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Chemical Constituents of the Stem Bark of *Prunus africana* and Evaluation of their Antibacterial Activity

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Abstract: Chromatographic separation of the methanolic extract of *Prunus africana* stem bark led to the isolation of five compounds (β- sitosterol (**1**), benzoic acid (**2**), two oleanolic derivatives (**3** and **4**), and *p*-hydroxybenzoic acid (**5**). The structures of compounds were elucidated based on 1D and 2D NMR spectral data and compared with reported literature values. This is the first report of benzoic acid and its derivatives from the genus *Prunus*. The crude extract and isolated compounds were evaluated for their antibacterial activity against five bacterial strains (*E. coli*, *S. aureus*, *S. flexineri*, *S.typhimurium,* and *P. aeruginosa*) using disc diffusion assay and showed good antibacterial activity against the tested strains. The crude extract showed potent activity (21.03 ± 0.05 mm) against *P. aeruginosa*, which is even greater than the reference drug gentamycin (14.06 ± 0.09 mm). The antibacterial activity of *P.africana* extract and isolated compounds supports its traditional use, suggesting that it could be considered as a source of bioactive compounds in antimicrobial drug development.

Keywords: *Prunus africana,* oleanolic acid derivatives, benzoic acid, *p*-hydroxybenzoic acid, β-sitosterol, antibacterial activity.

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INTRODUCTION

Prunus africana, belonging to the Rosaceae family, is the only species of the genus *Prunus*, which comprises more than 400 species found in Africa. It is widely used in traditional medicine in southern, eastern, and central African countries (1) to treat prostatic cancer and related conditions across various communities for many years (2-4). The stem bark is used by herbalists in the treatment of various ailments including prostate problems, stomach aches, urinary and bladder infections, chest pain, malaria, microbial infections, and renal disease (5-9). In Ethiopia, the traditional healers use infusions of leaves, decoctions of stem and root barks of *P. africana* to treat urinary disorders, diarrhea, stomach ache, wounds, and bacterial diseases (10). The pharmacological efficacy of the plant is believed to be due to its various phytochemical constituents (11). However, the phytochemical and bioactivity information pertaining to the stem bark of this plant are limited. Therefore, and as part of the ongoing search for new bioactive compounds from Ethiopian medicinal plants, the study was aimed at investigating the phytochemical constitution of the stem bark extract of *P. africana* and evaluating its antibacterial activity.

MATERIALS AND METHODS

General Method

Solvents and reagents used for extraction and purification of compounds are of analytical and HPLC grade. Analytical TLC pre-coated sheets

ALUGRAM®Xtra SIL G/UV_{254} (layer: 0.20 mm silica gel 60 with fluorescent indicator $UV_{F254/365}$) was used for purity analysis. For column chromatography, silica gel 100-200 mesh was used. Chromatograms were visualized on TLC by spraying with 10% H_2SO_4 and heating on hot plate. NMR spectra data were recorded on an Avance 600 MHz spectrometer (Bruker, Billerica, MA, USA, at 600 MHz (^{1}H) and 150 MHz (^{13}C). Chemical shifts were expressed in parts per million (ppm) downfield of trimethylsilane (TMS) as internal reference for ^{1}H resonances, and referenced to the central peak of the appropriate deuterated solvent's resonances (residual $CDCl_3$ and DMSO at δ_H 7.26 for protons and δ_C 77.0 for carbons). Whatman filter paper No.3, DMSO, Petri dishes, and gentamycin were used in antibacterial analysis.

Plant Material

The stem barks of *P. africana* were collected from Horro Buluk, Horro Guduru Wollega zone, Oromia regional state, Ethiopia in September, 2019. The plant material was identified by an expert in botany (Dr. Fekadu Gurmessa) and the voucher specimen (DAD003Pa) has been deposited in the Wollega University Herbarium, then washed thoroughly with tap water, cut into small pieces, and dried under shade.

Extraction and Isolation

The powdered stem barks of *P. africana* (1 kg) were extracted with methanol (3x3 L) at room temperature for 48 h with occasional shaking. The crude extract was filtered from marc using Whatman filter paper. The solvent was evaporated under reduced pressure using rotary evaporator at 40 °C. A dark brown residue was obtained (40 g, 4%). Thirty-eight grams of the sample were subjected to liquid-liquid partition successively with n-hexane, dichloromethane, and ethyl acetate. The resulting phases were concentrated and afforded, respectively, 5 g, 8 g, and 12 g of residues. The TLC analysis of dichloromethane and ethyl acetate extracts showed six similar spots with the same R_f value of 0.72, 0.60, 0.59, 0.45, 0.33, 0.30 in n-hexane/ethyl acetate (7:3) solvent system. The two extracts were then combined together and subjected to column chromatography on silica gel for isolation of the compounds. About 18 g of extract were adsorbed on 26 g of silica gel and subjected to column chromatography, packed with silica gel (320 g). The mobile phase consisted of hexane with an increasing gradient of ethyl acetate, starting with 10:0 hexane:ethyl acetate, then 9:1, to 8:2 until 4:6, 200 mL each. The elution rate was 10 drops per minute to afford 50 major fractions. The resulting fractions of similar TLC profiles were combined together. Fractions 5–10 (2% EtOAc in n-hexane) were combined afforded 15 mg and purified by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to give β-sitosterol (**1**, 12 mg) while fractions 15-20 (3% EtOAc in hexane) showed similar TLC profiles and were combined to give 30 mg and purified by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to give benzoic acid (**2**, 20 mg). Fractions 26-30 (5% EtOAc in hexane) showed similar spots combined together (afforded 35 mg) and purified by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to give a mixture of two oleanolic derivatives, **3** and **4** (30 mg). Fractions 35-40 (10% EtOAc in hexane) were similarly showed similar TLC profiles combined together (afforded 20 mg) and further purified by Sephadex LH-20 (eluting with CH_2Cl_2/MeOH; 1:1) to give *p*-hydroxy-benzoic acid (**5**, 15 mg).

Pathogenic Bacterial Strains

Five pathogenic bacterial strains were purchased from the Department of Biology of Wollega University and used for the evaluation of antibacterial activities. One gram-positive, *Staphylococcus aureus* (ATCC25923), and four gram-negative: *Escherichia coli* (ATCC25922); *Pseudomonas aeruginosa* (ATCC27853); *Salmonella typhimurium* (ATCC13311); *Shigella flexneri* (ATCC29903).

Antibacterial Activity Assay

The antibacterial activities of methanol extract and isolated compounds were tested against five bacterial strains using the disc diffusion method as described by Wayne (2012) with a slight modification (12). The test solutions were prepared with known weight of crude extract (1.5 mg) and isolated compounds were dissolved in 1 mL of DMSO. A 0.6 mm diameter, sterile Whatman test disk was placed on the surface of the inoculated Mueller Hinton Agar in a 90 mm petri dish and soaked with 0.0015 g/mL of the crude extract and the isolated compounds. The antibacterial activity was determined by measuring the zone of growth inhibition surrounding the disks. Gentamycin (10 µg) and DMSO were used as positive and negative controls, respectively. The tested samples were allowed to diffuse for 30 minutes, and the plates were then kept in an incubator at 37 °C for 48 h (13). The experiments were carried out in triplicate and the mean of inhibition zones' diameters were calculated.

RESULTS AND DISCUSSION

The stem barks of *P. africana* were extracted with methanol then sequentially partitioned in n-hexane, dichloromethane and ethyl acetate. The dichloromethane and ethyl acetate extracts were combined and subjected to column chromatography for further purification and afforded five compounds **1**-**5** (Figure 1).

Compound **1** was isolated as white crystalline substance with a melting point of 134-135 °C. ^{1}H NMR spectrum (Table 1) showed an olefinic proton at δ_H 3.54 (1H, tdd, 11.2, 6.5, 4.6 Hz) corresponds H-6 and oxymethine proton at δ_H 3.54 (1H, tdd,

11.2, 6.5, 4.6 Hz) for H-3. It also showed proton signals at δ_H 0.69 (3H, s), 1.02 (3H, s), 0.94 (3H,d, 6.5 Hz), 0.84 (3H, d, 6.8 Hz), 0.81 (3H, d, 6.8 Hz), 0.85 (3H, t, 7.2 Hz) for six methyl groups and were assigned to H-18, H-19, H-21, H-26, H-27 and H-29, respectively.

The ^{13}C NMR (Table 1) spectrum showed signals for 29 carbon atoms including signals for six methyl carbons (δ_C 19.8, 19.4, 19.1, 18.8, 11.9 and 11.8), eleven methylene carbons (δ_C 42.2, 39.8, 37.3, 33.9, 31.9, 31.6, 28.3, 26.1, 24.3, 23.1 and 21.1), nine methine carbons (δ_C 121.7, 71.8, 56.8, 56.1, 50.1, 45.8, 36.2, 31.9 and 29.2) and three quaternary carbon atoms (δ_C 140.7, 42.3 and 36.5). This was in agreement with existing literature spectra of typical β-sitosterol (14). Therefore, based on these spectral data and comparison with literature values, the compound was identified as β-sitosterol.

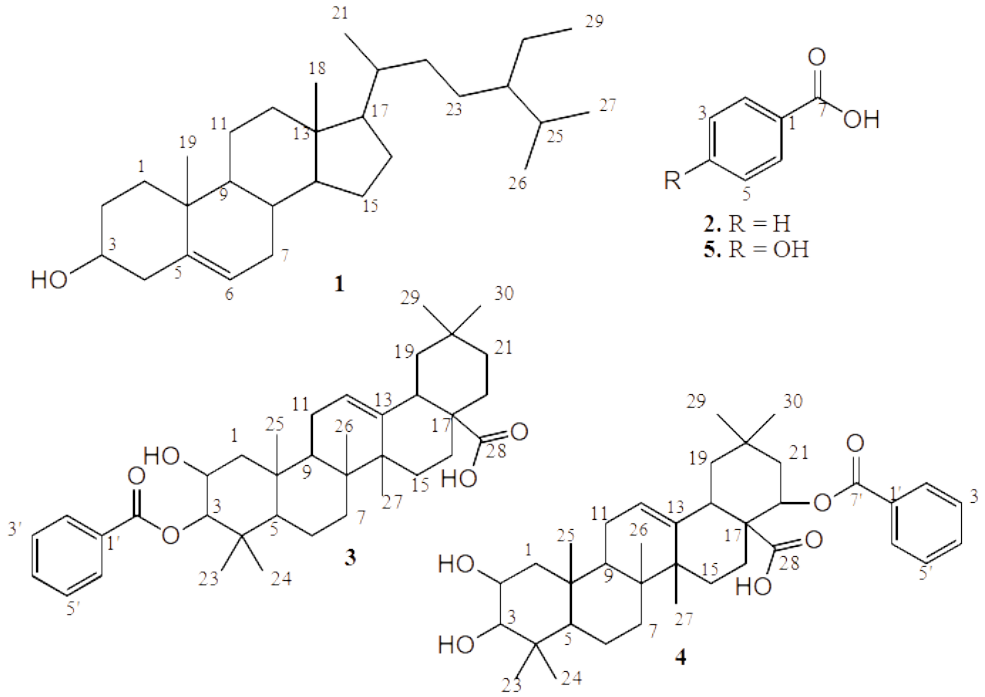

Figure 1: Structures of the isolated compounds from of *P. Africana* stem barks.

Compounds **2** and **5** were isolated as white crystalline solids. Their structures were readily indentified as benzoic acid and p-hydroxy-benzoic acid, respectively, on the basis of their ^{1}H and ^{13}C-NMR spectra data (Table 2). Five mutually coupled aromatic protons including the ortho and meta identical protons in the ^{1}H NMR spectrum along with seven carbon signals corresponding to five aromatic methines and two quaternary carbon atoms (one for carboxylic acid) in the ^{13}C NMR were observed for compound 2 in agreement with (15). However, the presence of only four aromatic protons in the ^{1}H NMR spectrum and four methine carbons and three quaternary carbon atoms including one oxygenated aromatic carbon in the ^{13}C-NMR for compound 5 reveled the structure of compound **5** as p-hydroxy-benzoic acid. It is worth mentioning that this is the first report of benzoic acid and its derivative from the genus Prunus. The co-occurrence of benzoic acid in this plant is then possible that the coupling could have occurred between oleanoic acid and benzoic acid to form compounds **3** and **4** through esterification.

Compounds **3** and **4** were co-isolated as a mixture (deduced from the difference in carbon signal intensities, proton intigration, carbon NMR spectral patterns, and 2D correlations) and obtained as a white amorphous solid. Their ^{1}H and ^{13}C NMR spectral data (Table 3) were virtually identical, with a few differences in the number of methine carbons and metheylene carbons. Based on the carbon signal intensities and exhustive analyses of 2D NMR correlations (^{1}H-^{1}H)-COSY, (^{1}H-^{13}C)-HMBC, (^{1}H-^{13}C)-HSQC and NOES, the two compounds were characterized as follows:

The ^{1}H NMR spectrum (Table 3) of compound **3** consists of signals at δ_H 0.91, 0.96, 0.95, 0.98, 0.94, 1.04 and 1.15 integrated for three protons each indicating the presence of seven methyl groups. It also showed an olefinic proton signal at δ_H 5.30 (1H, t, 3.7 Hz) and two oxy-methine protons at δ_H 4.02(1H, m,) and 3.45 (1H, m) and were assigned to H-12, H-2 and H-3, respectively. These arguments were corroborated by the presence of two olefinic (δ_C 122.6 and 143.7) and two oxygenated methine carbons (δ_C 66.6 and 78.9) carbon signals in the ^{13}C-NMR spectrum (Table 3).

The ^{13}C NMR and DEPT spectra exhibited 30 carbon resonances in the aliphatic and olefinic regions with the resonance at δ_C 183.8 attributed to carboxylic acid carbon. These NMR data and further analyses of HSQC, COSY and HMBC were in a good agreement with anoleanic-12-ene type pentacyclic triterpenoid derivative (16, 17).

The ^{1}H NMR spectrum showed proton signals in the aromatic region at 8.11 (2H, d, 7.5 Hz, H-2′ and H-6′), 7.62 (1H, t, 7.6 Hz, H-4′) 7.49 (2H, t, 7.8 Hz, H-3′ and H-5′) which corresponded to protons for mono-substituted benzene moiety. The ^{13}C-NMR and DEPT spectra also displayed the presence of seven carbon signals, accountable to five aromatic methine carbons (δ_C 130.2, 128.5, 133.8, 128.5, 130.2), aromatic quaternary (δ_C 132.5) and carboxylic (δ_C 171.2) carbon atoms, supporting the presence of benzoic ester moiety. The position of the benzoic ester group was established at C-3 (δ_C 78.9) of the oleanolic acid on the basis of HMBC correlation, indicating long-range cross coupling between H-3 (δ_H 3.45) and the carbonyl carbon (δ_C 171.2) of the benzoic ester group. Thus, based on the above spectroscopic evidence and compared with literature reports (16, 17), compound **3** was identified as oleanoic acid-3-benzoate.

The ^{1}H and ^{13}C NMR spectral data (Table 3) of compound **4** were also similar to those of compound **3** with the presence of seven methyl groups (at δ_H 0.94 (3H, H-26), 0.87 (3H, H-24), 1.20 (3H, H-29), 0.76 (3H, H-25), 0.92 (3H, H-30), 1.08 (3H, H-23), 1.10 (3H, H-27)), the carboxylic acid (δ_C 183.5) and olefinic (δ_H 5.27, H-12) groups of the oleanoic acid moiety. The presence of benzoic ester moiety was also evident that signals at δ_H 7.72 (2H, dd, 5.7, 3.3 Hz, H-3′ and H-5′), 7.62 (1H, t, 7.6 Hz, H-4′), 7.55 (2H, dd, 5.3, 3.3 Hz, H-2′ and H-6′) correspond to aromatic protons and signal at δ_C 167.8 for carbonyl carbon of the benzoic ester group as in compound **3**. The only notable difference is the chemical shift value for the methylene, 22-CH_2 (δ_H 1.85; δ_C 32.8), as would be in compound **3**, has been replaced by a set of signal corresponding to oxy-methine (δ_H 4.23; δ_C 68.2). In this case, the position of the benzoic ester group was established at C-22 following the long-rage HMBC coupling of H-22 (δ_H 4.23) with ester carbonyl carbon, C-7' (δ_C 167.8) (Figure 2). Therefore, based on the above spectroscopic evidence, compound **4** was identified as oleanoic acid-22-benzoate.

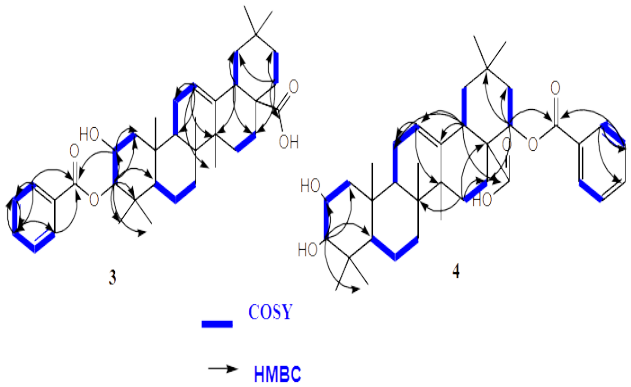

Figure 2: COSY and HMBC correlations of compound **3** and **4.**

The disk diffusion assay was employed to determine the antibacterial activities of the extract and isolated compounds against five bacterial species: *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Salmonella typhimurium* (ATCC13311) and *Shigella flexneri* (ATCC29903). The antibacterial activity test (Table 4) showed varying degrees of inhibition of bacterial growth. The crude extract showed considerable activity on both Gram-positive and Gram-negative bacterial strains with inhibition zones ranging from 9.67 ± 0.47 to 21.03 ± 0.05 mm. The highest activity (21.03 ± 0.05 mm) was observed against *P. aeruginosa*, which is even greater than that of the reference drug (gentamycin, 14.06 ± 0.09 mm) against the same strain (18). Whereas the isolated compounds showed moderate activities against all test strains. This variation in bacterial growth inhibition, by the extract and the isolated compounds, could be related to the synergistic effect of various compounds or to minor compounds present in the crude extract that were no isolated. In general, the remarkable activities of *P. africana* crude extract support its traditional use and suggest that it could be used as a potential candidate in the development of novel antibacterial agents.

Table 1: ^{1}H NMR (600 MHz) and ^{13}C NMR spectral data for compound **1** (in $CDCl_3$)

		Compound 1		β-sitosterol (14)	
No.	**δ_C**	**δ_H(m, J in Hz)**	**δ_C**	**δ_H(m, J in Hz)**	**Appearance**
1	37.3		37.3		CH_2
2	31.9		31.7		CH_2
3	71.8	3.54(1H, tdd, 11.2, 6.5,4.6 Hz)	71.8	3.53(1H, tdd, 4.5, 4.2, 3.8 Hz)	CH
4	42.2		42.3		CH_2
5	140.7		140.7		C
6	121.7	5.35(1H dd,5.0 ,2.3 Hz)	121.7	5.36 (1H, t, 6.4 Hz)	CH
7	31.6		31.7		CH_2
8	31.9		31.9		CH
9	50.1		50.2		CH
10	36.5		36.5		C
11	21.1		21.1		CH_2
12	39.8		39.8		CH_2
13	42.3		42.3		C
14	56.1		56.8		CH
15	24.3		24.6		CH_2
16	28.3		28.3		CH_2
17	56.8		56.1		CH
18	11.9	0.69(3H, s)	11.9	0.63(3H, s)	CH_3
19	19.4	1.02(3H, s)	19.4	1.01(3H, s)	CH_3
20	36.2		32.5		CH
21	18.8	0.94(3H,d, 6.5 Hz)	18.8	0.93 (3H, d, 6.5 Hz)	CH_3
22	33.9		33.9		CH_2
23	26.1		26.1		CH_2
24	45.8		45.9		CH
25	29.2		28.9		CH
26	19.8	0.84(3H, d, 6.8 Hz)	19.8	0.83 (3H, d, 6.4 Hz)	CH_3
27	19.1	0.81(3H, d, 6.8, Hz)	18.8	0.81 (3H, d, 6.4 Hz)	CH_3
28	23.1		23.1		CH_2
29	11.8	0.85(3H, t, 7.2 Hz Hz)	11.9	0.84 (3H, t, 7.2 Hz)	CH_3

Table 2: ^{1}H and ^{13}C NMR (600 MHz) spectral data for compound **2** (in $CDCl_3$) and **5** (DMSO).

		Compound 2			Compound 5	
No.	**δ_C**	**δ_H(m, J in Hz)**	**Appearance**	**δ_C**	**δ_H(m, J in Hz)**	**Appearance**
1	129.2	-	C	121.8	-	C
2 & 6	130.1	8.16(2H,d,7.2 Hz)	CH	131.9	7.79(2H,d, 8.7Hz)	CH
3 & 5	128.3	7.49(2H,t,7.8Hz)	CH	115.6	6.82(2H,d,8.7Hz)	CH
4	133.7	7.64(1H,t,7.4Hz)	CH	162.1	-	C
7	172.6	12.50(1H,br.s)		167.6	12.43(1H,br.s)	

Table 3: ^{1}H and ^{13}C NMR (600 MHz) spectral data for compound **3** and **4** (in $CDCl_3$)

		Compound 3			**Compound 4**	
No.	**δ_C**	**δ_H(m, J in Hz)**	**Appearance**	**δ_C**	**δ_H(m, J in Hz)**	**Appearance**
1	41.7		CH_2	40.9		CH_2
2	66.6	4.02(1H,ddd,4.8, 9.6,11.4Hz)	CH	66.6	4.02(1H,ddd,4.8, 9.6,11.4Hz)	CH
3	78.9	3.45(1H,d,2.5Hz)	CH	78.8	3.45(1H,d,2.5 Hz)	CH
4	39.0		C	38.8		C
5	52.4	2.22(1H,dd,13.3,1.8Hz)	CH	48.1	1.23(1H,dd,13.3,1.8 Hz)	CH
6	18.0		CH_2	17.9		CH_2
7	32.4		CH_2	32.7		CH_2
8	39.5		C	39.7		C
9	47.3	1.62 (1H, d, 7.6 Hz)	CH	47.3	1.62 (1H, d, 7.6 Hz)	CH
10	38.4		C	38.3		C
11	23.4		CH_2	23.3		CH_2
12	122.6	5.30(1H,t,3.7Hz)	CH	125.6	5.27(1H,t,3.7 Hz)	CH
13	143.7		C	138.0		C
14	41.9		C	42.0		C
15	29.7		CH_2	27.9		CH_2
16	22.9		CH_2	23.8		CH_2
17	47.9		C	48.1		C
18	41.6	2.85 (1H, dd, 13.7,4.7 Hz)	CH	40.9	2.84 (1H, dd,13.7, 4.7 Hz)	CH
19	45.9		CH_2	46.5		CH_2
20	30.6		C	30.7		C
21	33.8		CH_2	38.7		CH_2
22	32.8		CH_2	68.2	4.23(1H,dd,11.4,5.9 Hz)	CH
23	28.9	1.04(3H,s)	CH_3	28.5	1.08(3H,s)	CH_3
24	21.2	0.96(3H,s)	CH_3	17.0	0.87(3H,s)	CH_3
25	16.3	0.98(3H, s)	CH_3	16.5	0.76(3H, s)	CH_3
26	10.9	0.91(3H, s)	CH_3	14.1	0.94(3H, s)	CH_3
27	26.1	1.15(3H,s)	CH_3	24.1	1.10(3H,s)	CH_3
28	183.8		C=O	183.5		C=O
29	33.1	0.95(3H, s)	CH_3	32.4	1.20(3H, s)	CH_3
30	23.6	0.94(3H, s)	CH_3	23.7	0.92(3H, s)	CH_3
1′	132.5		C	132.5		C
2′	130.2	8.11(1H, d, 7.5Hz)	CH	130.9	7.55(1H,dd, 5.7,3.3Hz)	CH
3′	128.5	7.49(1H,t, 7.8Hz)	CH	128.8	7.73(1H, dd,5.7,3.3Hz)	CH
4′	133.8	7.62(1H,t, 7.6Hz)	CH	133.7	7.62(1H,t, 7.6Hz)	CH
5′	128.5	7.49(1H,t, 7.8Hz)	CH	128.8	7.75(1H, dd,5.7,3.4Hz)	CH
6′	130.2	8.11(1H, d, 7.5Hz)	CH	130,9	7.53(1H,dd, 5.7,3.3Hz)	CH
7′	171.2		C=O	167.8		C=O

Table 4: Antibacterial activities of crude extract and isolated compounds from *P.africana.*

Sample	Bacteria inhibition zone (mm)				
	E.coli	***S. aureus***	***S. flexineri***	***S.typhimurium***	***P. aeruginosa***
Extract	9.67±0.47	11.10+0.08	14.23±0.21	10.03±0.05	21.03±0.05
1	7.13+0.12	8.25+0.20	7.08+0.06	8.13+0.10	7.66+0.24
2	7.50±0.08	8.08±0.12	13.00±0.08	12.58±0.31	10.08±0.12
Mixture (3&4)	8.07+0.05	7.66+0.24	8.04+0.06	13.41+0.12	7.05+0.07
5	9.02+0.02	8.48+0.02	11.07+0.05	14.41+0.12	9.33+0.47
Gentamycin	22.13±0.05	19.05±0.04	20.03±0.05	20.10±0.03	14.06±0.09
DMSO	-	-	-	-	-

CONCLUSIONS

Phytochemical investigation of methanol extract of stem barks of *P.africana* led to the isolation of five compounds; β-sitosterol (**1**), benzoic acid (**2**), two oleanolic derivatives (**3** and **4**) and p-hydroxy-benzoic acid (**5**). Compound **2** and **5** are reported for the first time in the genus *Prunus*. The crude extract showed strong activity against *P. aeruginosa*, whereas the isolated compounds showed moderate activity against the tested strains. The antibacterial activity displayed by the extract support the traditional use of this plant against various ailments caused by bacteria. Further comprehensive evaluations, including *in vivo* activity and cytotoxicity tests could be done for conclusive decision on the potential candidacy of P*. africana* for formulation and medicinal uses.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

Availability of Data and Material

NMR data of compounds are available and attached as supporting information.

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Supplementary Material

Chemical Constituents of the Stem Bark of *Prunus africana* and Evaluation of their Antibacterial Activity

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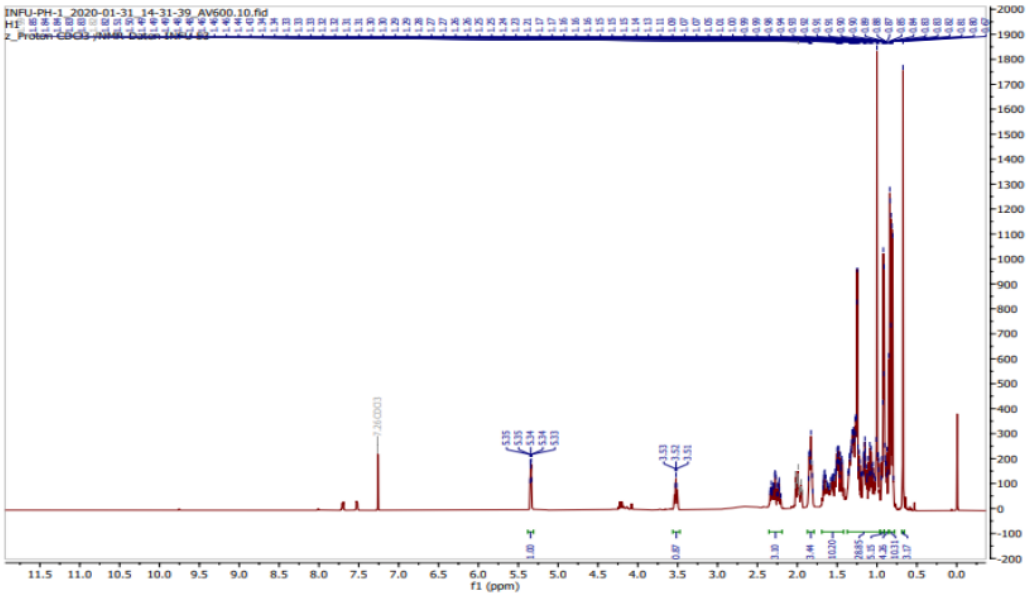

Figure S1: The ^{1}H NMR spectrum of PH-1(1) observed at 500 MHz in $CDCl_3$ at 25 °C. Assignments are given in Table 1.

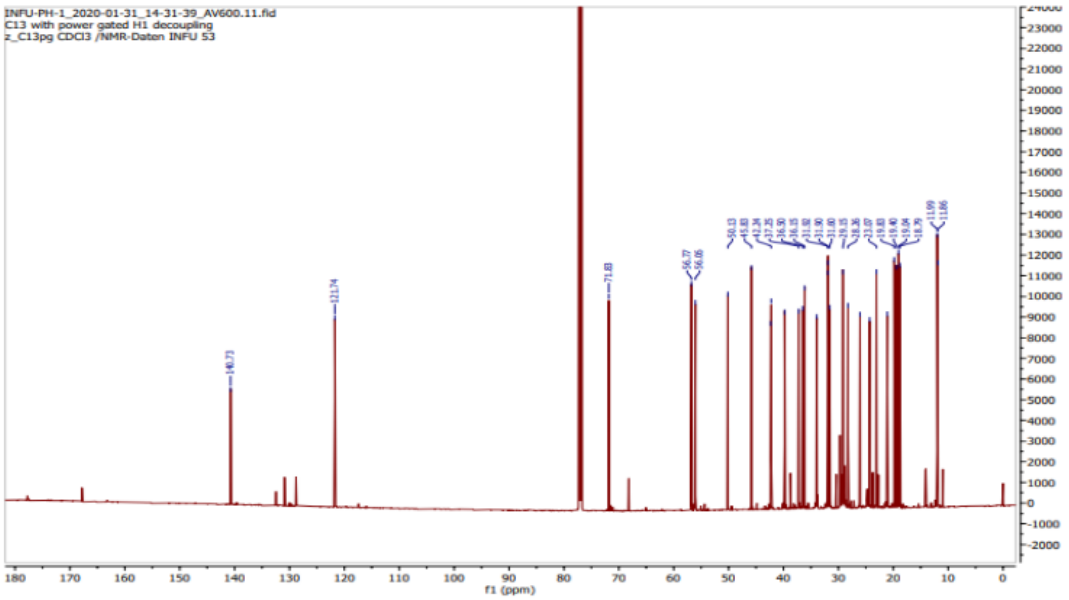

Figure S2: The ^{13}C NMR spectrum of PH-1(1) observed at 125 MHz in $CDCl_3$ at 25 °C. Assignments are given in Table 1.

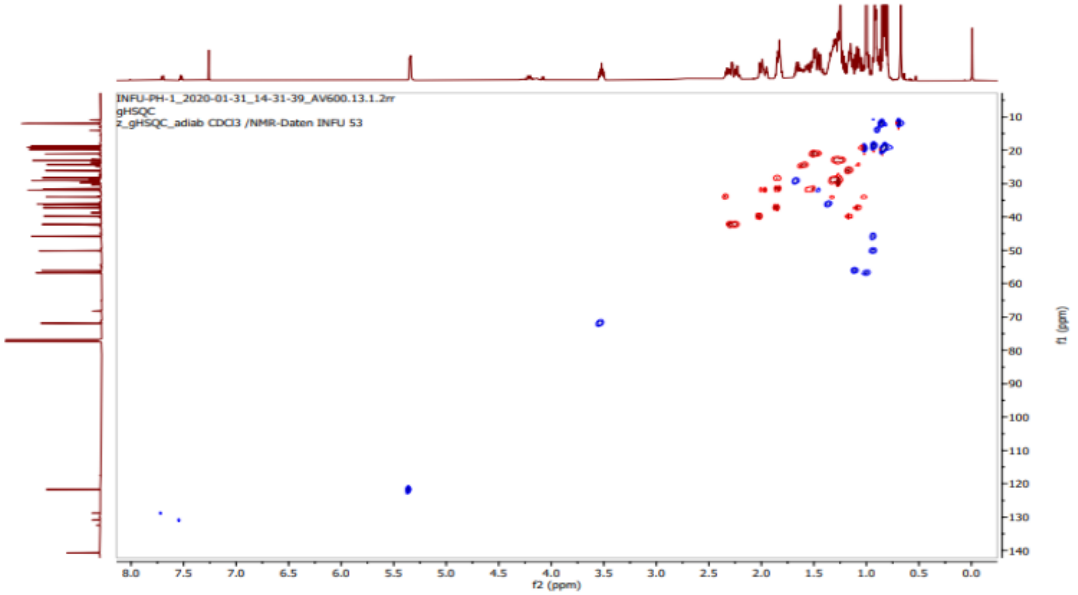

Figure S3: The HSQC spectrum of PH-1(1) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

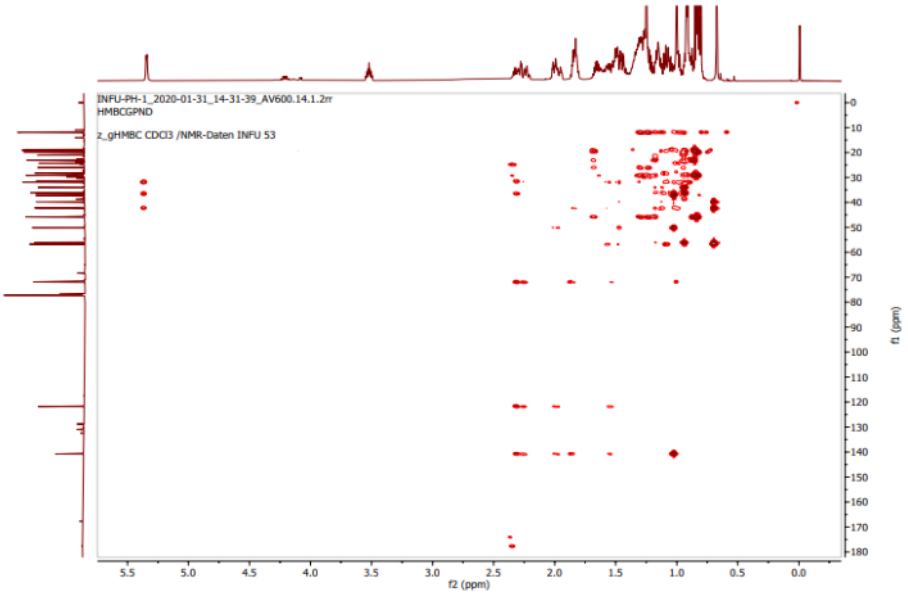

Figure S4: The HMBC spectrum of PH-1(1) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

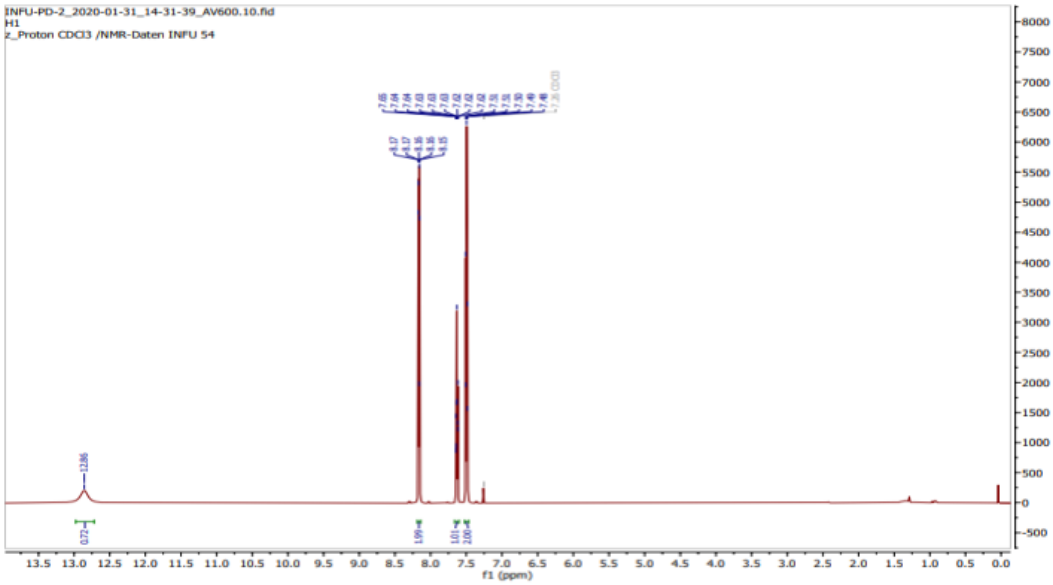

Figure S5: The ^{1}H NMR spectrum of PD-2(2) observed at 500 MHz in $CDCl_3$ at 25 °C. Assignments are given in Table 2.

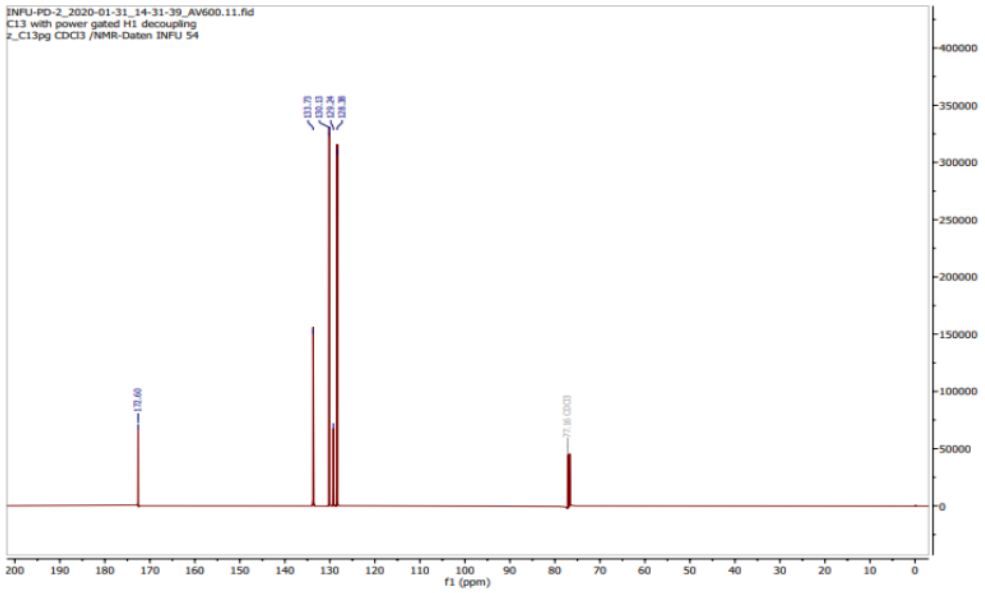

Figure S6: The ^{13}C NMR spectrum of PD-2(2) observed at 125 MHz in $CDCl_3$ at 25 °C. Assignments are given in Table 1.

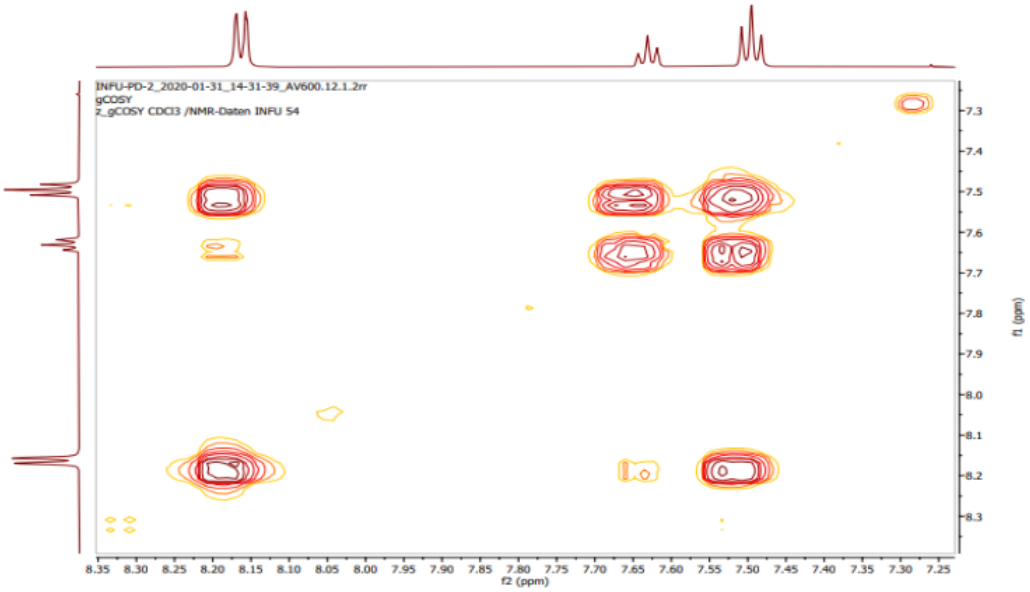

Figure S7: The COSY spectrum of PD-2(2) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

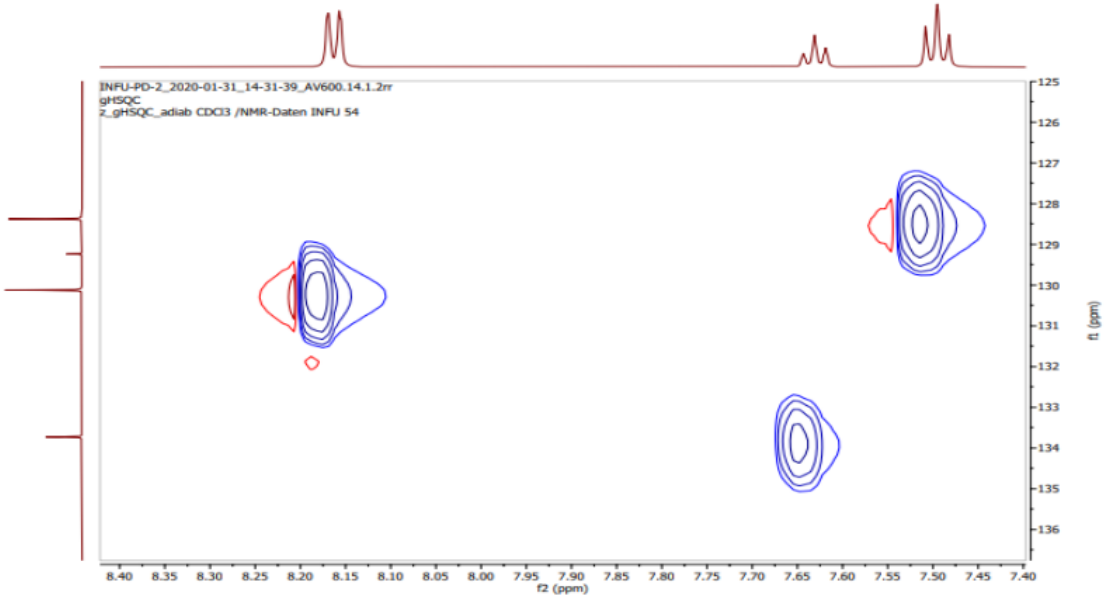

Figure S8: The HSQC spectrum of PD-2(2) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

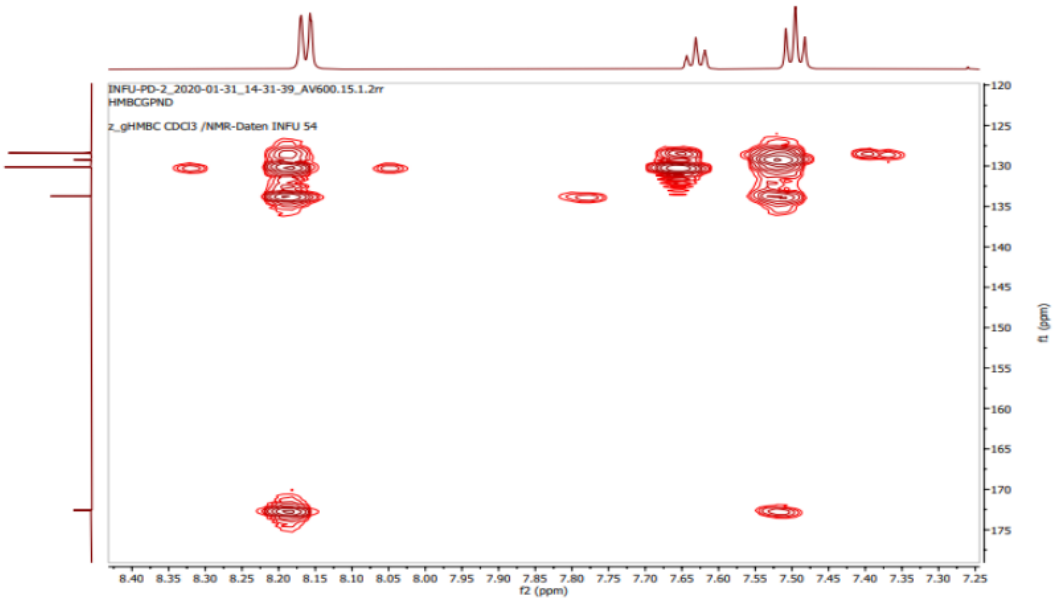

Figure S9: The HMBC spectrum of PD-2(2) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

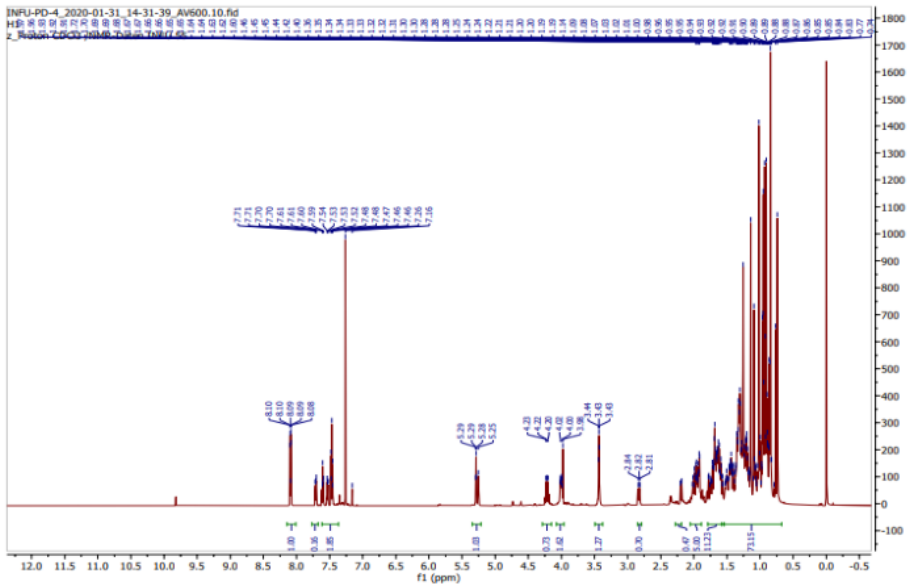

Figure S10: The 1H NMR spectrum of PD-4(3 and 4) observed at 500 MHz in CDCl3 at 25 °C. Assignments are given in Table 3.

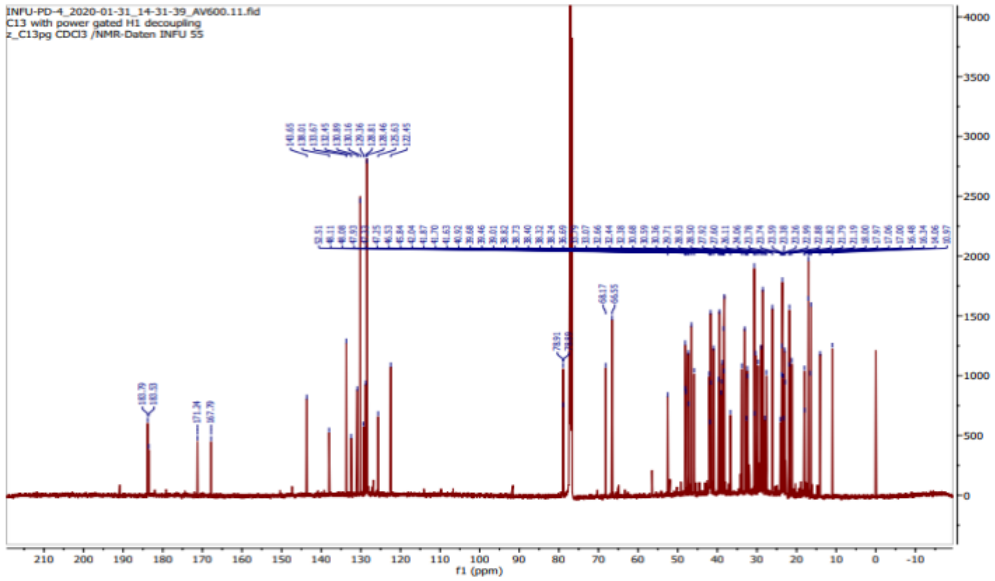

Figure S11: The ^{13}C NMR spectrum of PD-4(3 and 4) observed at 125 MHz in $CDCl_3$ at 25 °C. Assignments are given in Table 3.

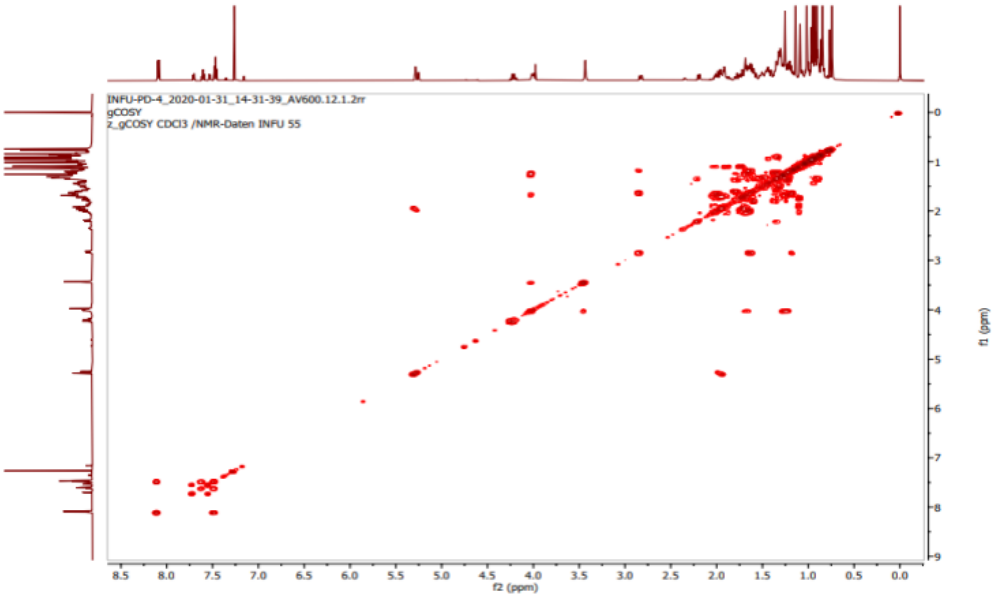

Figure S12: The COSY spectrum of PD-4(3 and 4) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

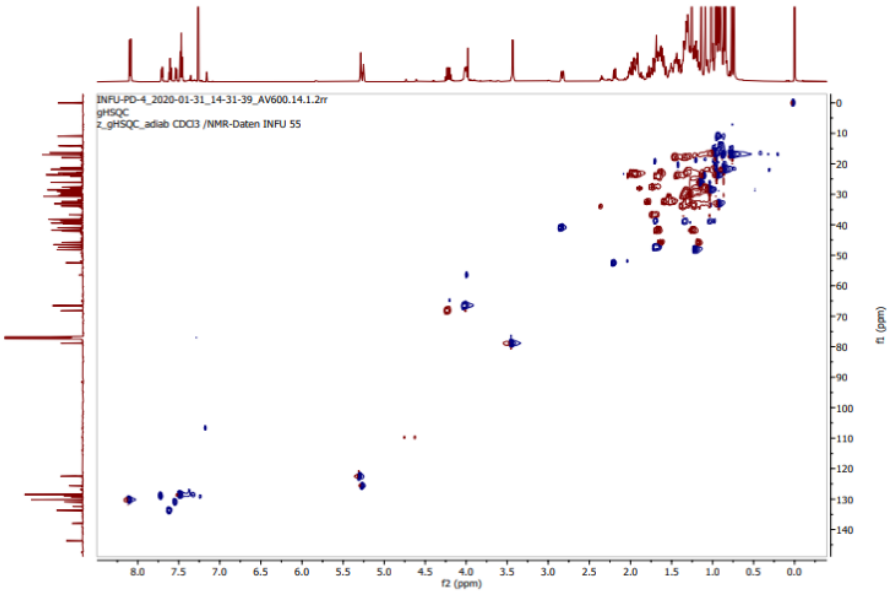

Figure S13: The HSQC spectrum of PD-4(3 and 4) observed at 500 MHz for $CDCl_3$ solution at 25 °C.

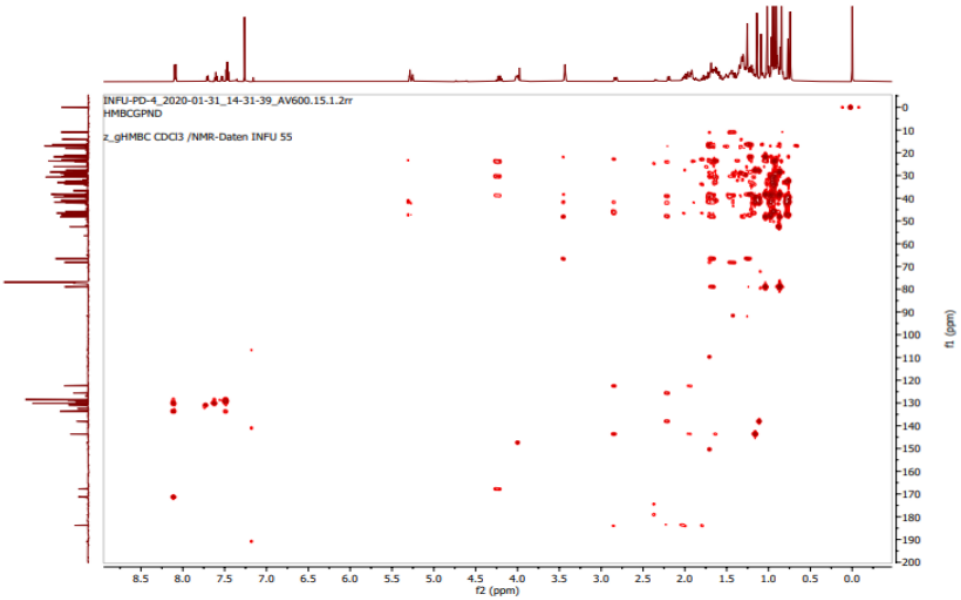

Figure S14: The HMBC spectrum of PD-4(3 and 4) observed at 500 MHz for CDCl3 solution at 25 °C.

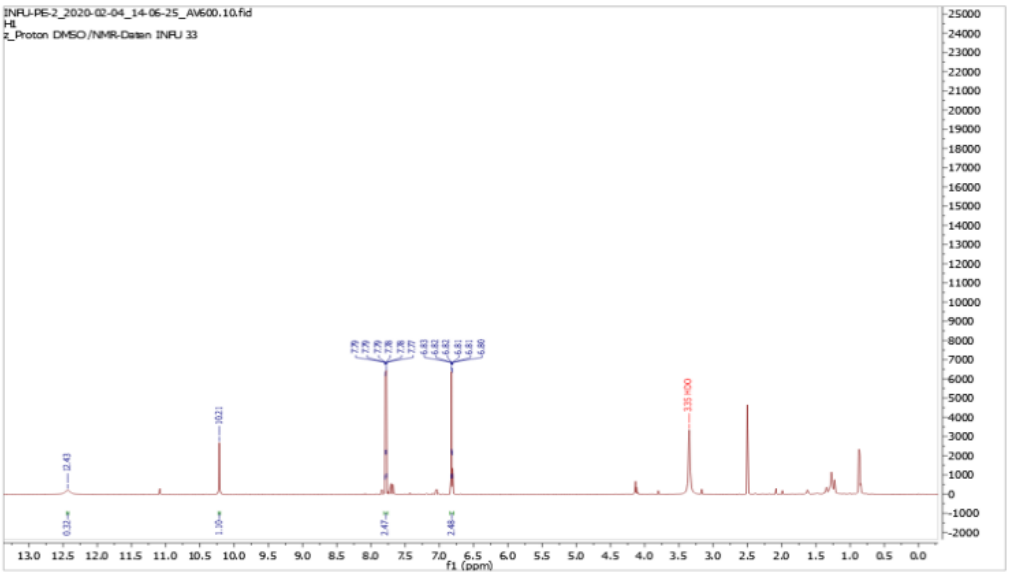

Figure S15: The 1H NMR spectrum of PE-2(5) observed at 500 MHz in DMSO at 25 °C. Assignments are given in Table 2.

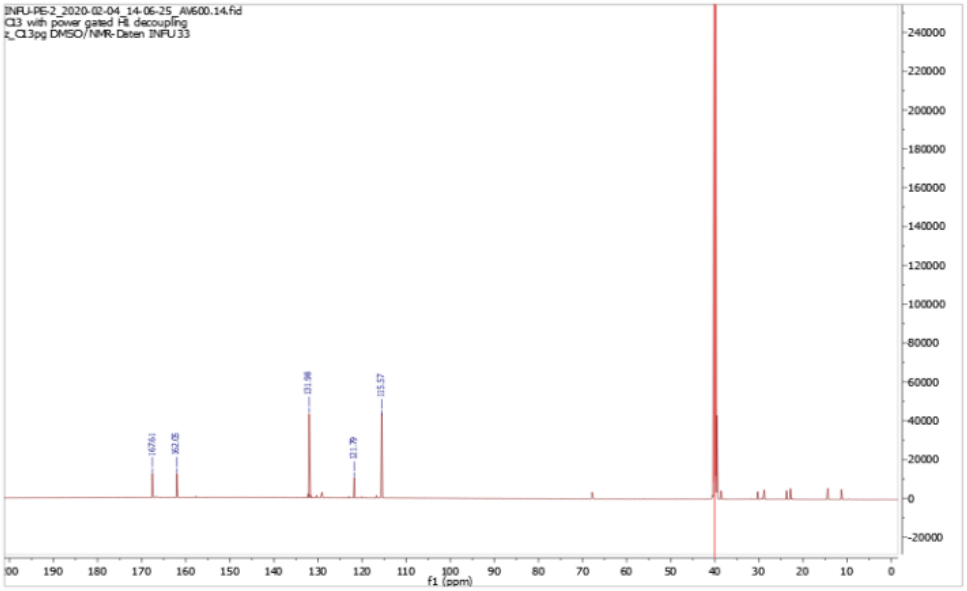

Figure S16: The 13C NMR spectrum of PE-2(5) observed at 125 MHz in DMSO at 25 °C. Assignments are given in Table 2.

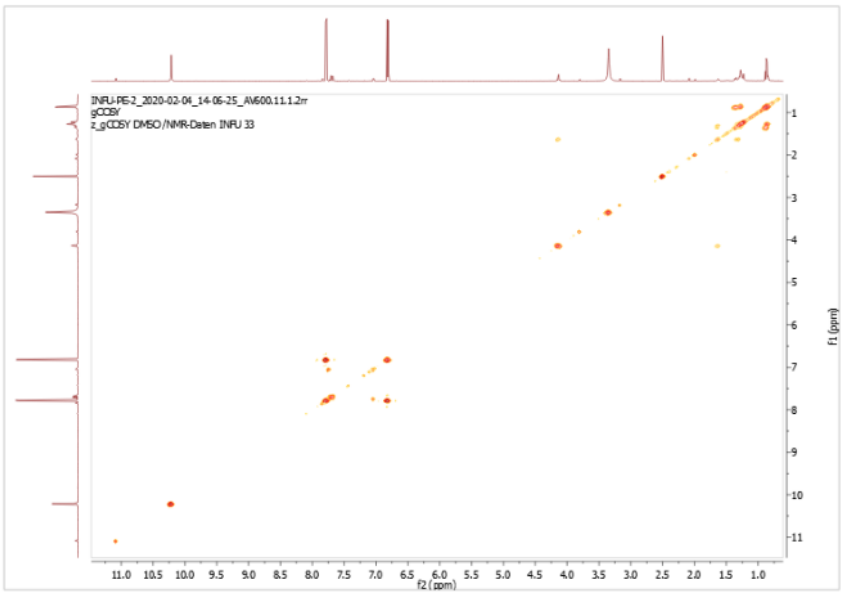

Figure S17: The COSY spectrum of PE-2(5) observed at 500MHz for DMSO solution at 25 °C

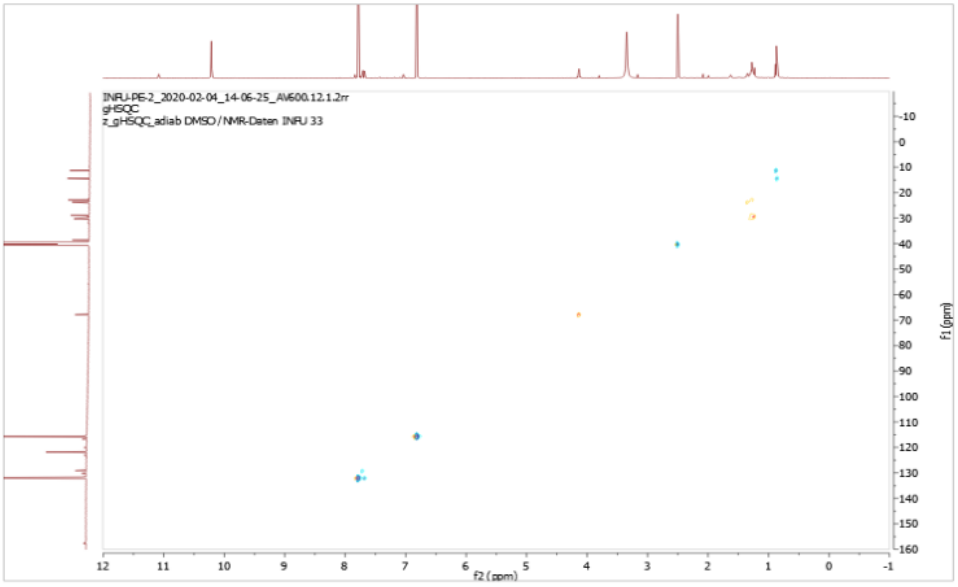

Figure S18: The HSQC spectrum of PE-2(5) observed at 500 MHz for DMSO solution at 25 °C.

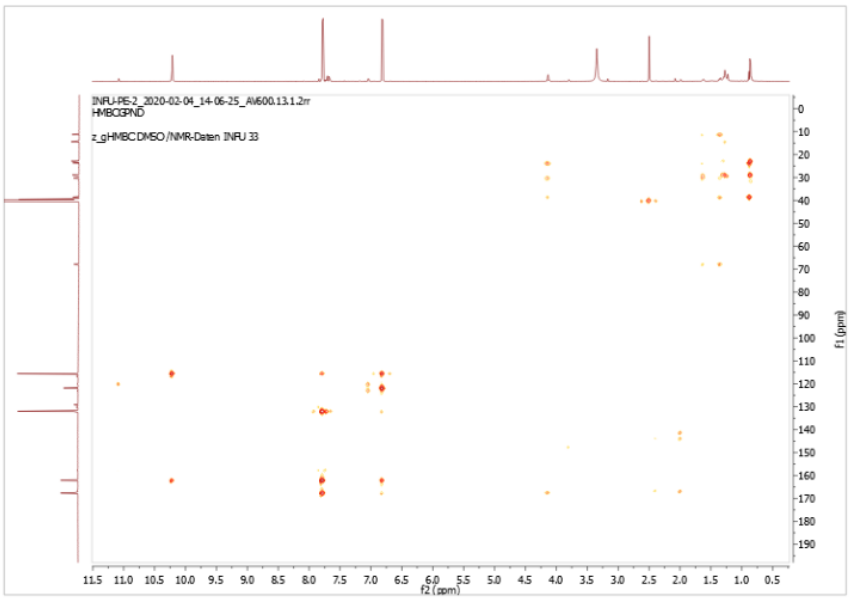

Figure S19: The HMBC spectrum of PE-2(5) observed at 500 MHz for DMSO solution at 25 °C

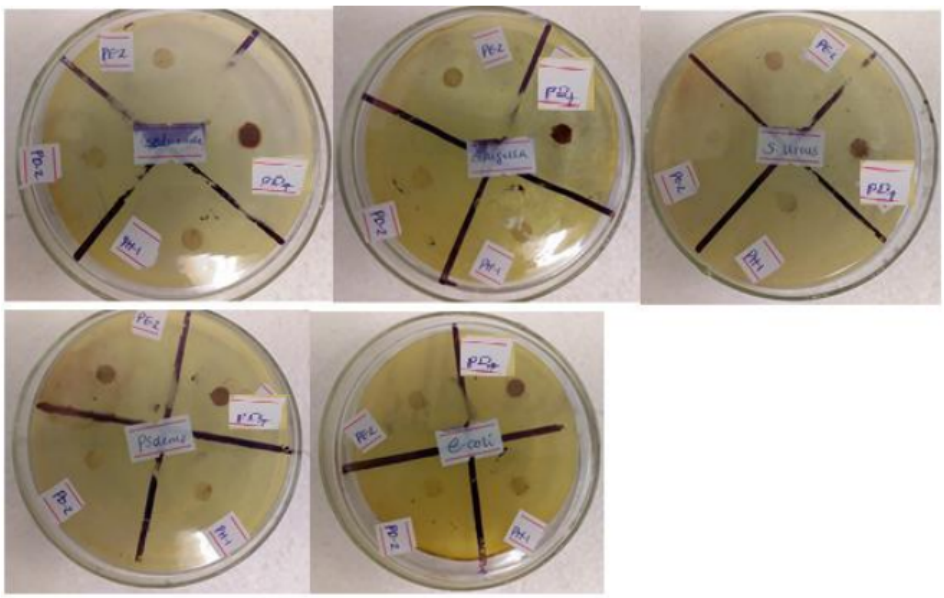

Figure S20: Antibacterial analysis of Crude Extracts, isolated compounds and gentamycin at 10 µg/mL concentration. Key 1: -: PH-1(1) PD-2(2), PD-4(3 and 4), PE-2(5)

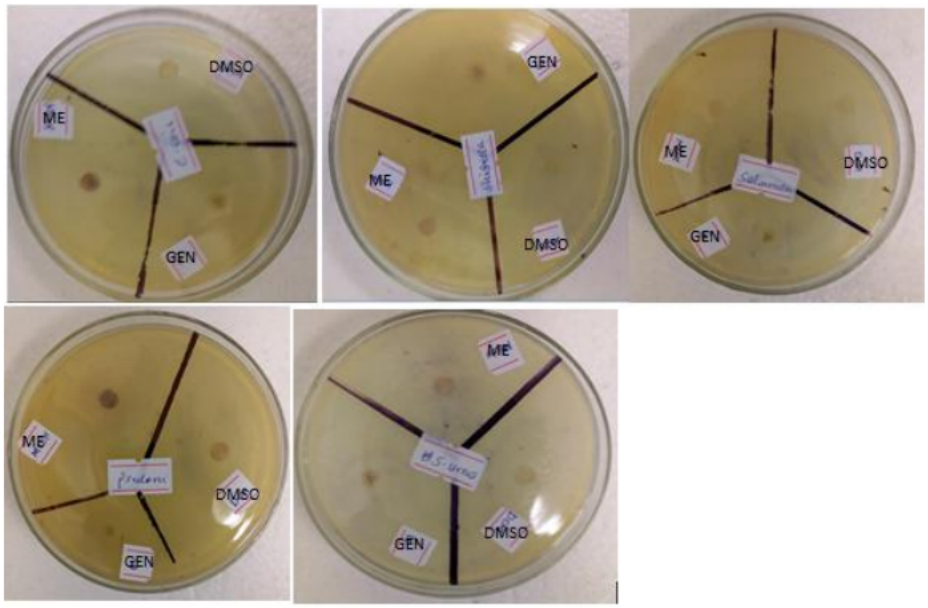

Figure S21: Antibacterial analysis of Crude Extracts, isolated compounds and gentamycin at 10 µg/mL concentration. Key 2: -: GEN-Gentamycin, DMSO-Dimethylsulfoxide, ME-Methanol extract.