of a tri-substituted olefinic bond were observable in the ^{1}H-NMR spectrum of the isolated compound **2**.

^{13}C-NMR spectrum of the isolated compound **2** revealed to consist of thirty (30) carbon atoms which were further classified to eight (8) CH_3, ten (10) CH_2, five (5) CH, and seven (7) quaternary carbon atoms at C-23, C-24, C-25, C-26, C-27, C-28, C-29, and C-30; C-1, C-2, C-6, C-7, C-11, C-15,

Table 5. ^{13}C spectroscopic data of isolate **2** compared with literature (400 MHz, $CDCl_3$)

Carbon Position	^{13}C-NMR δ_{ppm} Isolated compound 2	^{13}C-NMR δ_{ppm} *β-amyrin*, from literature [33]
1	37.7	37.80
2	33.1	28.10
3	79.1	78.80
4	38.0	38.40
5	55.5	55.60
6	17.5	18.80
7	35.8	33.10
8	38.8	38.20
9	49.3	47.80
10	37.7	37.20
11	25.9	23.40
12	116.9	121.40
13	158.1	145.600
14	39.0	41.20
15	28.0	26.80
16	33.4	27.30
17	35.1	32.20
18	48.8	47.20
19	41.3	46.60
20	33.7	31.10
21	36.7	34.20
22	37.6	37.30
23	21.3	28.90
24	18.8	15.50
25	15.4	15.60
26	15.5	16.70
27	27.2	26.40
28	28.8	28.80
29	29.9	33.40
30	29.8	23.80

C-16, C-19, C-21, and C-22; C-3, C-5, C-9, C-12, and C-18, and also C-4, C-8, C-10, C-13, C-14, C-17, and C-20, respectively. These carbon signals were measured in ppm and compared to data from the literature (Table 5).

^{1}H- and ^{13}C-NMR chemical shift values were assigned to all the protons and carbons based on spectroscopic information. A literature search revealed that isolated compound **2**'s spectral data supported the structure of an oleanane triterpenoid framework (*β*-amyrin) with an OH at the C-3 position and an oleifinic bond at C-12 and C-13 position [33], this was additionally confirmed via a crucial COSY and HMBC correlations as presented in **Figure S12**. Consequently, structure of isolated compound **2** (Scheme 1) was found to be in line with information found in the previously reported work [33]. Triterpenoids (pentaccyclic triterpenes) with thirty (30) carbons skeleton, represent a varied class of natural products which are produced by arrangement of squalene epoxide molecules [35,49-50]. The triterpenoids, lupeol, and *β*-amyrin are powerful bioactive [10,33,51] and strong insecticidal and acaricidal compounds [20,30,33] found in diverse medicinal plants. Lupeol and *β*-amyrin have a wide range of bioactivities and bioassays that have been studied [52], suggesting that they have beneficial therapeutic characteristics with a variety of actions against different diseases. These compounds were reported to be acaricidal [20], antiangiogenic [53], antioxidative [38,54], and anti-inflammatory in nature [51,55]. They inhibit early stages of benzoyl peroxide-induced tumor development [56]. They are also crucial for normalizing the lipid profile [45], the activity of wound healing [36], the preventive impact against hypercholesterolemia linked to kidney damage, and the suppression of immunological factors [49].

In vitro antimicrobial activity

Antimicrobial activity (*in vitro*) of crude ethyl acetate rhizome's extract and isolated compounds of *D. pachyrhizus* was determined by the use of agar well diffusion method against eight pathogenic strains of bacteria: *Bacillus cereus*, *Staphylococcus aureus, Streptococcus pneumonia*, and *Staphylococcus saprophyticum* (Gram positive bacteria), *Escherichia. coli*, *Selmonella. Typhi, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* (Gram negative bacteria), and *Candida albicans, Chrysosporium merdarium,* and *Trichophyton rubrum* (Fungal isolates), and also compared to Ciprofloxacin and Flucunazole as commercial antibiotic and antifungal.

The biological activity of the test extract and isolated compounds was expressed in clear inhibition zone (mm) at various concentrations of the crude extract. The inhibition zone of the extract increases with increasing concentration (20 and 40 mg/mL) against the tested microbes. The results in Table 2 revealed that isolated compound **1** (Lupeol) showed highest inhibition zone against *E. coli, S. typhi, C. merdarium*, and *T. rubrum* 29, 32, 25, and 29 mm, respectively. These higher zones of inhibition recorded from lupeol were in agreement with what was reported by Babii *et al.* [50] which also agrees with the herbal use of the plant rhizome in the management of typhoid, diarrhea, and urinary tract infections. These values were high than the zone of inhibitions recorded from the crude ethyl acetate extract, *β*-amyrin, and the standard used antibiotic drug. *β*-amyrin, on the other hand, was found to be highly active against *P. aeruginosa* and *Candida albicans* with the highest inhibition zone of 29 and 28 mm, respectively, compared to crude extract, lupeol, and ciprofloxacin. These high zones of inhibition corroborate with the zones of inhibition reported from *Neorautanenia mitis* aqueous extract [51] and lower than the values reported from crude methanolic extract of *Dolichos erosus* [52,53]. These are also in line with the local use of the rhizome in managing

pneumonia, urinary tract, and vaginal infections [54]. Same activity index (30 mm) was recorded from crude ethyl acetate and *β*-amyrin isolated from the plant against *S. saprophyticus* which were all greater than the zone of inhibition recorded from the standard antibiotic (29 mm) used and also agrees with the local use of the rhizome in the treatment of vaginal infections in women and urethritis in herbal traditional practice [54]. Similar zones of inhibition with standard drug were determined from the isolated compounds (25, 25, and 26 mm) against *S. aureus,* (30, 31, and 32 mm) against *S. aureus* and (27, 28, and 29) against *K. pneumonia* for lupeol, *β*-amyrin, and ciprofloxacin, respectively. These values prove active potentials of the isolated compounds in developing new antibiotic for treating pneumonia, meningitis, vomiting, and diarrheal syndrome as well as dermal and epidermal tissues problems [55]. The crude ethyl acetate extract shows the highest inhibition zone (30 mm) against *S. pneumonia*. The value is greater than the value recorded for the two isolated compounds and the standard drug (28, 27, and 25 mm) lupeol, *β*-amyrin, and ciprofloxacin, respectively, and showed synergistic potential of the combination therapy of the compounds.

MIC which is the lowest concentration of a substance or drug that inhibit growth of microorganism and MBC/MFC as a minimum concentration of a substance or drug that completely kills microorganisms are presented in Table 3. The MIC and MBC/MFC values of the crude extract and isolated compounds against test microorganisms ranged from 2.5-40 mg/mL. Least MIC values (2.5 mg/mL) obtained showed potential property of the crude extract and compounds compared to the antibiotic drug used as control against *S. aureus, S. saprophyticus, E. coli, S. typhi*, and *P. aeruginosa* which also correspond to the high zones of inhibition against the microbes and ethnomediinal importance of the plant [56]. The high MIC and MBC/MFC recorded revealed moderate to low active the drugs are and determined their lower zones of inhibitions.

The highest zones of inhibition (24-32 mm), at 10 mg/mL of the isolated compounds revealed full susceptibility against the tested bacterial strains. These high zones of inhibition recorded were higher than some reported inhibition zone against *E. coli, S. saprophyticus*, and *S. typhi* [31,51], lower than the values reported from *Dolichos fiblorus* [23], *Dolichos mitis* [43], and wood of *Eucalyptus urograndis* and *Mimosa tenuiflora* [34]. Values also recorded against *S. pneumonia, S. saprophyticum, K. pneumonia, S. typhi*, and *P.aeruginosa* (gram positive and negative bacterial strains) were greater than the values obtained from the positive control (ciprofloxacin) against the tested bacteria. These high activity observed from the study could possibly be associated with the use of the rhizome in the treatment of syphilis, cough, and typhoid in ethnomedicine. The isolated compounds, on the other hand, such as Lupeol and *β*-amyrin from the rhizome of the plant could be potential antibiotic agents susceptible against the bacteria and proved its folklore uses.

Conclusion

In conclusion, this study isolated and characterized two triterpenoid from ethyl acetate extract of *Dolichos pachyrhizus* rhizomes. The extract and isolated compounds exhibited significant activity to both test bacteria and fungi. These findings supported traditional use of *D. pachyrhizus* in treating various microbial infections. The bioactive compounds identified in this study hold promise for further exploration and development as potential antimicrobial agents. Future studies should focus on the purification, synthesis, and evaluation of these compounds for their therapeutic applications in the pharmaceutical industry.

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Disclosure statement

The authors declare that they have no conflict of interest.

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