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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*

1. Introduction

Antimicrobial resistance (AMR) is a growing global concern fueled by several interconnected factors. Poor infection control practices in hospitals and clinics, the accumulation of antibiotics in the environment, and the widespread use of antibiotics in the animal and agri-food sectors all contribute to the emergence and spread of resistant microorganisms. Since the discovery of penicillin, resistance has been progressively reported with each new class of antibiotics, including vancomycin, methicillin, and others. This trend underscores the urgent need for coordinated action to preserve the effectiveness of existing antimicrobial agents [1]. Some artificial intelligence-based studies are being conducted on animal proteins and peptides to target the phenomenon of AMR. Other methods include immune assistance, combination therapies, efficient diagnostics, and natural products [2]. According to reports, about 3% of natural antibiotics come from plants [3]. Though this percentage is insignificant, some plants provided with active skeletons and chemical modifications were processed to discover active lead compounds. For instance, quinolones initially isolated from *Cinchona* plants served as reference molecules to synthesize many quinolone antibiotics still in use [4,5]. Recent research on natural products derived from fungi and bacteria highlights their potential as a valuable source of novel antimicrobial compounds [6]. These bioactive substances could play a crucial role in addressing the antimicrobial resistance (AMR) crisis by providing new therapeutic options against resistant pathogens. The continued exploration of natural products is therefore essential in the global effort to combat AMR. Many challenges are sometimes faced in search of natural antibiotics from plants, including the low amount of samples isolated, the poor solubility of compounds, and the unavailability of infrastructure to undertake proper antimicrobial assays [7].

Previous chemical investigations on the medicinal plant *Olax subscorpioidea* revealed that it is a source of bioactive compounds like saponins, diterpenoids, and flavonoids. The plant itself is used in traditional medicine in Western Africa to treat numerous ailments such as venereal diseases, jaundice, cancer, and microbial infections [8]. As part of our ongoing investigation into bioactive secondary metabolites from medicinal plants [9,10], we are reporting here the isolation and characterization of secondary metabolites from the stem bark of *Olax subscorpioidea* and their semisynthetic derivatives, along with their antibacterial activity against some clinically important bacteria.

2. Materials and methods

2.1. Instruments and chemicals

One-dimensional NMR (^{1}H, ^{13}C NMR, and DEPT 135) and two-dimensional NMR (^{1}H-^{1}H COSY, HSQC, and HMBC) data resulted from the analysis performed on a BRUKER DRX-500 spectrometer at 500 MHz (^{1}H data) and 125 MHz (^{13}C data). For the environment conditions, the temperature was 298 K, and exclusively deuterated solvents were used for analysis. These data were expressed in terms of chemical shifts (δ) relative to the internal standard (TMS), and the J values were expressed in Hz. The mass spectra were recorded in FABMS (+) using a JEOL-600-H2 and JEOL HX 110 apparatus. In regard to the isolation of chemical constituents, 70–230 and 230–400 nm mesh silica gel 60 (Merck) and Sephadex LH-20 gel were used as stationary phases in column chromatography. TLC was carried out on percolated silica gel 60 F254 (Merck) aluminum plates and monitored by UV-visible lamp multiband UV-54/365 nm (model UVGL-58 Upland CA 91786, USA) and/or by spraying with 10% sulfuric acid, followed by warming at 90 °C. The extracts, fractions, sub-fractions, and isolated compounds were dried in a desiccator (Desiccator PC 250mm) at a temperature ranging from 40 to 60 °C.

2.2. Plant material

The plant material was collected in Didango village (Koutaba), Noun Division, Cameroon, western region in November 2018. A sample of this plant was then authenticated at the Cameroon National Herbarium, where it was compared to a voucher specimen registered with the reference number 3528/SRFK.

2.3. Extraction

The stem barks of *O. subscorpioidea* were cut, dried away from direct sunlight, and ground to yield 4.25 kg of powder, which was extracted three times (3 × 24 hours) with 10 L of ethanol at room temperature. The filtrate obtained was evaporated to dryness under reduced pressure to obtain 600 g of extract. A portion of this extract (550 g) was suspended in distilled water (500 mL) and then successively extracted with EtOAc (2 × 500 mL) and *n*-BuOH (2 × 500 mL). Each solution was evaporated to dryness, resulting in 114 g and 203 g of EtOAc and *n*-BuOH fractions, respectively.

2.4. Isolation of compounds

A mass of 200 g of the *n*-butanol soluble fraction was subjected to column chromatography over silica gel using a mixture of EtOAc:MeOH from 100:00 to 70:30 and then with the mixture EtOAc:MeOH:H_2O (70:30:10) to afford three main sub-fractions: A (37.8 g), B (24.8 g), and C (46.5 g). An amount of A (30 g) was submitted to Sephadex LH-20 column chromatography eluted with MeOH in order to remove gums, followed by chromatography on silica gel as the stationary phase and the mixture n-hexane:EtOAc (30:70) to afford 7 sub-fractions (A1 to A7). Further purifications of the resulting sub-fractions led to isolation of **2** (523.0 mg), **5** (19.7 mg), **1** (1100 mg), and **6** (12.7 mg). Fraction B (20 g) was chromatographed on Sephadex LH-20 using MeOH and on silica gel by using the mixture EtOAc:MeOH:H_2O (95:5:2) to lead to the three main subfractions B1 (8 g), B2 (2 g), and

B3 (4.2 g). 970 mg of B3 was then chromatographed on medium pressure column chromatography (MPLC) by using reverse-phase silica gel; the eluent consisted of the mixture of H_2O:MeOH (83:17). A volume of 50 mL was collected, and this separation afforded compound **3** (28.7 mg) as a brown gum.

2.5. Allylation of compound 1

The process used here was the one carried out by Tsafack et al. (2018) [11] with slight modifications.

Compound **1** (250.0 mg, 0.53 mmol) and K_2CO_3 (302.0 mg, 2.2 mmol) were dissolved in DMSO (4.0 mL), and the reaction medium was allowed to stir (by using a magnetic stirrer) at room temperature. After two hours, allyl bromide (187.0 µL) was added dropwise using a micropipette under continuous stirring of the reaction medium; the mixture was allowed to stir continuously for three additional hours. The reaction was monitored using TLC until the substrate was completely consumed. The next step consisted of pouring 50 mL of distilled water into the reaction medium in order to stop the reaction. The mixture was then transferred into the separatory funnel and extracted twice with 100 mL of *n*-butanol. The organic phase was evaporated.

Further separation was achieved in an open column chromatography on silica gel and eluted with the solvent mixture *n*-hexane-EtOAc (50:50). 100 mL was collected, and this separation led to the isolation of compound **4** (128 mg, 36%).

2.6. Methanolysis of compound 1

The substrate (200.0 mg, 0.4 mmol) was dissolved in 4.0 mL of methanolic HCl (3.5:0.5) (1.5 N, 0.75 mmol), and the mixture was refluxed at 90 °C. After 2 hours, the reaction medium was checked by using a TLC plate, and the substrate was completely consumed. The reaction medium was evaporated to dryness to eliminate MeOH. The residue obtained was dissolved with 50 mL of distilled water and liquid-liquid extracted into a separatory funnel with 100 mL of EtOAc. The organic phase was collected two times and evaporated to dryness, and the final residue was collected with EtOAc. The reaction product precipitated as a beige amorphous powder. This product was filtered and washed several times with methanol to obtain compound **2** (102.0 mg, yield 74.3%).

2.7. Spectroscopy data of isolated compounds

Olasubscorpioside A (**1**)
Beige amorphous powder; ^{1}H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectroscopic data, see **Table 1**. HR-ESI-MS m/z: 489.1363 $[M+Na]^+$ (Calcd for $C_{22}H_{26}O_{11}Na$: 489.1367) [12]
4'-*O*-methylgallocatechin (**2**)
Beige amorphous powder; ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) in CD_3OD spectroscopic data, Table S1 [13].
Olasubscorpioside C (**3**)
Brown gum (MeOH), $[\alpha]_D^{25}$ + 11.6 (c = 1, MeOH); UV (MeOH) λ_{max} 205.58 nm; IR (KBr) ν_{max} 3253, 2917 cm^{-1}; CD (c = 1, MeOH) λ_{max} 216 (-3.59), 205 (-1.17) and 199 (-8.30); ^{1}H and ^{13}C NMR; see Table S2; FABMS(+) m/z 785 $[M+H]^+$, FABMS (-) m/z 783 $[M-H]^-$, HRFABMS m/z 785.2272

$[M+H]^+$ (Calcd for $C_{38}H_{41}O_{18}^+$: 785.2215) [14].

(2*R*,3*S*) 5,7,3',5'-tetra-*O*-allyl-4'-*O*-methylgallocatechin 3-*O*-*α*-L-rhamnopyranoside (**4**)

Greenish gum, soluble in MeOH. FAB-MS (+) (Figure S1): $[M+H]^+$ *m/z* 627 (calc. for $C_{34}H_{42}O_{11}$, 627). ^1H (500 MHz) and ^{13}C (125 MHz) NMR data in CD_3OD data, see Table 1.

Olasubscorpioside B (5a) and isoolasubcorpioside B (**5b**)

Orange oil; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD) spectroscopy data, Table S3. HR-ESI-MS m/z: 519.0899 $[M+K]^+$ (Calcd for $C_{22}H_{24}KO_{12}$: 519.0899) [12].

Glyceryl-1-eicosanoate (**6**)

White amorphous powder, ^1H (400 MHz) and ^{13}C (100 MHz) NMR data in in acetone-d_6, spectroscopy data, Table S4 [15]

Table 1. ^1H (500 MHz) and ^{13}C (125 MHz) data of compound **4** in CD_3OD compared to ^{13}C NMR (100 MHz) chemical shifts of compound **1** in CD_3OD.

Positions	**4**		**1**	**Position**	**4**	
	δ_C	δ_H **(multiplicity, *J* in Hz)**	δ_C		δ_H **(multiplicity, *J* in Hz)**	
2	81.0	4.75 (d, 7.5)	79.3	**Allyl-5**		
3	75.7	4.01 (td, 7.8, 5.5)	74.2	**1'''**	69.9	4.50 (m)
4	27.7	2.88 (dd, 16.5, 5.7)	26.0	**2'''**	134.8	6.04 (m)
		2.72 (dd, 16.4, 8.1)		**3'''**	117.4	5.39 (m); 5.23(m)
5	158.9	/	156.2	**Allyl-7**		
6	94.3	6.15 (d, 2.3)	95.1	**1''''**	69.8	4.47 (m)
7	160.2	/	156.5	**2''''**	134.8	6.04 (m)
8	95.5	6.10 (d, 2.3)	94.1	**3''''**	117.2	5.39 (m); 5.23 (m)
9	156.4	/	155.2	**Allyl-3' and 5'**		
10	103.2	/	99.1	**1'''''**	70.9	4.55 (m)
1'	136.0	/	135.0	**2'''''**	134.8	6.04 (m)
2'	107.3	6.70 (s)	105.7	**3'''''**	117.8	5.39 (m); 5.23 (m)
3'	153.5	/	150.2			
4'	139.9	/	135.2			
5'	153.5	/	150.2			
6'	107.3	6.70 (s)	105.7			
OCH_3-4'		3.81 (s)	59.5			
Rha						
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			

Note: d = doublet, dd = doublet of doublets, s = singlet, m = multiplet, $\boldsymbol{\delta_H}$ = chemical shift of proton, δ_C = chemical shift of carbon 13.

2.8. Antibacterial assay

2.8.1. Bacterial isolates, strains, and culture media

The antibacterial effect of the samples was tested on four bacterial species as follows:

Staphylococcus aureus MRSA3 (clinical isolate, resistant to Ofxar, Kan^r, Tet^r and Erm^r) [16] and *Staphylococcus aureus* MRSA6 (clinical isolate, resistant to Kan^r, Tet^r, Cyp^r, Chl^r, Amp^r) [16,17];

Providencia stuartii NEA16 (clinical isolate that overexpresses efflux pumps) [18] and *Providencia stuartii* PS2636 (isolate overexpressing efflux pumps associated to porines) collected from the Laboratoire UMR-MD1 de l'Université de la Méditerranée, Marseille, France;

Pseudomonas aeruginosa PA01 (isolate) [18] and *Pseudomonas aeruginosa* PA124 (multiresistant clinical isolate) [19];

Escherichia coli AG102 (isolate overexpressing efflux pumps as resistant mechanism) [20] and *Escherichia coli* ATCC10536 (strain) [18];

Where:

Chl^r: resistant to chloramphenicol; Erm^r: resistant to erythromycin; Kan^r: resistant to kanamycin; Tet^r: resistant to tetracycline; Amp^r: resistant to ampicillin; $Ofxa^r$: resistant to ofloxacin; and Cyp^r: resistant to cyprofloxacin.

Mueller Hinton broth (MHB) and Mueller Hinton agar (MHA) were used for the bacterial culture. The bacteria were conserved on an agar slant at 4 °C. Prior to any antibacterial test, each colony was collected and once again cultured freshly on an agar plate for 24 h. The MHB was used to determine the MIC and MBC.

2.8.2. Preparation of mother solutions of samples

Mother samples were prepared at a concentration of 4096 µg/mL by dissolving 5.0 mg of crude extract in 100 µL of DMSO and then completed with 1.121 mL of culture medium to get 1.221 mL of solution. Mother solutions of isolated products were prepared at a concentration of 1024 µg/mL by dissolution of 1.0 mg of the sample. Each of these mother solutions was then diluted to get several concentrations: 1024, 512, 256, 128, 64, 32, 16, and 8 µg/mL.

2.8.3. Determination of minimal inhibitory concentration (MIC)

The inhibitory potential of microbial growth of extracts and isolated compounds was determined by the microdilution method as reported by Mativandlela et al. (2006) [21]. In each well of a 96-well microplate, 100 µL of broth was introduced. Then, 100 µL of extract or isolated compound solution was added to the first well line in triplicate. The negative control was the culture medium in solution with DMSO. After incubation, 40 µL of aqueous solution of paraiodonitrotetrazolium chloride (INT) 0.2% was added to the wells. This chemical is colorless in an oxidized form; when bacteria produce NADH during growth, it reduces INT and turns it pink.

The MIC is the lowest concentration of a compound that is able to inhibit 99.99% of microbial growth; the MBC refers to the lowest concentration at which no growth is obtained. The experiment was repeated three times, and the results were the average of the three trials.

3. Results

3.1. Structure elucidation and identification of secondary metabolites

The investigation of chemical constituents of the *n*-butanol soluble fraction resulting from the ethanol extraction of *O. subscorpioidea* stem barks led to the isolation and characterization of five flavonoids and one monoglyceride. These compounds included olasubscorpioside A (**1**) [12], 4′-*O*-methylgallocatechin (**2**), olasubscorpioside C (**3**) [14], olasubscorpioside B (**5a**), isoolasubscorpioside B (**5b**) [12], and glyceryl-1-eicosanoate (**6**) [15]. The allylation of olasubscorpioside A (**1**) led to the formation of tetra-*O*-allyl olasubscorpioside A (**4**) (**Figure 1**), and the hydrolysis of the same compound **1** led to 4′-*O*-methylgallocatechin (**2**), which was equally isolated from the plant extract [22].

The semisynthetic product **4** was obtained as a greenish gum that was soluble in MeOH. It had a positive result on the Molisch test, indicating that the carbohydrate moiety was not affected by the allylation reaction. Its FAB-MS positive mode **(Figure S1)** displayed the protonated molecular ion $[M+H]^+$ at m/z 627 corresponding to the molecular formula $C_{34}H_{42}O_{11}$, with 14 degrees of unsaturation. However, the mass difference between the substrate (**1**) (m/z 466) [12] and the allylated product (**4**) was 160 a.m.u, corresponding to four allyl units, a possible reaction mechanism of allylation is proposed in **Figure 2**.

The ^{1}H NMR spectrum **(Figure S2)** showed signals of four allyl units at δ_H 4.47 (2H, m, H-1‴), 4.50 (2H, m, H-1⁗), and 4.55 (4H, m, H-1⁗′), as well as those of the olefinic methines between δ_H 5.98 and 6.08 (4H, m, H-2‴/2⁗/2⁗′) and resonances of exomethylene protons at δ_H 5.23 (4H, m, H-3‴/3⁗) and 5.40 (4H, m, H-3⁗′), indicating the allylation of four hydroxyl groups. It also displayed the signals of flavan-3-ol skeleton with the characteristic signals at δ_H 4.75 (2H, d, 7.5, H-2), 4.01 (1H, td, 7.8, 5.5, H-3), 2.88 (1H, dd, 16.5, 5.7, H-4a), 2.72 (1H, dd, 16.4, 8.1, H-4b), 6.15 (1H, d, 2.3, H-6), 6.10 (1H, d, 2.3, H-8), and 6.70 (2H, s, H-2'/6'), as well as the signals of α-L-rhamnopyranoside moiety at δ_H 4.36 (1H, d, 1.3, H-1"), 3.52 (1H, m, H-2"), 3.56 (1H, m, H-3"), 3.31 (1H, m, H-4"), 3.63 (1H, m, H-5"), and 1.23 (3H, d, 6.3) [12]. The ^{13}C NMR spectrum **(Figure S3)** showed the signals of four oxymethylene allyl units at δ_C 69.8 (C-1‴), 69.9 (C-1⁗), and 2 × 70.9 (C-1⁗′), which were attributable to carbons at C-1 of each allyl unit. The HSQC spectrum (**Figure S4**) assisted in establishing direct correlations from protons to carbons. The NOESY spectrum (**Figure S5**) suggested the fixation of allyl groups to the substrate through the correlations between the protons at δ_H 4.50 (H-1‴) and 6.15 (H-6), δ_H 4.47 (H-1⁗) and 6.10 (H-8), and δ_H 4.55 (H-1⁗′) and 6.70 (H-1’/6’). The connectivity of each allyl unit to the substrate was confirmed by HMBC correlations of the signals at δ_H 4.55 (H-1⁗′) and 6.70 (H-2') to the carbons at δ_C 153.5 (C-3'/5'), the ones from the resonances at δ_H 4.50 (H-1‴) and 6.15 (H-6) to the carbon at δ_C 158.9 (C-5), and finally, the HMBC connectivities from the protons at δ_H 4.47 (H-1⁗) and 6.10 (H-8) to the carbon at δ_C 160.2 (C-7) (**Figure S6**). The chemical shifts are presented in **Table 1,** together with those of the substrate (**1**).

Thus, the structure of **4** was established as (2*R*,3*S*) 5,7,3',5'-tetra-*O*-allyl-4'-*O*-methylgallocatechin 3-*O*-*α*-L-rhamnopyranoside **(4),** semi-synthesized and characterized in this work for the first time, and trivially named tetra-*O*-allylolasubscorpioside (**4**).

Compound **2** was obtained as a beige amorphous solid, soluble in MeOH. It gave a negative result to the Molisch test, thus suggesting that the carbohydrate moiety was removed during the reaction. By comparing its ^{1}H NMR and ^{13}C NMR data with reported data, it was identified as 4'-*O*-methylgallocatechin, previously isolated from *Panda oleosa* [13]. The structures of compounds **1**–**4**

are presented in **Figure 1**. The NMR data of **2**, **3, 5,** and **6** are presented in Tables S2–4 (Supplementary data).

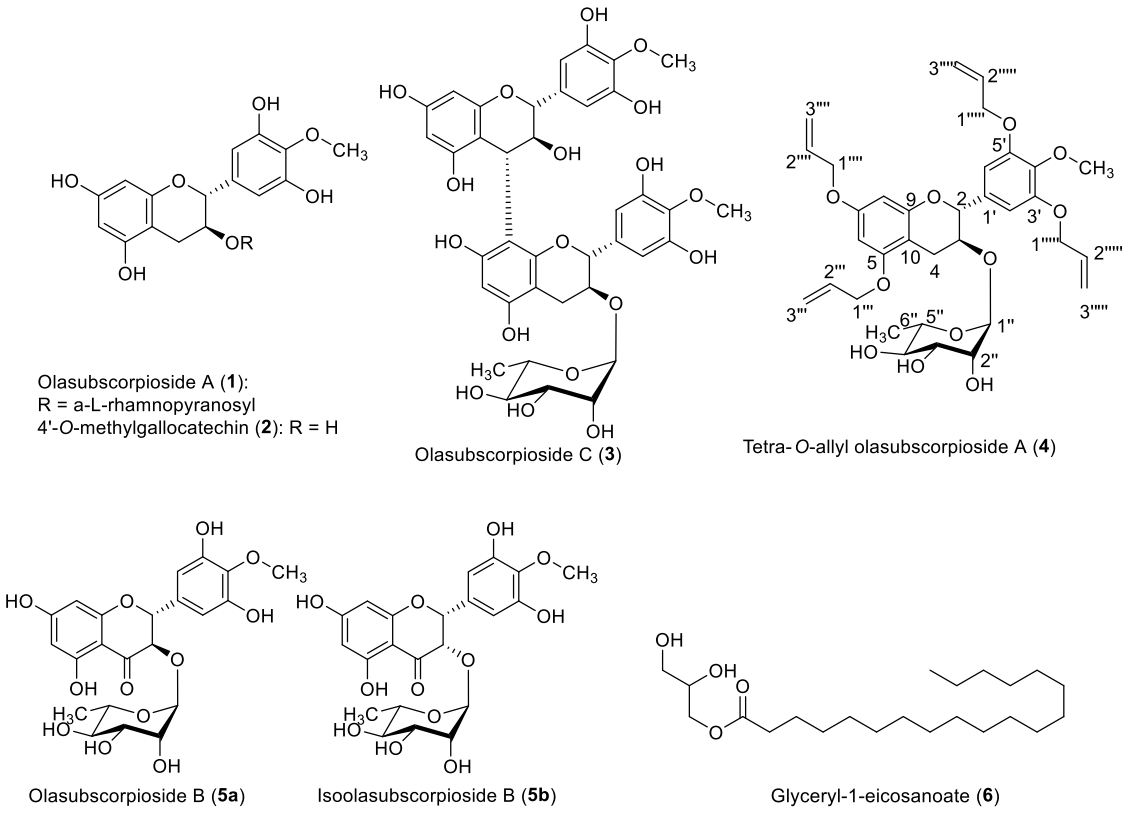

Figure 1. Structures of compounds **1–6**.

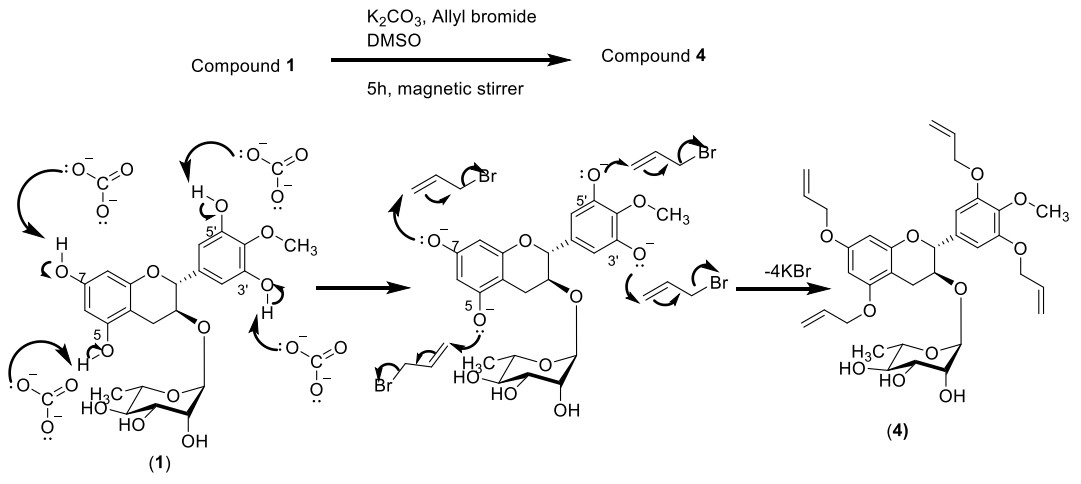

Figure 2. Possible mechanism of the allylation reaction.

3.2. Antibacterial activity

The ethanol extract of *O. subscorpioidea* displayed a moderate antibacterial effect against the isolates of *Staphylococcus aureus*, *Providencia stuartii* PS2636, and *Pseudomonas aeruginosa* PA01 (256 < MIC < 512 µg/mL). Conversely, this antibacterial activity was promising, with individual compounds suggesting an antagonist effect of the constituent in the extract. According to the scale of interpretation of antibacterial activity of isolated compounds [23], olasubscorpioside A (**1**) and 4'-*O*-methylgallocatechin (**2**) displayed a significant antibacterial activity against methicillin-resistant *Staphylococcus aureus* MRSA3 (isolate) with an MIC value of 8 µg/mL (17.2 µM for **1** and 25.0 µM for **2**) (**Table 2**).

Olasubscorpioside A (**1**) equally showed a moderate antibacterial activity against methicillin-resistant *S. aureus* MRSA6 (isolate), *Providencia stuartii* PS2636 (isolate), *Pseudomonas aeruginosa* PA01 (isolate), and *Escherichia coli* ATCC 10356 with MICs of 16, 16, 64, and 32 µg/mL (38.6, 38.6, 137.3, and 68.7 µM, respectively). Hydrolysis enhanced the activity against some bacteria on which olasubscorpioside A (**1**) had barely inhibited growth. This included the multiresistant *P. aeruginosa* (isolate) and *E. coli* (ATCC10356) (strain), suggesting the importance of OH-3 in the antibacterial activity of 4'-*O*-methylgallocatechin (**2**) against multiresistant *P. aeruginosa* and *E. coli*.

Olasubscorpioside C (**3**) showed moderate antibacterial activity against methicillin-resistant *S. aureus* (isolate), *P. stuartii* NEA16 (isolate), and *P. aeruginosa* PA01 (isolate), with MICs ranging from 16 to 64 µg/mL (20.4–81.6 µM). This compound was more active than olasubscorpioside A (**1**) and 4'-*O*-methylgallocatechin (**2**) individually on the clinical isolate of *P. aeruginosa* PA124. The activity of compound **1** against the multiresistant *Pseudomonas aeruginosa* PA124 (isolate) and *Escherichia coli* AG102 (isolate) was improved by the allyl groups; in fact, compound **4** showed a MIC of 128 µg/mL (204.5 µM) relative to the MIC > 256 µg/mL (549.4 µM) in compound **1** in *P. aeruginosa* and 64 µg/mL (102.25 µM) relative to 128 µg/mL (274.7 µM) in *E. coli*.

The increased activity of compound **4** against some selected isolates compared to the starting substrate **1** is not surprising. Indeed, lipophilicity is a determinant parameter for the metabolism of a drug candidate through the membrane of a microbe. Non-polar chemical functions such as allyl or prenyl moieties have been demonstrated to improve the access and attraction or inhibit RAS transformation [24–26]. However, the resulting activities were moderate and/or weak, but this could be considered an enhancement of the antibacterial effect of some ingredients against certain bacteria. When an MBC is less than or equal to four-fold the MIC, the antibacterial agent is considered bactericidal; otherwise, the molecule is said to be bacteriostatic [27]. Compounds **1–4** showed a bactericidal effect on the two isolates of *S. aureus,* except for compound **2,** which had a bacteriostatic effect on methicillin-resistant *S. aureus* MRSA6. Finally, compounds **1** and **2** showed a bacteriostatic effect on *P. stuartii* PS2636, *E. coli* ATCC 10356, and *P. aeruginosa* PA01.

Table 2. Antibacterial activity of isolated and semisynthetic compounds of *O. subscorpioidea* stem bark.

Samples		Cr EE	1	2	3	4	Chl
Sa 1	MIC	256	8	8	16	16	64
	MBC	512	32	32	64	32	64
Sa 2	MIC	512	16	16	32	16	128
	MBC	1024	32	128	128	64	256
Ps 1	MIC	1024	128	128	32	128	256
	MBC	-	-	-	128	-	-
Ps 2	MIC	256	16	16	256	32	128
	MBC	-	256	128	-	-	256
Pa 1	MIC	>1024	>256	32	128	128	64
	MBC	-	-	-	-	-	256
Pa 2	MIC	512	64	32	64	128	32
	MBC	-	-	256	128	-	128
Ec 1	MIC	1024	32	>256	128	>256	256
	MBC	>1024	128	-	-	-	-
Ec 2	MIC	>1024	128	32	>256	64	128
	MBC	-	-	-	-	-	256

Note: MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration) in µg/mL; 1: olasubscorpioside A; 2: 4′-*O*-methylgallocatechin; 3: olasubscorpioside C; 4: tetra-*O*-allyl olasubscorpioside A; *Sa* 1: Methicillin-resistant *Staphylococcus aureus* MRSA3; (isolate) *Sa* 2: Methicillin-resistant *Staphylococcus aureus* MRSA6 (isolate); *Ps* 1: *Providencia stuartii* NEA16 (isolate); *Ps* 2: *Providencia stuartii* PS2636 (isolate); *Pa* 1: Multi-resistant *Pseudomonas aeruginosa* PA124 (isolate); *Pa* 2: *Pseudomonas aeuriginosa* PA01 (isolate); *Ec* 1*: Escherischia coli* ATCC 10356 (strain); *Ec* 2: *Escherischia coli* AG102 (isolate); Cr EE: Crude ethanol extract; Chl: Chloramphenicol; - : not determined

4. Discussion

Under the reserve of the action mechanism studies, these results suggest that allylation and glycosylation may decrease the antibacterial activity in some bacteria and increase it in other bacteria. The resistance mechanism of the bacteria used in this assay has been reported in previous studies [16–20]. More precisely, one report in 2022 evidenced the efflux pumps associated with the mechanism of resistance of *P. stuartii* and *E. coli* [28]. 4'-*O*-methylgallocatechin (**2**) and 5,7,3',5'-tetra-*O*-allyl-4'-*O*-methylgallocatechin 3-*O*-*α*-L-rhamnopyranoside (**4**), due to their low polarity, could be more resistant to efflux rejection by *Escherichia coli* (isolate) than **1** and **3**; as such, the mechanism of antibacterial activity of flavonoids may largely depend on the specific bacterium. In general, this mechanism of action includes the deactivation of nucleic synthesis, cytoplasmic membrane function, and energy sources [29]. The hydroxyl groups at 5, 7, and 3' contribute to the antibacterial activity of flavonoids [30]; glycosylation affects the bacterial DNA [29]. The antimicrobial activity of certain polyphenolic compounds is thought to be associated with their ability to precipitate proteins within the bacterial cytoplasm. This has been found in several phenolic acids and flavonoids, which mainly act by perforating the cell membrane and/or making the membrane more viscous, which leads to the

destruction of the cell membrane [31]. More specifically, quercetin penetrates inside the bacterial cell and inhibits the synthesis of nucleic acids and proteins and energy production by mitochondrial cells in fungi [32]. Therefore, it could be possible that the polar compounds **1, 2**, and **3** disrupted the peptidoglycan layer in *S. aureus,* whereas their interactions with the lipopolysaccharide layer of Gram-negative bacteria were poor. This is supported by the bactericidal effect of these compounds (**1**−**4**) and the crude extract on *S. aureus*.

5. Conclusions

The chemical investigation of the *n*-butanol soluble fraction of *O. subscorpioidea* led to the isolation of antibacterial secondary metabolites against several clinical bacterial isolates. Higher activity was obtained with compounds **1** and **2** against *S. aureus*. More importantly, allylation enhanced the activity of compound **1** against the multiresistant species *P. aeruginosa* and *E. coli*, which overexpress efflux pumps as their resistance mechanism. The crude ethanol extract showed moderate-to-weak activity with these bacteria (250 < MIC < 1024 μg/mL). Therefore, this study exposes some of the chemical constituents that could be responsible for the use of the stem bark of *Olax subscorpioidea* in traditional medicine to fight microbial infections. The cytotoxicity of these compounds was not evaluated in the study; as such, this aspect should be considered in future investigations to fully support the contribution of these results in the fight against AMR. Further research on individual bioactive compounds is strongly encouraged. It is recommended to assess the antibacterial activity of each compound against both the same and different bacterial strains, as resistance mechanisms may vary. Additionally, evaluating the Absorption Distribution Metabolism Excretion and Toxicity (ADMET) properties of these compounds through *silico* methods will help to predict their metabolic behavior and pharmacokinetic profiles.

Author contributions

B.T. performed the chemical studies, elucidated the structures of chemical compounds, conceptualized and wrote the manuscript; V.M carried out the biological assays and wrote the manuscript; B.T.T, S.G.M, and X.S.N. did the spectroscopic analysis, elucidated the structures of chemical compounds, and prepared the manuscript, R.B.T. and B.K.P. supervised the chemical studies and prepared the manuscript; X.S.N., V.K.,L.A.T., and R.B.T. supervised and reviewed the manuscript. All authors have revised and provided their agreement to the published version of the manuscript.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflicts of interest

The authors declare no conflicts of interest in this paper.

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