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Full Length Research Paper

In vitro antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* L'Herit, ex Dc

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Chemical investigation of ethyl acetate leaf-extract of *Pterocarpus santalinoides* led to isolation of a friedelane-terpenoid. Structure elucidation was done by comparison of the Nuclear Magnetic Resonance (NMR) spectral features with literature data and was consistent with reported values for friedelan-3-one. The following microorganisms (Clinical isolates): methicillin resistant *Staphylococcus aureus* (MRSA), *S. aureus*, *Corynebacterium ulcerans*, *Streptococcus pneumoniae*, *Campylobacter jejuni*, *Helicobacter pylori*, *Escherichia coli*, *Shigella dysenteriae*, *Candida tropicalis* and *Candida krusei* were used for the *in vitro* antimicrobial activity. The isolated compound had Minimum Inhibition Concentration (MIC) value of 10 µg/ml for MRSA, *H. pylori*, *E. coli* and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of 40 µg/ml for *E. coli*; 20 µg/ml for MRSA, *H. pylori* and *C. krusei*; 10 µg/ml for *S. aureus, S. pneumonia* and *C. tropicalis.* It showed moderate antimicrobial activity against most of the pathogens tested. This is also the first report of the isolation of friedelan-3-one from this species.

Key words: Friedelan-3-one, methicillin resistant *Staphylococcus aureus* (MRSA), *Helicobacter pylori, Escherichia coli*.

INTRODUCTION

Plant based traditional medicine systems have continued to play an essential role in the health care of many cultures. Medicinal plants have remained vital in man's fight against disease (Phillipson, 2001). *Pterocarpus santalinoides* is a plant used by Igede people of North-Central Nigeria for its antidiarrheal properties (Nworu et al., 2009; Odeh and Tor-Anyiin, 2014). Phytochemical and antimicrobial activities of its leaf-extracts have been reported (Odeh and Tor-Anyiin, 2014).

The plant *P. santalinoides* (L'Herit ex Dc), family Papilionoideae is a tree that grows along riverine forests of Africa and tropical South America and is a native of Brazil, Cameroon, Ghana, Nigeria, and Senegal (Osuagwu and Akomas, 2013). *P. santalinoides* is

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monoecious, flowering from December to March, fruit ripening between March and April. The Nigerian species are trees with light yellow flowers and they usually have alternate leaflets (Keay, 1989; Agroforestry Tree Database, 2011; Osuagwu and Akomas, 2013).

Extracts from this plant have been utilized for their antifungal and antibacterial activities among the Igede people of Benue State, Nigeria in treatment of inflammation of lower abdomen, stomach ache, and other infectious diseases (Igoli et al., 2003). In this study, isolation, characterization, and anti-microbial properties of friedelan-3-one from the leaf-extracts of the plant are reported.

MATERIALS AND METHODS

Plant

Leaves of *P. santalinoides* L'Hér. ex DC. Papilionoideae were collected in March, 2014 from Anyuwogbu-Ibilla, Oju L.G.A, Benue State, Nigeria. The plant was identified and authenticated by Dr. S. A. Shomkegh of Department of Social Forestry and Environmental Protection, University of Agriculture, Makurdi; a voucher specimen was also deposited. All solvents reagents used for this work were purchased from Sigma-Aldrich® with the exception of Silica gel 60 F_{254} Thin Layer Chromatography Plates and Silica gel G which were purchased from Merck® KGaA, Germany. Solvents though of analytical grade were redistilled to remove possible traces of plastic.

Preparation of extracts

The leaves were air-dried at room temperature in the chemistry laboratory, University of Agriculture, Makurdi. The powder was obtained using mortar and pestle. The powdered plant material (1 kg) was extracted with hexane (2.5 L), ethyl acetate (2.5 L) and methanol (2.5 L) for 8 h successively using a Soxhlet apparatus. The extracts were concentrated and allowed to dry in a fume hood to give the crude extracts, and were coded: PSH, PSE, and PSM for hexane, ethyl acetate, and methanol, respectively. Their yields were recorded.

Phytochemical screening

The crude extracts were subjected to various phytochemical tests to identify the chemical constituents (Sofowora, 1982; Hassan et al., 2004; Edeoga et al., 2005; Anowi et al., 2012a; Anowi et al., 2012b).

Fractionation and isolation

The ethyl acetate extract was pre-adsorbed onto Celite prior to purification by Vacuum Liquid Chromatography (VLC) on silica gel. The column was eluted with gradient mixtures of hexane-ethyl acetate, starting from [100% hexane: 0% ethyl acetate] to [0% hexane: 100% ethyl acetate] and ethyl acetate-methanol ranging from [100% ethyl acetate: 0% methanol] to [0% ethyl acetate: 100% methanol] as previously described by Tor-Anyiin et al. (2015). Eluents were analyzed by thin layer chromatography (TLC). Fraction PS vlc-1 was obtained as a white solid [with melting point (260 to 265°C), R_f × 100 (76)].

Antimicrobial activity of PS vlc-1

Cultivation and standardization of test organisms

Tests were carried out using the following microorganisms (Clinical isolates): methicillin resistant *Staphylococcus aureus* (MRSA), *S. aureus*, *Corynebacterium ulcerans*, *Streptococcus pneumoniae*, *Campylobacter jejuni*, *Helicobacter pylori*, *Escherichia coli*, *Shigella dysenteriae*, *Candida tropicalis*, and *Candida krusei.* The test microbes were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. Pathogens were checked for purity and maintained in slants of Agar.

Test organisms (loop full) were taken from agar slants and sub cultured into test tubes containing Sterile Nutrient Agar (Oxoid Limited, England) for bacteria and Sabouraud Dextrose Agar (Titan Biotech Limited, Rajasthan, India) (20 ml) for fungi. Test tubes were incubated for 48 h at 37°C. The broth culture was standardized using Sterile Normal Saline (prepared from NaCl, bought from Sigma-Aldrich Chemical Company Inc, USA) to obtain a density of 106 CFU/ml for bacteria. A sporulated test fungal spore was harvested with 0.05% TWEEN® 80 in Sterile Normal Saline and standardized to 106 spores/ml.

Antimicrobial profile

PS vlc-1 (0.004 mg) was weighed and dissolved in DMSO (Sigma-Aldrich Chemical Company Inc., USA) (10 ml) bringing it to a concentration of 40 µg/ml in preparation for antimicrobial assay. All media were prepared according to manufacturer instruction, sterilized at 121°C for 15 min and were poured into sterile petri dishes, allowed to cool, and solidify. Disc diffusion method was used for screening of the extract. Sterilized media were seeded with standard inocula (0.1 ml) of test microbes, Mueller-Hinton Agar (Oxoid Limited, England) for the bacteria and Sabourand Dextrose Agar for the fungi. Inocula were evenly spread over the surface of media using sterile swabs. For each inoculated medium, a well (6 mm) was cut at the centre using a standard cork-borer (6 mm diameter).

Agar diffusion method was used for antibacterial screening. Solutions of PS vlc-1 (0.1 ml) were introduced into each well of inoculated media. Inoculated media were incubated at 37°C for 24 h for bacteria and at 30°C for 7 days for the fungi, after which plates were observed for zones of inhibition of growth. Diameter of zone of inhibition was measured with a transparent ruler and the result was recorded in millimetres.

Determination of minimum inhibitory concentration (MIC)

MIC was carried out using broth dilution method. Mueller Hinton and Sabouraud Dextrose broth were prepared according to the manufacturer's instruction. The broth (10 ml) was dispensed into test tubes, separated, and was sterilized at 121°C for 15 min and allowed to cool. Mc-Farland's turbidity standard scale number of 0.5 was prepared to give a turbid solution. Normal saline was prepared and was used to make a turbid suspension of the microbes. The Normal Saline (10 ml) was dispensed into test tubes and test microbes were inoculated and incubated for 6 h at 37°C. Dilution of micro-organisms in Normal Saline was continuously done until the turbidity (1.5×10^6 CFU/ml) matched that of the Mc-Farland scale by visual comparison. Two-fold serial dilution of extract in sterile broth gave concentrations of 20, 10, 5, and 2.5 µg/ml. Having obtained different concentrations of extracts in the broth, a standard inoculum (0.1 ml) of the microbes was inoculated into the different concentrations. Incubation for bacteria was at 37°C for 24 h and at 30°C for one week for fungi. Test tubes were then observed for

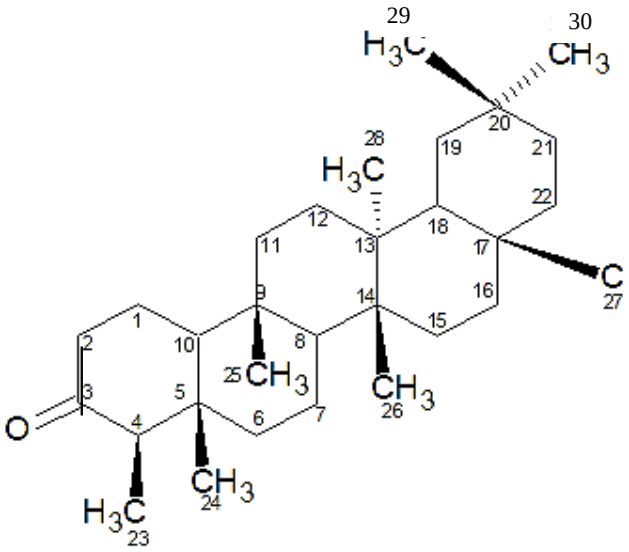


Figure 1. Structure of friedelan-3-one isolated from leaves of *Pterocarpus santalinoides.*

turbidity. The lowest concentration of fraction in the broth for without turbidity was recorded as the MIC.

Minimum bactericidal concentration (MBC)/Minimum fungicidal concentration (MFC)

MBC/MFC was carried out to determine whether test microbes were killed or only their growth was inhibited. Mueller Hinton and Sabouraud Dextrose Agar were prepared according to manufacturer's instruction, sterilized at 121°C for 15 min, and poured into sterile petri-dishes. A loop-full of the test mixture was removed from each plate, incubated at 37°C for 24 h for bacteria, 7 days for fungi, and was observed for the presence of colonies which indicated growth. MBC/MFC was the lowest concentration of extracts that showed no bacterial or fungal growth.

RESULTS AND DISCUSSION

Previous investigations of leaf-extracts of *P. santalinoides* showed that the leaf-extracts exhibited broad growth inhibition against microbes; these studies found extracts to be active against *E. coli, S. typhi, S. aureus, Shigella flexneri, Alcaligenes faecalis, Enterobacter. aerogenes, Pseudomonas aeruginosa* (Osuagwu and Akomas, 2013; Odeh and Tor-Anyiin, 2014). The antimicrobial activities results showed ethyl acetate extract to have the highest zone of inhibition (35 mm) at 20 mg/ml. It showed moderate activity against Gram-negative strains of bacteria that are frequently reported to be less sensitive to plant extracts (Eze et al., 2012). Thus, further purification via VLC of this ethyl acetate extract yielded 11 mg of a white non-crystalline substance (PS vlc-1). Comparison of the NMR spectral features of this substance with existing literature (Igoli and Gray, 2008; Abdullahi et al., 2011) suggests that the isolated compound (PS vlc-1) is a pentacyclic triterpene (Table 1). The ^{1}H spectrum showed a proton quartet at δH 2.25 (H-4) and a methyl doublet at 0.87 (H-23). Other methyls appeared as singlets at 0.71 (H-24), 0.86 (H-25), 1.06 (H-26), 1.04 (H-27), 1.17 (H-28), 0.99 (H-29), and 0.94 (H-30) (Table 1). Its ^{13}C spectrum gave a carbonyl signal at 213 ppm typical of a saturated ring ketone; other peaks (Table 1) were also in good agreement with reported data. The spectra data of PS vlc-1, shown in Table 1, were used together with literature reports (Igoli and Gray, 2008; Abdullahi et al., 2011) to identify PS vlc-1 as friedelan-3-one. Identification was further corroborated using its 2-D spectra (Figures 2 and 3). For instance in its proton-proton correlation spectroscopy (^{1}H-^{1}H COSY) (Figure 2), the compound gave a correlation between the protons at δH 2.25 H-4 (1 H,q) and 0.87 H-23 (3H, d) indicating a methyl substituent at position C-4 typical of a friedelane moiety (Abdullahi et al., 2011). In its Heteronuclear Multiple Bond Correlation (HMBC) spectrum (Figure 3), the methyl at 0.87 (H-23) gave correlation to the carbonyl at C-3. The proton at δH 2.25 H-4 also gave correlation to C-3 (Figure 3), a confirmation of a ring typical of friedelan-3-one ring A (Figure 1).

This is the first report of the presence of friedelan-3-one in *P. santalinoides*. The isolated compound (PS vlc-1) was investigated for antimicrobial activity against *MRSA, S. aureus*, *C. ulcerans*, *S. pneumoniae*, *C. jejuni*, *H. pylori*, *E. coli*, *S. dysenteriae*, *C. tropicalis* and *C. krusei.* and had MIC values of 5 µg/ml for most pathogens (Table 3). For MBC/MFC, it showed moderate antimicrobial activity against most of the pathogens tested (Table 4). The highest diameter of zone of inhibition was against *S.*

Table 1. Spectral data of PS vlc-1 in $CDCl_3$ compared with literature reports.

Position of H/C	Experimental data (PS vlc-1)		Literature data (Abdullahi et al., 2011)		Literature data (Igoli and Gray, 2008)	
	$^1H(\delta)$	$^{13}C(\delta)$	$^1H(\delta)$	$^{13}C(\delta)$	$^1H(\delta)$	$^{13}C(\delta)$
	δ H	δ C	δ H	δ C	δ H	δ C
1	1.96,1.67	22.3(t)	1.95, 1.71 (2H, add)	22.3(t)	1.97	22.5
2	2.37, 2.29	41.6(t)	2.37,2.27 (2H,add)	41.5(t)	2.40, 2.28	41.8
3	-	213.3(s)	-	213.2(s)	-	213.0
4	2.25	58.3(d)	2.25 (1Hq)	58.2(d)	2.28	58.5
5	-	42.2(s)	-	42.1(s)	-	42.7
6	*	41.3(t)	1.74,1.28 (2H,d)	41.3(t)	*	41.6
7	1.74	18.3(t)	1.74, 1.36 (2H,m)	18.2(t)	*	18.5
8	1.38	53.1(d)	1.38 (1H,dd)	53.1(d)	*	53.4
9	-	37.5(s)	-	37.4(s)	-	37.7
10	1.53	59.5(d)	1.53 (1H,m)	59.5(d)	*	59.6
11	1.47	35.6(t)	1.45, 1.26 (2H,m)	35.6(t)	*	35.9
12	*	30.5(t)	1.33, 1.32 (2H,m)	30.5(t)	*	30.8
13	-	39.7(s)	-	39.7(t)	-	40.0
14	-	38.3(s)	-	38.5(s)	-	38.6
15	*	32.5(t)	1.47,1.27 (2H,m)	32.6(t)	*	32.7
16	1.59	36.0(t)	1.58,1.35 (2H,m)	36.0(t)	*	36.3
17	-	30.0(s)	-	30.0(t)	-	29.9
18	*	42.8(d)	1.56 (1H,m)	42.8(d)	*	43.1
19	*	35.4(t)	1.37,1.22 (2H,m)	35.3	*	35.6
20	-	28.2(s)	-	28.2(s)	-	28.4
21	1.53,1.38	32.3(t)	1.50.1.31 (2H,m)	32.8(t)	*	32.3
22	0.94(m)	39.3(t)	1.51.0.95 (2H,m)	39.2(t)	*	39.5
23	0.87(d)	6.9(q)	0.88 (3H,d)	7.0(q)	0.89(d) *J*=2.7	7.04
24	0.71(s)	14.2(q)	0.73 (3H,s)	14.6(q)	0.74(s)	14.3
25	0.86(s)	18.1(q)	0.87 (3H,s)	17.9(q)	0.88(s)	18.2
26	1.06(s)	20.3(q)	1.01 (3H,s)	20.2(q)	1.01(s)	20.5
27	1.04(s)	18.7(q)	1.05 (3H,s)	18.6(q)	1.02(s)	18.9
28	1.17(s)	32.1(q)	1.18 (3H,s)	32.1(q)	1.06(s)	32.2
29	0.99(s)	35.1(q)	1.00 (3H,s)	35.0(q)	1.19(s)	32.0
30	0.94(s)	31.8(q)	0.94(3H,s)	31.8(q)	0.96(s)1.26(s)	35.2

*Overlapping signals; $^{\delta}$Chemical shift; - = No hydrogen.

pneumonia (32 mm), while the least was against *E. coli* (22 mm) (Table 2).

The isolated compound (friedelan-3-one), a terpene, has been reported to exhibit antifeedant and anti-inflammatory activities (Duke, 1992). Friedelan-3-one has been found to show hepatoprotective activity (Džubák et al., 2006). The mode of action of terpenes is speculated to involve the disruption of the cell membrane activity (Cowan, 1999). In the present study, friedelin demonstrated antibacterial (MRSA, *S. aureus, S. pneumonia, H. pylori*, and *E. coli*) and anticandidal activity (*C. tropicalis*); a previous study by Kuete et al. (2007) showed friedelin to exhibit antibacterial (*Enterococcus faecalis, S. aureus* and *P. aeruginosa*) and anticandidal (*C. albicans*, *C. krusei* and *Candida glabrata*) activities.

In a study by Ee et al. (2005), friedelin was reported to exhibit growth inhibitory activities against (human oestrogen receptor-negative breast cancer) MBA-MD-231 cells.

The foregoing account of medicinal properties of friedelin, indicate that it is an important medicinal compound. Its isolation from the leaves of *P. santalinoides* demonstrates it to be a leading medicinal principle behind traditional/folkloric applications of the leaves in antibacterial, antifungal, anti-inflammatory, antidiarrheal, and other infectious diseases.

This study demonstrates the leaves of *P. santalinoides*

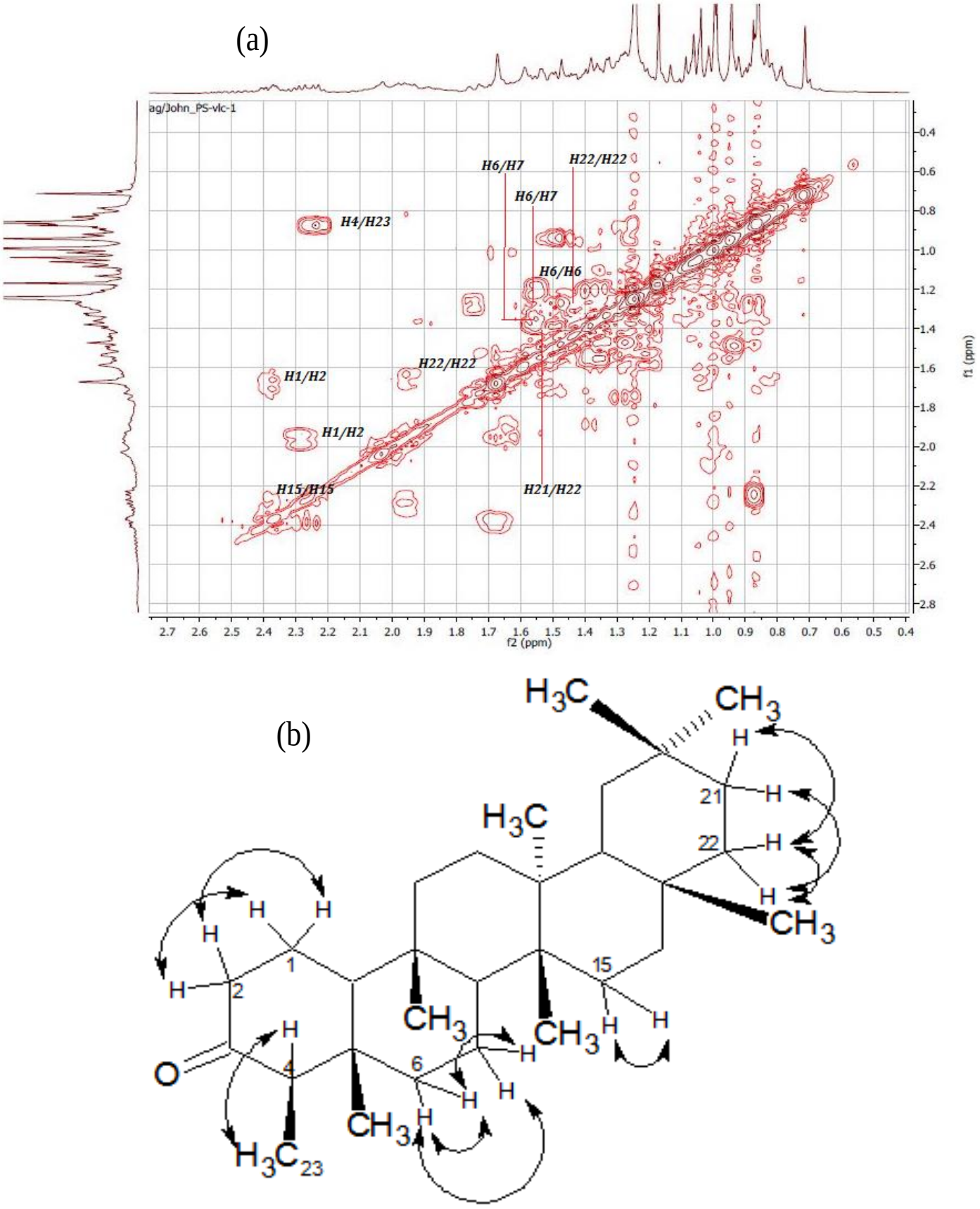
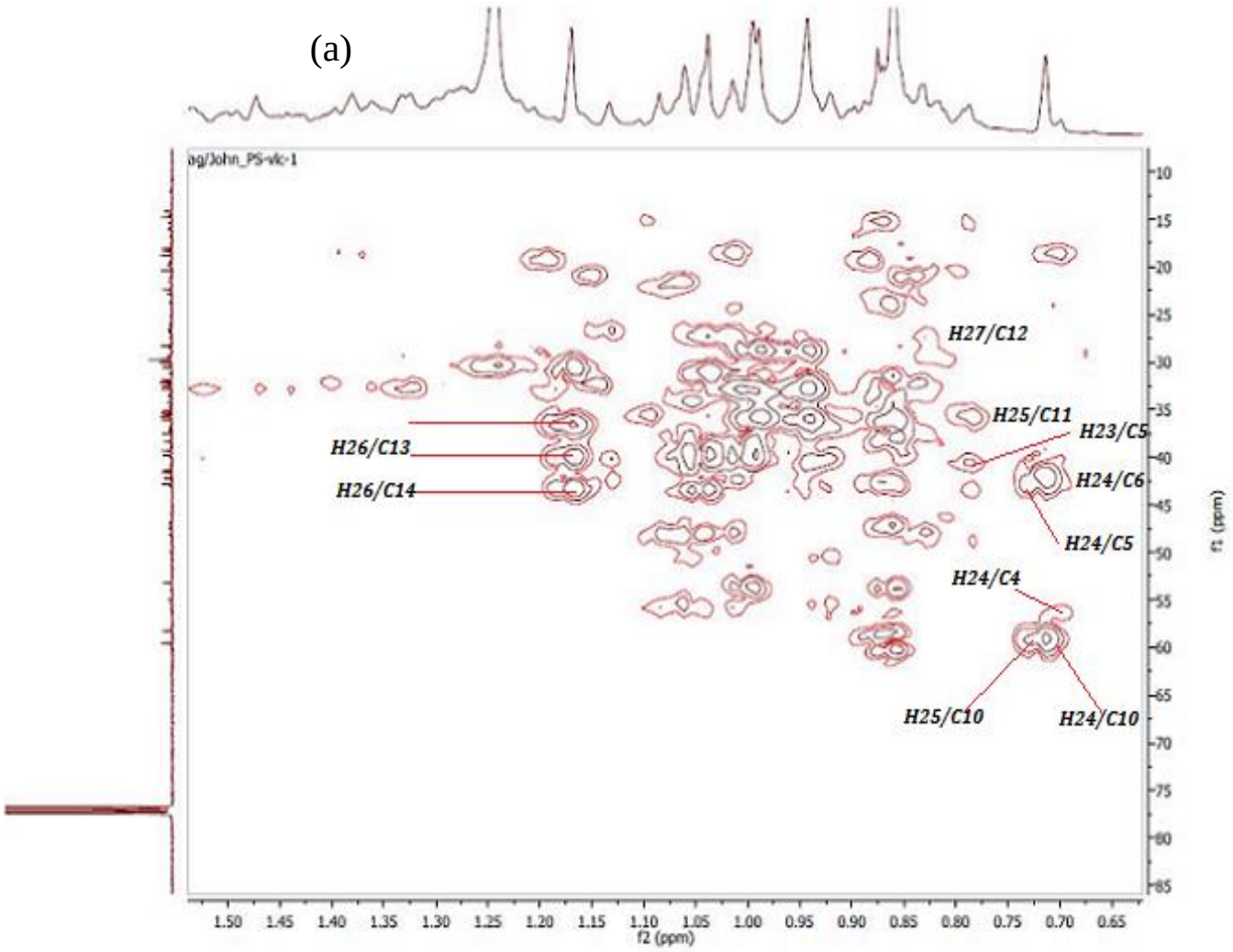


Figure 2. Prominent ^{1}H-^{1}H COSY labels (a) and correlations (b) for isolated compound.



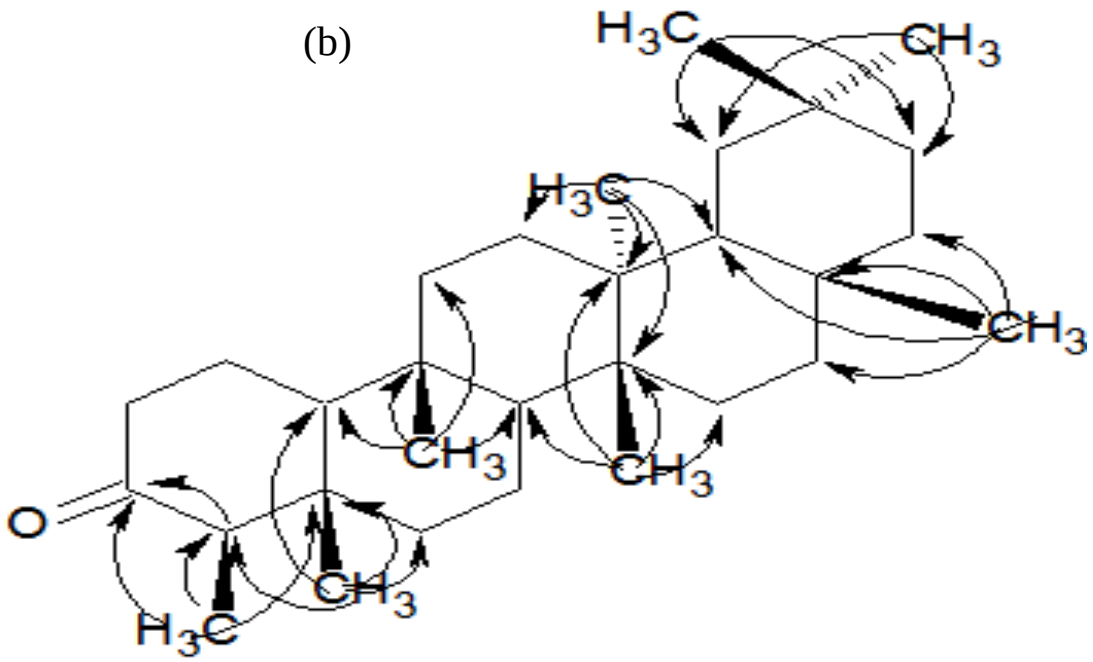


Figure 3. Prominent HMBC labels (a) and correlations (b) for isolated compound.

Table 2. Antimicrobial sensitivity and diameter of zone of inhibition.

Test microorganism	Extract		Vended antimicrobials	
	PS-vlc-1	Sparfloxacin	Ciprofloxacin	Fluconazole
MRSA	S (26)	S (37)	R	R
S. aureus	S (29)	S (38)	S (35)	R
C. ulcerans	R	S (35)	S (37)	R
S. pneumoniae	S (32)	S (42)	S (40)	R
C. jejuni	R	S (37)	S (32)	R
H. pylori	S (24)	R	S (34)	R
E. coli	S (22)	S (40)	S (37)	R
S. dysenteriae	R	S (39)	S (40)	R
C. tropicalis	S (30)	R	R	S (35)
C. krusei	S (27)	R	R	S (37)

S: Sensitive; R: Resistance; PS-vlc-1 concentration = 40 µg/ml; Drug concentration (Positive Control) =5 µg/ml; Numeric value in brackets = Diameter of zone of inhibition in millimetres.

Table 3. Minimum inhibition concentration (MIC) of PS-vlc-1 against test microorganism.

Test microorganism	Extract concentration (µg/ml)				
	40	20	10	5	2.5
MRSA	-	-	∞	+	++
S. aureus	-	-	-	∞	+
C. ulcerans					
S. pneumoniae	-	-	-	∞	+
C. jejuni					
H. pylori	-	-	∞	+	++
E. coli	-	-	∞	+	++
S. dysenteriae					
C. tropicalis	-	-	-	∞	+
C. krusei	-	-	-	∞	+

- = No turbidity (no growth), ∞= MIC, +=turbidity (light growth) ++=moderate turbidity. MRSA: Methicillin Resistant *Staphylococcus aureus.*

Table 4. Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of PS vlc-1 against test micro-organisms.

Test microorganism	Extract concentration (µg/ml)				
	40	20	10	5	2.5
MRSA	-	∞	+	++	+++
S. aureus	-	-	∞	+	++
C. ulcerans					
S. pneumoniae	-	-	∞	+	++
C. jejuni					
H. pylori	-	∞	+	++	+++
E. coli	∞	+	++	+++	++++
S. dysenteriae					
C. tropicalis	-	-	∞	+	++
C. krusei	-	∞	+	++	+++

- = No colony growth, ∞=MBC/MFC, + = scanty colony growth, ++ = moderate colonies growth, +++ = heavy colony growth.

to be a veritable source for this compound with a promising antimicrobial activity.

Conflict of Interests

The authors have not declared any conflict of interests.

Abbreviations

MRSA, Methicillin resistant *Staphylococcus aureus*; **MIC,** minimum inhibition concentration; **MBC,** minimum bactericidal concentration; **MFC,** minimum fungicidal concentration; **VLC,** vacuum liquid chromatography; **TLC,** thin layer chromatography; **PS-vlc-1,** fraction of active compound isolated; **DMSO,** dimethylsulphoxide.

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