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Research article

Gallocatechin analogues from *Olax subscorpioidea* Oliv. against multiresistant clinical bacterial isolates

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Abstract: The recurrence of bacterial infections is commonly associated with the multiple interactions between humans and their environment. These interactions have progressively contributed to the development of antimicrobial resistance among numerous clinically important pathogenic bacteria. The present study targeted the antibacterial activity of natural products derived from the stem bark of *O. subscorpioidea* against clinical isolates of bacteria. This included the Gram-positive bacterium *Staphylococcus aureus* and three Gram-negative bacteria, *Pseudomonas aeruginosa, Providencia stuartii,* and *Escherichia coli.* The plant material was extracted by maceration, and chromatography was performed to isolate the chemical constituents from plant extracts as well as from the reaction medium. The resulting compounds were characterized using spectroscopic and spectrometric techniques. The antibacterial activity was assessed by the microdilution method. Five compounds were isolated, including olasubscorpioside A (**1**), 4′-*O*-methylgallocatechin (**2**), olasubscorpioside C (**3**), olasubscorpioside B (**5a**), isoolasubcorpioside B (**5b**), and glyceryl-1-eicosanoate (**6**). The semisynthetic derivatives 4′-*O*-methylgallocatechin (**2**) (also isolated from the plant extract) and tetra-*O*-allyl olasubscorpioside A (**4**) were prepared and characterized. Interestingly, olasubscorpioside A (**1**)

and 4′-*O*-methylgallocatechin (**2**) displayed a significant antibacterial effect (MIC = 8 µg/mL) against the pathogenic isolate *Staphylococcus aureus* resistant to methicillin, ofloxacin, kanamycin, tetracycline, and erythromycin. Compound **2** had a better activity relative to **1** against *Pseudomonas aeruginosa* (from MIC > 256 to 128 µg/mL) and *Escherichia coli* (from MIC = 128 to 64 µg/mL). Gallocatechins from the stem bark of *Olax subscorpioidea* are promising bioactive molecules to fight against multiresistant bacteria.

Keywords: antibacterial activity; clinical isolates; bacterial resistance; gallocatechin; olasubscorpioside; *Olax subscorpioidea*

Appendix

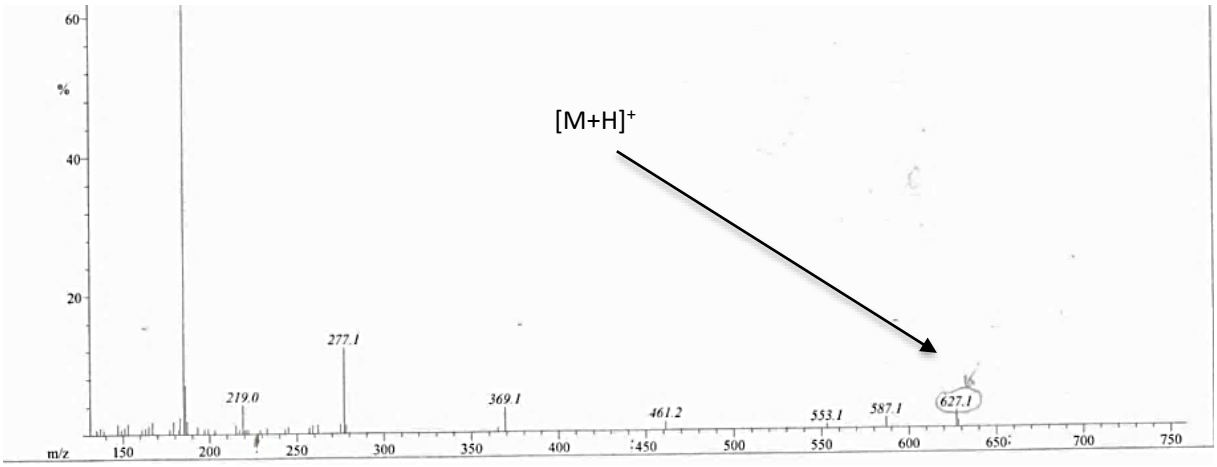

Figure S1. FAB mass spectrum of compound **4**.

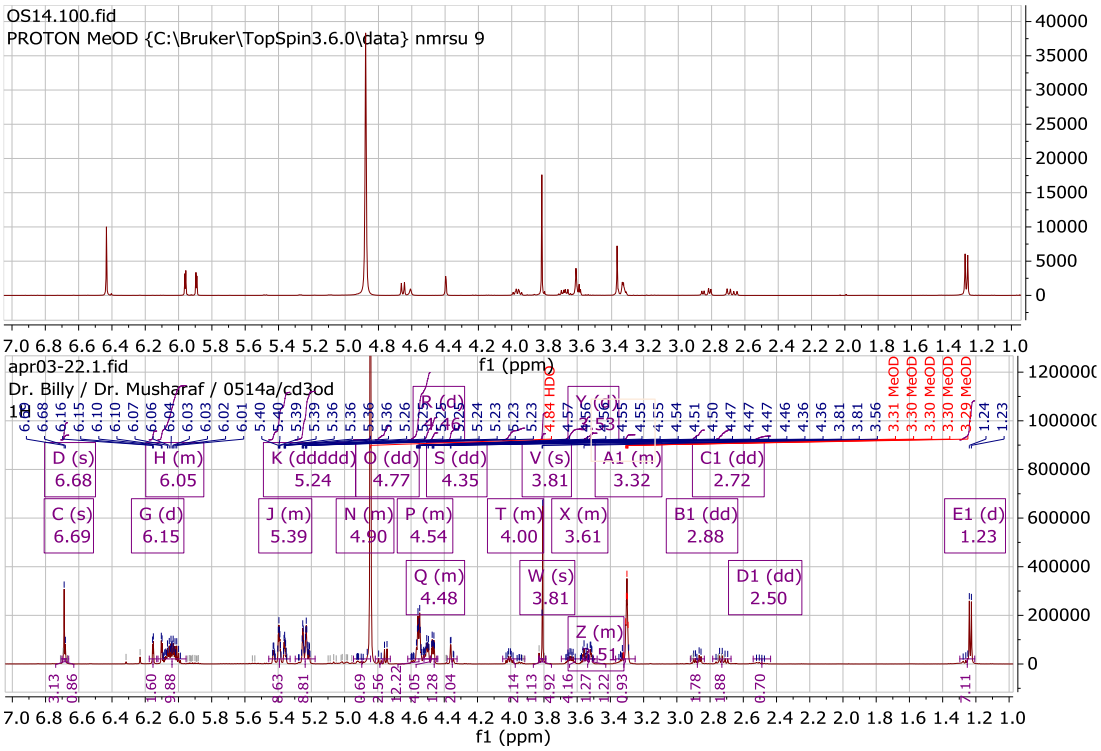

Figure S2. ^{1}H NMR spectrum of compound **4** in CD_3OD, 500 MHz, compared to the spectrum of compound **1**.

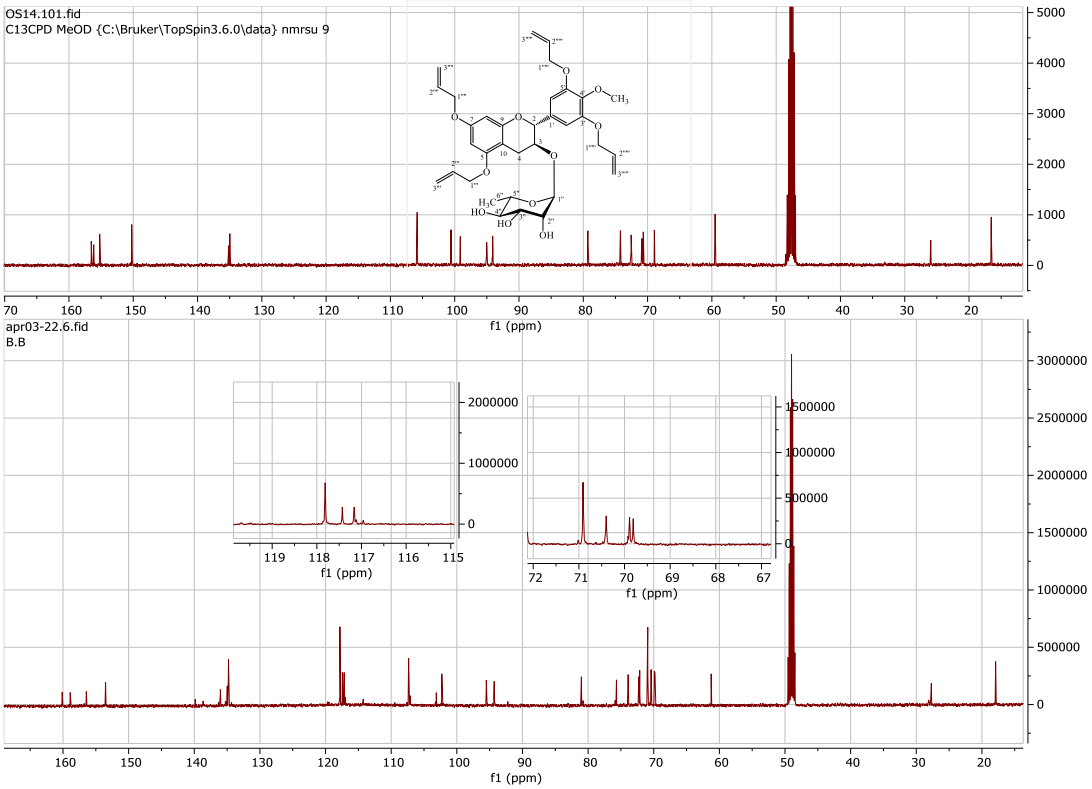

Figure S3. ^{13}C NMR spectrum of compound 4 in CD_3OD, 125 MHz, compared to the spectrum of compound 1.

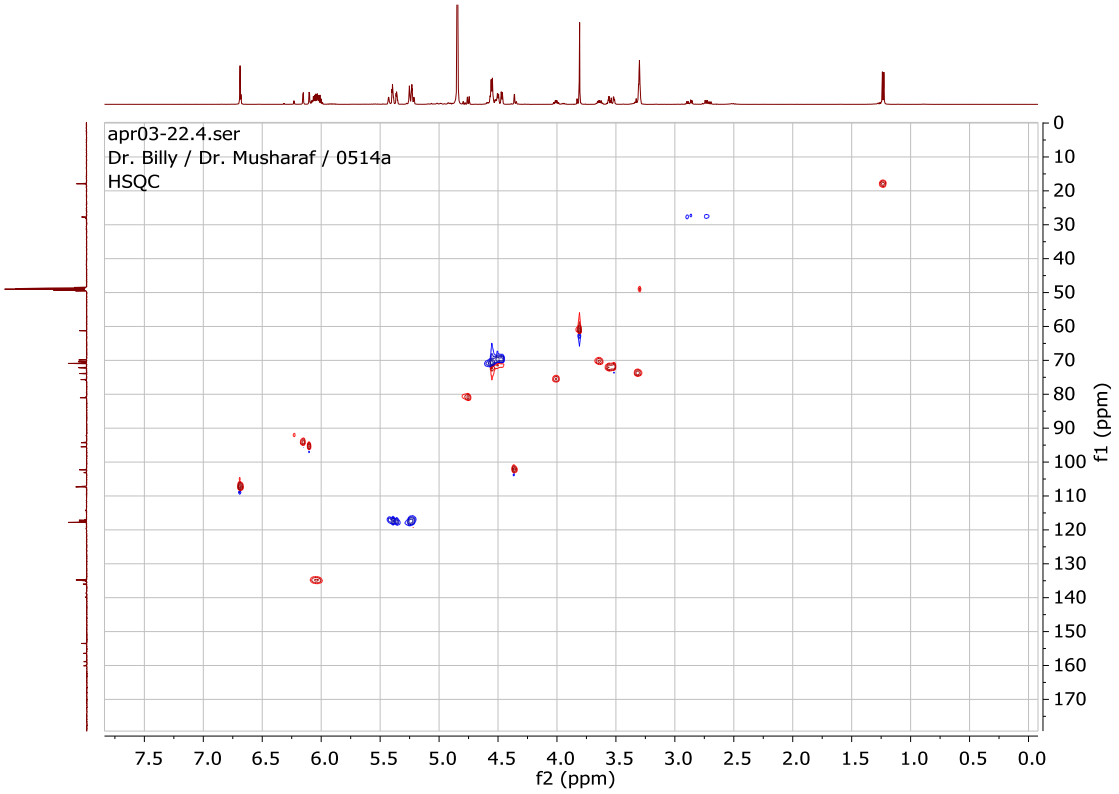

Figure S4. HSQC spectrum of compound **4**.

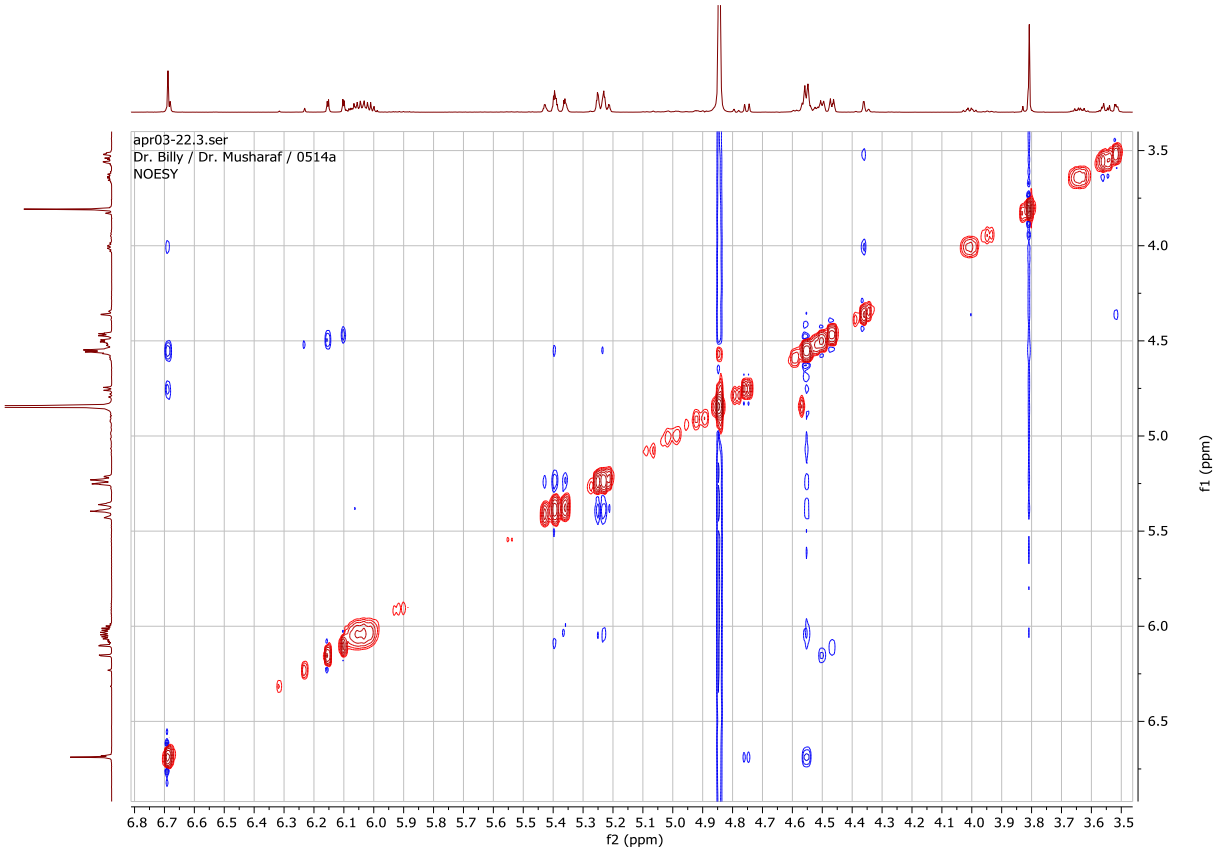

Figure S5. NOESY spectrum of compound **4**.

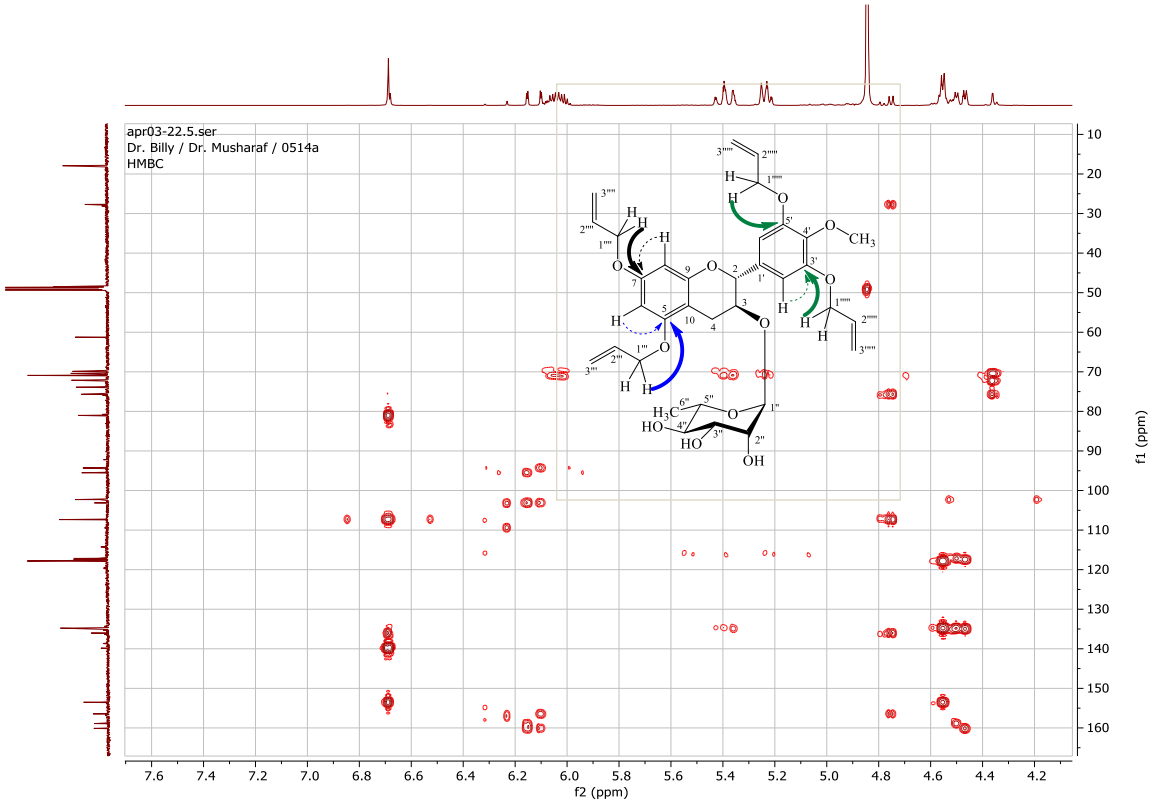

Figure S6. HMBC spectrum of compound **4**.

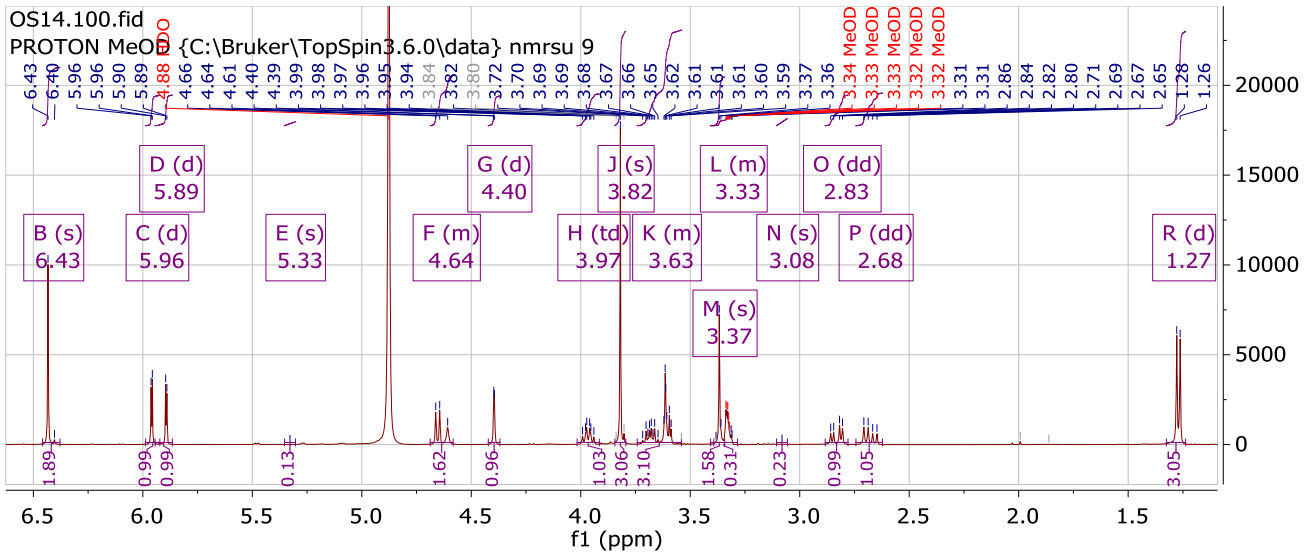

Figure S7. ^{1}H NMR spectrum of compound **1** in CD_3OD, 400 MHz.

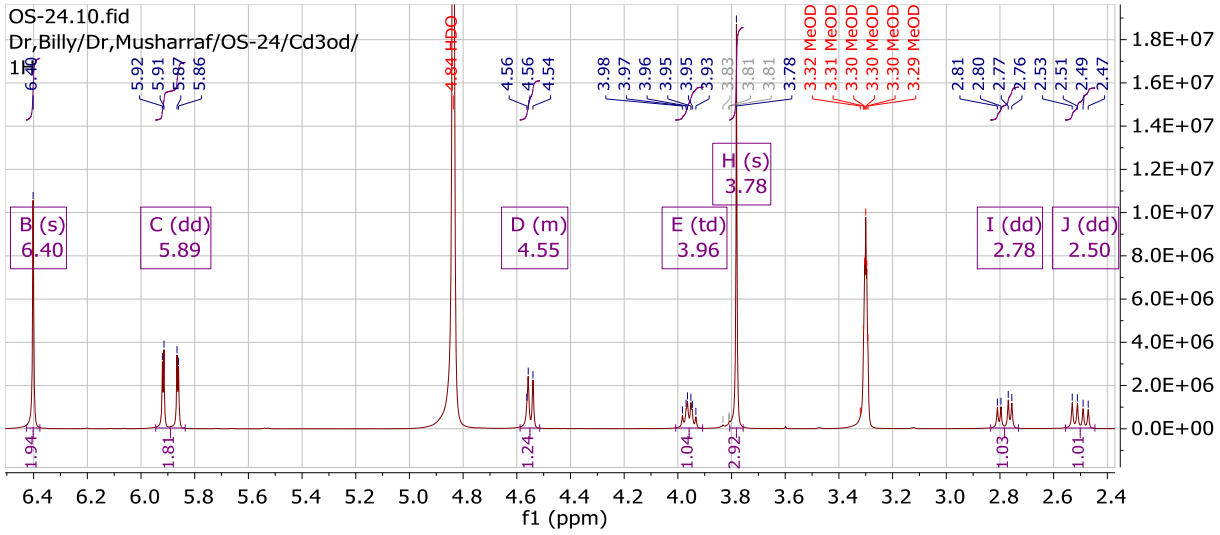

Figure S8. ^{1}H NMR spectrum of compound **2** in CD_3OD, 500 MHz.

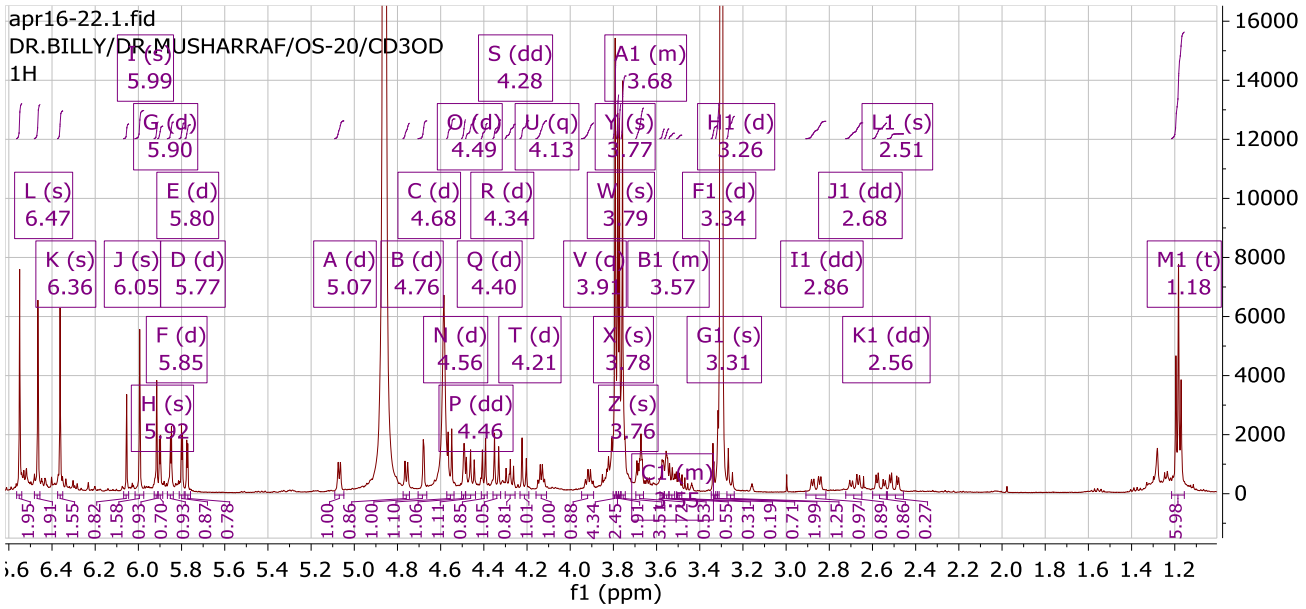

Figure S9. ^{1}H NMR spectrum of compound **3** in CD_3OD, 500 MHz.

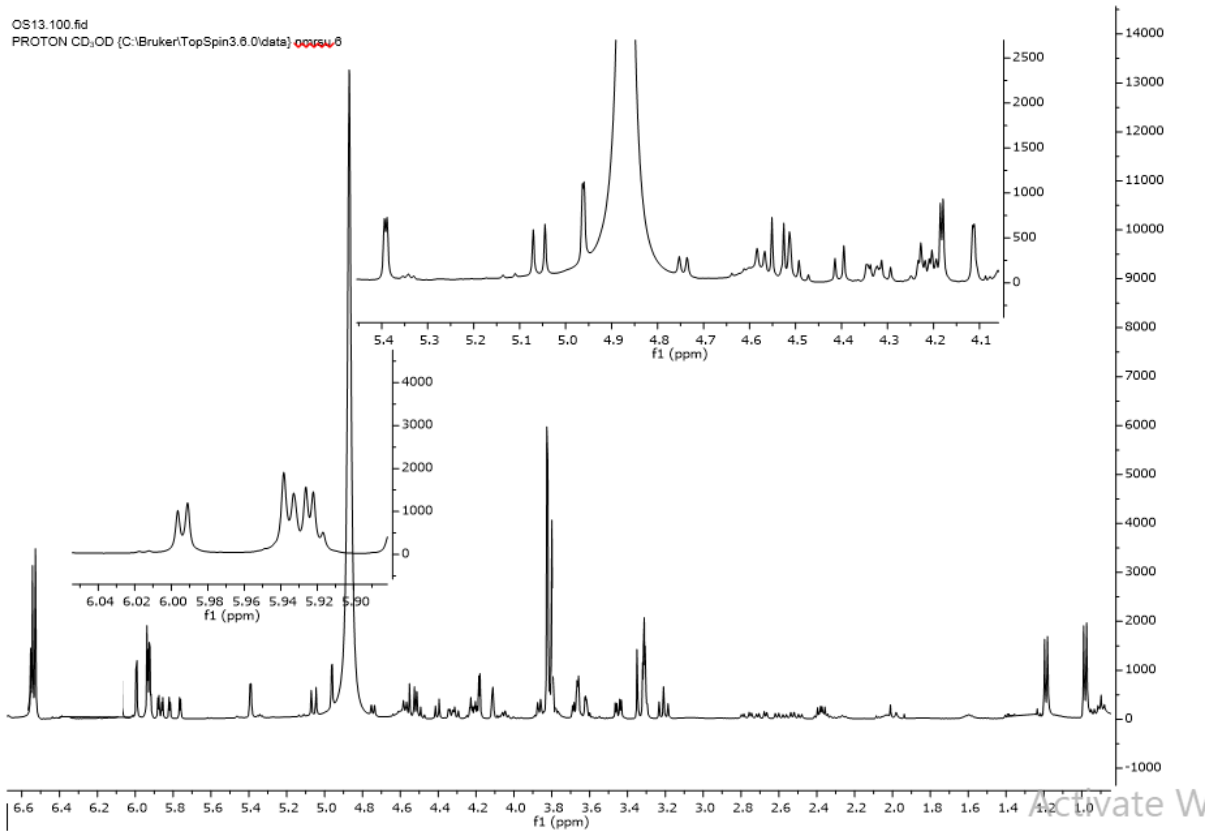

Figure S10. ^{1}H NMR spectrum of compound **5** in CD_3OD, 400 MHz.

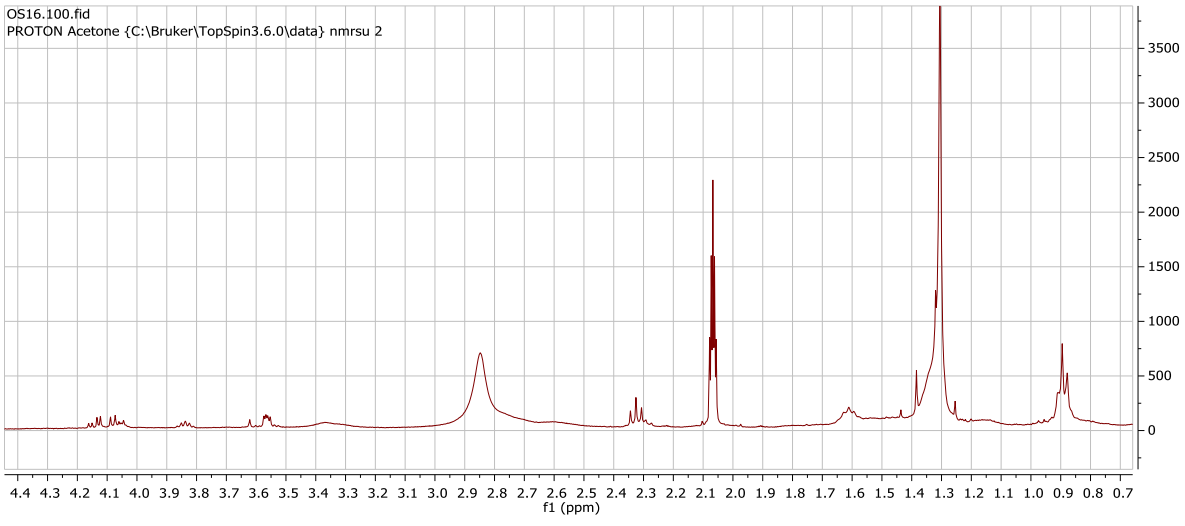

Figure S11. ^{1}H NMR spectrum of compound **6** in CD_3OD, 400 MHz.

Table S1. NMR data of compound **4** comparatively to the data of compounds **1** and **2**.

Positions	4		1	2
	δ_C	δ_H (multiplicity, *J* in Hz)	δ_C	δ_C
2	81.0	4.75 (d, 7.5)	79.3	82.8
3	75.7	4.01 (td, 7.8, 5.5)	74.2	68.6
4	27.7	2.88 (dd, 16.5, 5.7) 2.72 (dd, 16.4, 8.1)	26.0	28.1
5	158.9	/	156.2	157.4
6	94.3	6.15 (d, 2.3)	95.1	96.5
7	160.2	/	156.5	157.9
8	95.5	6.10 (d, 2.3)	94.1	95.6
9	156.4	/	155.2	156.7
10	103.2	/	99.1	100.8
1'	136.0	/	135.0	136.7
2'	107.3	6.70 (s)	105.7	107.4
3'	153.5	/	150.2	151.7
4'	139.9	/	135.2	136.4
5'	153.5	/	150.2	151.7
6'	107.3	6.70 (s)	105.7	107.4
OCH_3-4'		3.81 (s)	59.5	61.0
1''	102.3	4.36 (d, 1.3)	100.6	
2''	72.1	3.52 (m)	70.9	
3''	72.3	3.56 (m)	70.6	
4''	73.9	3.31 (m)	72.6	
5''	70.4	3.63 (m)	69.0	
6''	17.9	1.23 (d, 6.3)	16.5	
1'''	69.9	4.50 (m)		
2'''	134.8	6.04 (m)		
3'''	117.4	5.39 (m); 5.23(m)		
1''''	69.8	4.47 (m)		
2''''	134.8	6.04 (m)		
3''''	117.2	5.39 (m); 5.23(m)		
1'''''	70.9	4.55 (m)		
2'''''	134.8	6.04 (m)		
3'''''	117.8	5.39 (m); 5.23(m)		

Table S2. ^1H (500 MHz) and ^{13}C (125 MHz) NMR data of compound **3** in CD_3OD.

Positions	3			
	Rotamer 1		Rotamer 2	
	δ_C	δ_H (multiplicity, *J* in Hz)	δ_C	δ_H (multiplicity, *J* in Hz)
Upper Unit				
2C	83.9	4.34 (d, 9.7)	84.0	4.21 (d, 9.8)
3C	73.7	4.47 (dd, 9.8, 8.3)	74.2	4.28 (dd, 9.8, 7.4)
4C	38.4	4.56 (d, 8.3)	38.6	4.40 (d, 7.4)
5A	157.3		156.8	

Positions	3			
	Rotamer 1		Rotamer 2	
	δ_C	**δ_H (multiplicity, *J* in Hz)**	**δ_C**	**δ_H (multiplicity, *J* in Hz)**
6A	97.7	5.80 (d, 2.3)	97.5	5.90 (d, 2.3)
7A	158.5		158.6	
8A	96.4	5.85 (d, 2.4)	97.0	5.77 (d, 2.4)
9A	155.8		155.6	
10A	107.1		107.4	
1'B	136.7		136.8	
2'B	108.5	6.55 (s)	108.7	6.36 (s)
3'B	151.4		151.2	
4'B	136.9		136.7	
5'B	151.4		151.2	
6'B	108.5	6.55 (s)	108.7	6.36 (s)
4'B OCH_3	60.8	3.79 (s)	60.8	3.78 (s)
Lower Unit				
2F	79.7	5.07 (d, 5.2)	79.8	4.76 (d, 6.2)
3F	75.1	4.13 (q, 5.1)	74.7	3.92 (q, 6.1)
4F	25.0	2.86 (dd, 16.3, 5.5)	26.2	2.68 (dd, 16.4, 6.5)
		2.50 (dd, 16.5, 4.5)		2.56 (dd, 16.4, 5.1)
5D	155.7		155.6	
6D	97.6	5.92 (s)	96.1	6.06 (s)
7D	158.5		158.7	
8D	108.3		108.7	
9D	154.4		154.2	
10D	99.8		101.7	
1'E	136.4		136.1	
2'E	106.5	6.46 (s)	107.1	6.00 (s)
3'E	151.6		151.0	
4'E	135.9		136.6	
5'E	151.6		151.0	
6'E	106.5	6.46 (s)	107.1	6.00 (s)
4'E OCH_3	60.9	3.76 (s)	60.8	3.77 (s)
α-L-Rhamnopyranosyl				
1"	101.6	4.68 (d, 1.7)	101.3	4.49 (d, 1.7)
2"	72.4	3.76 (m)	72.3	3.67 (m)
3"	72.2	3.56 (m)	72.2	3.55 (m)
4"	74.0	3.30 (m)	74.1	3.32 (m)
5"	70.2	3.56 (m)	70.3	3.50 (m)
6"	18.0	1.19 (d, 6.2)	18.0	1.18 (d, 6.2)

Table S3. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data of compounds **5a** and **5b** in CD_3OD.

Positions	**Olasubscorpioside B**		**Isoolasubscorpioside B**	
	δ_C	δ_H **(multiplicity, *J* in Hz)**	δ_C	δ_H **(multiplicity, *J* in Hz)**
2	83.6	5.06 (d, 10.3)	81.7	5.39 (d, 2.4)
3	78.4	4.54 (d, 10.3)	75.1	4.18 (d, 2.4)
4	195.6	/	193.9	/
5	164.2	/	164.3	/
6	97.4	5.94 (o)	97.4	5.94 (o)
7	168.7	/	168.8	/
8	96.3	5.92 (d, 2.3)	96.2	5.99 (d, 2.2)
9	163.8	/	163.8	/
10	102.4	/	101.7	/
1'	133.4	/	133.1	/
2'	107.8	6.53 (s)	106.9	6.54 (s)
3'	151.9	/	151.8	/
4'	137.3	/	136.8	/
5'	151.9	/	151.8	/
6'	107.8	6.53 (s)	106.9	6.54 (s)
4' OCH3	60.9	3.82 (s)	61.0	3.83 (s)
α-L-Rhamnopyranoside				
1"	102.0	4.11 (d, 1.7)	99.9	4.96 (d, 1.6)
2"	72.2	3.62 (m)	71.9	3.66 (m)
3"	72.1	3.35 (m)	71.8	3.45 (m)
4"	73.7	3.31 (m)	73.1	3.21 (m)
5"	70.5	4.23 (m)	70.4	2.37 (m)
6"	17.8	1.19 (d, 6.2)	17.8	0.98 (d, 6.2)

Table S4. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR data in of compound **6** in Acetone-d_6.

Positions	**6**	
	δ_C	**δ_H (multiplicity, *J* in Hz)**
1	65.2	4.14 (dd, 11.2, 4.6)
		4.06 (dd, 11.2, 4.6)
2	70.1	3.83 (m)
3	63.2	4.05 (m)
		3.56 (o)
1'	173.4	/
2'	33.7	2.32 (t, 7.4)
3'	25.0	1.62 (m)
4'-18'	28.2-30.7	1.25-1.31 (30H, br d)
19'	22.5	1.31 (m)
20'	13.5	0.89 (t, 6.8)