Original Article

Microbial Biosystems 9(1) (2024) 2024. 1102
10.21608/MB.2024.290495.1102

Contents lists available at Egyptian Knowledge Bank
Microbial Biosystems
Journal homepage: http://mb.journals.ekb.eg/

Synthesis, characterization and antibacterial properties of some halogenated glucose derivatives

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ARTICLE INFO

Article history
Received 17 May 2024
Received revised 3 June 2024
Accepted 7 June 2024
Available online 19 June 2024

Corresponding Editors:
Al-Abed, HF.
Abdel-Azeem, MA.

Keywords :
Antibacterial agents,
antimicrobial activity,
carbohydrates,
Escherichia coli,
Staphylococcus aureus.

ABSTRACT

We synthesised a series of glucose derivatives containing different halogens and studied their biological activity on two species of pathogenic bacteria. The synthesis requires multiple steps, starting with methyl-α-D-glucopyranoside, which reacts with benzaldehyde dimethyl acetal to protect the hydroxyls at 4- and 6-carbons to obtain compound **2**. We treated compound **2** with bis(2-chloroethyl) ether under the Williamson etherification condition, in the presence of phase transfer catalysts, to produce compound **3**. We treated compound **3** with sodium iodide under Flenksteien reaction conditions to obtain derivative **4**. We characterized all compounds using spectroscopic instruments. Finally, we studied the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. It is evident that the chlorine and iodine atoms significantly contribute to the antibacterial activity against the bacteria. After penetration, the sugar derivatives inside the bacterial cells degrade, releasing free chlorine or iodine ions that kill the bacteria.

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Introduction

Carbohydrates are poly-hydroxyl alcohol that contain at least one carbonyl residue, in their structures. The general formula of these compounds are represented by $C_n(H,O)_n$ (Su et al. 2021). Carbohydrates are the major source of energy in mammals (Wang et al. 2024) .They considered as the largest biomolecules beside lipid, protein and nucleic acid that found in the living cells that play significant roles(Aghdam et al. 2018) .Their chiral centers elect them to be an excellent biologically active materials towards microbes such as viruses, fungi and bacteria (Dubbu et al. 2018) .With some limitation. However, the interest to synthesis newly sugar derivatives are increasing when new protection deprotection strategies were developed to overcome the chemoselectivity that accompanied these type of transformations(Ágoston et al. 2015). In this context, these derivatives have to pass the principles of medicines that include the right identifications, prevention and treatments of certain diseases upon study (Maciej Studzian et al. 2021).The simplest form of carbohydrates are the monosaccharides like glucose. The simplicity make them easy to modify in comparison with disaccharides or others(Lim &Pullicin 2019).Therefore, several materials have been produced based on a structure-activity relationship in active sites of cells and a drugs functionality, which rise understanding of how they are developed as effective therapeutic agents (Song & Zheng 2017). Indeed, the halogenated derivatives of many of monosaccharides are showed reasonable biological activities (Jani 2019). Towards bacteria, especially, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*). These species are the most harmful microbiota (Jumina et al. 2019) .That cause a lot of infections to almost all cells of organs in the human (Pavesi et al. 2018; Regina & Silva 2021; Pokharel et al. 2023; Jopp et al. 2023). Hence, our attention has been driven to investigate two types of halogens derivatives, i.e. chlorine and iodine, to study the effects of each derivative upon attached in the same positions on sugar molecules.

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We choose the glucose as a starting building block due to its low cost, availability, and simple modification methodologies. Furthermore, the biological effects of these derivatives on two bacterial taxa (*S. aureus* and *E. coli*) have been performed.

Materials and Methods

All chemicals and solvents are purchased and used without further purifications. The methyl α-D-glucopyronside, benzaldehyde dimethyl acetal, bis-(2-chloroethyl) ether, sodium bicarbonate and sodium hydroxide, tetra butyl ammonium bromide, sodium iodide, sodium sulfate anhydrous and sulphonic acid monohydrate, acetone, chloroform, ethanol, ethyl acetate, N,N-Dimethyl formamide, n-hexane and toluene are obtained from Sigma Aldrich as analytical grades. ^{1}H and ^{13}C NMR spectra were recorded on Bruker advance NEO at 400 MHz and 100 MHz respectively using $CDC1_3$, using TMS as internal standard. FT-IR spectra were recorded by alpha-Broker (Germany). Anti-bacterial study was estimated by agar well diffusion method according to(Ahmad & Hawaiz 2024).

Synthesis of Methyl-4,6-O-benzylidene- α-D-glucopyronside (2)

The synthesis was followed standard procedures with some modification where, Methyl -α-D-glucopyranoside ***1*** (30 g, 154.6 mmol) was dissolved in 100 ml of DMF and (30ml,30.4g, 199 mmol) benzaldehyde dimethyl acetal was added. The reaction was promoted by an acid catalyst of sulphonic acid monohydrate (0.1g). The reaction was heated under vacuum using a rotary evaporator. The progress of reaction was monitored by thin layer chromatograph (TLC) which indicated that most starting materials are consumed after 4 hours. The DMF was omitted under vacuum, and the remaining semi soled was extracted in chloroform/ saturated sodium bicarbonate solution to ensure removing of trace of acid. The chloroform was dried by Na_2SO_4, filtered a removed by rotary evaporator (Isolab, Germany). The pure material have been obtained after chromatography (27.6g:63.3%) (Sabah & Hashim 2013).

Synthesis of methyl-4,6-O-benzylidene-2,3-bis[(2-chloroethoxy) ethyl]-α-D-glucopyranoside (3)

Compound ***2*** (30 g, 106.038 mmol) was dissolved in bis-(2-chloroethyl) ether (250 mL), then (30 g, 93 mmol) TBAB and 50% NaOH solution (50 mL of 50% w/v) were added. The mixture was stirred at room temperature for 72 hours. The reaction was monitor by TLC which indicated there is no starting sugar derivative left, the excess of solvent was evaporated under vacuum. The residue was dissolved in chloroform and extracted extensively with water. The organic layer was dried by Na_2SO_4, filtered and the solvent evaporated under vacuum. The pure material have been obtained after column chromatography to get compound ***3*** (37.8g: 71.80%)(Bakó & Töke 1995).

FTIR data (cm^{-1}): 3459.5, 3351.5(OH groups).3061.8, 3034.9 (**H**-aromatic), 2911.5, 2867.0 (**H**-aliphatic), 2000.0-1600,0(overtone bands),1454.2, 1371.6 (**C=C** aromatic),1298.2 (**CH_2-Cl**).

^{1}H -NMR (400 MHz, $CDCl_3$) δ 3.45–3.46 (m, **1H**), 3.46 – 3.47 (m, **1H**), 3.48 – 3.49 (m, **1H**), 3.49 – 3.54 (m, **1H**), 3.56 – 3.59 (m, **4H**), 3.62 – 3.79 (m, **8H**), 3.80 – 3.84 (m,**4H**), 3.86 – 4.09 (m, **5H**), 4.38 (dd, J = 10.5, 5.0 Hz, **1H**), 4.87 (d, J = 7.7 Hz, **1H**), 5.57 s, **1H**), 7.28 (tdd, J = 4.7, 3.5, 1.5 Hz, **3H**), 7.39 – 7.49 (m,**2H**). ^{13}C- NMR (101 MHz, $CDCl_3$) δ 39.71, 39.99, 55.42, 62.38, 69.01, 69.79, ,71.24, 76.71, 77.03, 77.23, 77.34, 77.89, 80.44, 81.42, 98.31, 101.95, 126.17, 128.44,129.40, 136.99.

Synthesis of methyl-4,6-O-benzylidene-2,3-bis[(2-iodoethoxy) ethyl]-α-D-glucopyranoside (4)

Compound ***3*** (75 g, 151 mmol) was dissolved in ethanol (250 mL), then NaI (63 g, 420.3 mmol) was added, and the solution was refluxed for 30 hours. The solid was filtered and then the solution was concentrated.

The residue was dissolved in ethyl acetate, filtered and then washed with water. The organic phase was dried over sodium sulfate and evaporated in vacuum. The pure material have been obtained after chromatography to get compound ***4*** (94.5 g,: 92%)(Pham et al. 2017).

FTIR data (cm^{-1}): 3063, 3036 (**H**-aromatic), 2911.6, 2865.1 (**H**-aliphatic), 1453 (**C=C** aromatic) ,1185.4 (**CH2-I**).

^{1}HNMR (400 MHz, $CDCl_3$) δ 7.28–7.42 (m, 5H, **Ph**) , 5.45 (d, J = 0.9Hz, 1H, Ph**CH** benzylidene) , 4.41 (m , 1H, **H-1**), 4.39 (dd, J = 11.4, 1.6 Hz, 1H, **H-6**), 4.27 (m, 1H, **H-3**) , 4.26 (m, 1H, **H-4**), 4.23 (dd, J= 11.5, 4.4 Hz, 1H, **H-6**), 3.85–3.96 (m, 17H , H-2 , 6 O**CH_2**, 2 **CH_2**I) , 3.62-3.66 (m, 1H ,**H-5**), 3.42-3.55 (d, J = 1.5 Hz, 3H, O**CH_3**). ^{13}CNMR (125 MHz, CDCl3) δ 137.23, 126.03, 128.26, 129.06 (**Ph**), 103.78 (Ph**CH** benzylidene), 101.30 (**C-1**), 82.68 – 80.75 (**C-4**, **C-3**, **C-2**) , 72.45-66.11 (6 **C**H_2O ,**C-5**, **C-6**), 50.95(**C**H_3) , 4.85 , 4.82 (2**CH_2-I**).

Antibacterial activity

The antibacterial activities of compounds (B1 (***3***) and B2 (***4***) were tested using agar well diffusion methods against two types of bacteria (*E. coli* and *S. aureus*) isolated from burn infections were obtained from Al-Sadr Medical City, Najaf Alshraf, Iraq. Five pure isolated colonies of fresh

culture were suspended in five milliliters of brain heart infusion broth (BHIB) on agar plate's surface of Mueller Hinton agar (MHA) and incubated at 37°C for four to eight hours (Abdel-Moneim et al. 2022). The turbidity produced by growth culture was calibrated with sterile broth to get an optical density comparable to the 0.5 McFarland requirements. A sterile cotton swab was dipped into the suspension. The dipping cotton swab was used to streaking the entire surface. Four wells (7 mm diameter each) were filled with B1 and B2 (100 uL) in four concentrations (125, 250, 500 and 1000 µg/ml) and the fifth well filled with Ampicillin, as control. The Petri-dishes were then incubated at 37°C for 24 hours. The diameter of the growth inhibition zones in millimeters was measured by metric ruler to determine antimicrobial inhibition activity (Vahdati & Moghadam 2020).

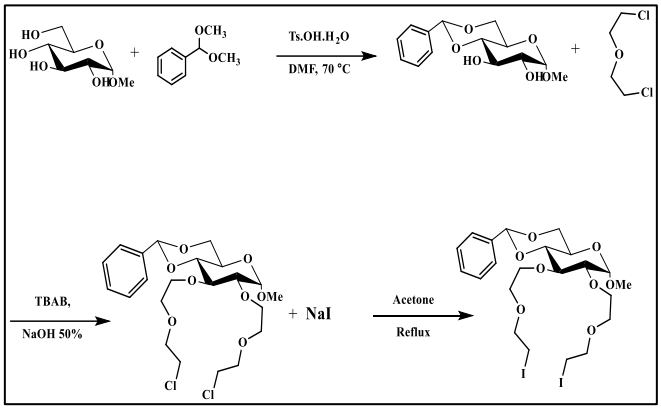
Ethical disclosures

In this research the procedures followed were following the regulations of the Clinical Research Ethics Committee and the Code of Ethics of the World Medical Association (Declaration of Helsinki) and declare that they have followed the protocols of their work center on the publication of patient data. The authors declare that they have not used any type of generative artificial intelligence for the writing of this paper.

Results and Discussion

*Synthesis of derivatives **3** and **4*** (Scheme 1)

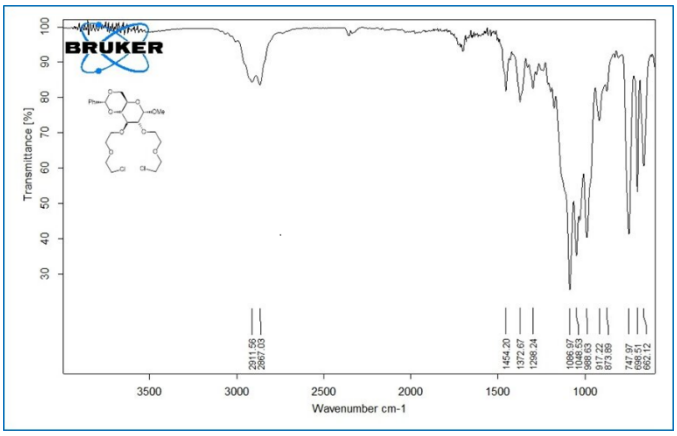
The 4 and 6 positions are easily to protect with benzylidene acetal due to its high stability and yield as well the easy work-up after reaction completing. The treatment of glycoside ***1*** with benzaldehyde dimethyl acetal catalyzed by H_2SO_4 in DMF was furnish the 4,6-O-benzylidene-α-D-glucopyranoside ***2*** in good yield. The next step is modifying the free hydroxyl groups at carbons 2- and 3- positions of compound ***2*** are ready to react via Williamson etherification reaction with bis-(2-chloroethyl) ether in basic conditions. Although, the ethers could be synthesized with sodium hydride in DMF or THF easily, we prefer to do the reaction under phase transfer catalysts for the following aspects. First, in this reaction must apply the bi(chloroethyl) ether in access mainly without any solvent needed so the sodium hydride couldn't apply. Seconds, the work-up the reaction is simple so the desired product could be obtained without any further purification, mainly column chromatography.



Scheme 1. Synthesis of derivatives ***3*** and ***4***

The FT-IR spectrum (Fig. 1) of compound ***3*** shows the disappearing of the stretching vibration bands in the range of (3450-3350 cm^{-1}) due to the absorption of stretching vibration of (OH- group at C-2 and C-3). In addition, the new signal in the region (747 cm^{-1}) which can be attributed to the stretching vibrations of (CH_2-Cl) bond.

Fig1. FTIR spectrum of compound ***3***



The ^{1}H-NMR spectrum of compound ***3*** indicated that few multiplets at the range of 3-4 ppm, which are arising from the protons of methylene two groups of (Cl-CH_2CH_2- CH_2CH_2) in the 2- and 3- carbons of the glucopyarnoside ring. The integration value of hydrogens is increasing by 16 in comparison with the integration of compound **3** (Fig. 2).

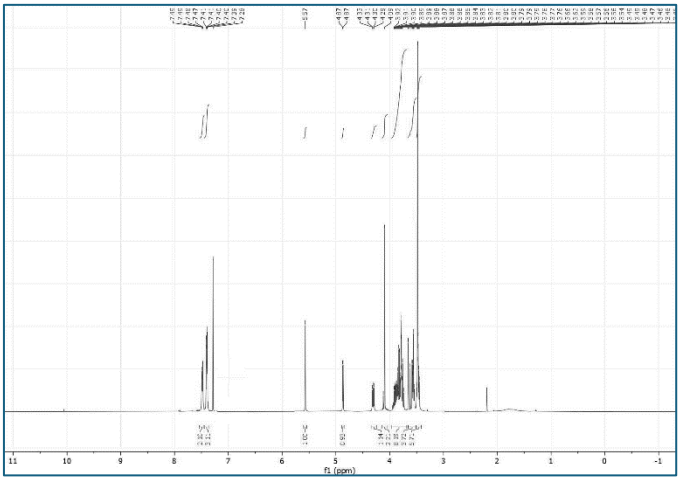


Fig 2. ^{1}HNMR spectrum of compound ***3***

The decoupled ^{13}CNMR spectrum of compound ***3*** shows some of new characteristic signals at the range of 72- 67 ppm which could be assigned to the carbons attached directly to the oxygen in the groups ($OCH_2CH_2OCH_2$-). In the same manner, the new two signals at 39.89 and 39.71 ppm are attributed to the carbons that attached directly to chlorine atoms (-CH_2Cl) as represented in (fig.3).

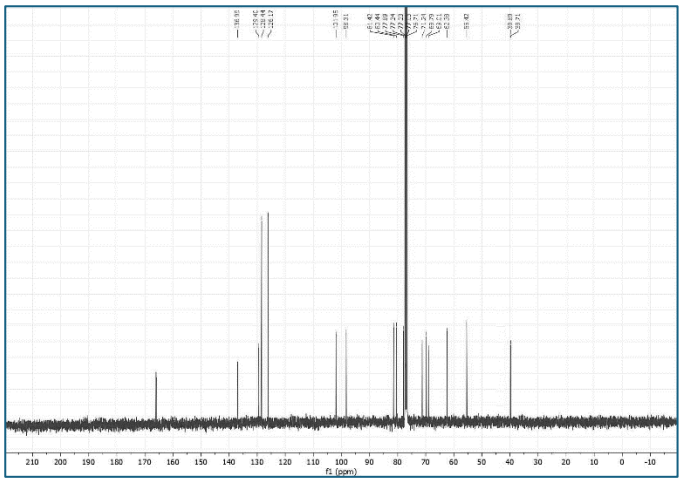


Fig 3. ^{13}CNMR spectrum of compound ***3***

To assigns all the protons of multiplets in the proton NMR spectrum, the assistant of two-dimensional NMR may be allowing the possibility to assign them. To do so, the HSQC-edited GP experiment (Fig.4), is implemented to assist the assignment of all carbons and protons as it correlation with attachment of each proton to its corresponding carbons. Furthermore, this experiment gives cross-spots with two different colors, one is for the (CH) and (CH_3) carbons and the other for (CH_2), while the quaternary carbon do not appear as spots.

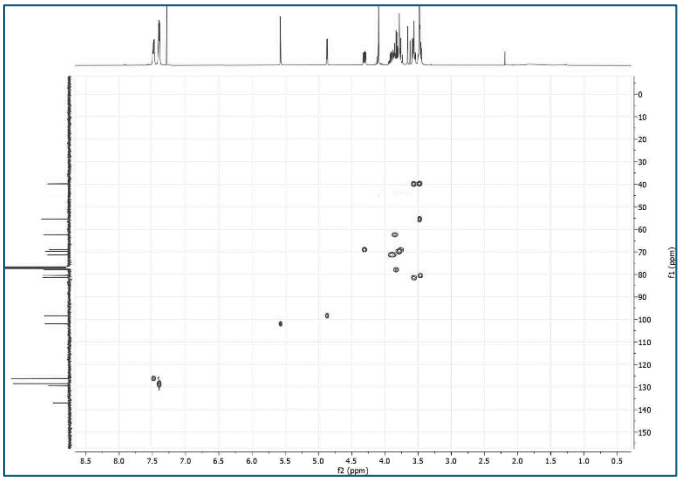


Fig 4. HSQC spectrum of compound ***3***

The conversion of compound ***3*** to the corresponding iodide analogue ***4*** can increase the electrophilicity of carbon atoms attached directly to the halide. Such reaction can be replaced the chlorine atom with more reactive iodide in simple reaction condition using acetone and sodium iodide. The sodium iodide will be dissolved with heating and the corresponding sodium chloride will be formed during the progress of reaction. The FT-IR spectrum of compound ***4*** (Fig.5), shows the absorption bands that characterize the product, in which the band at 617 cm^{1} can be attributed to the stretching vibration of the carbon-iodine bond.

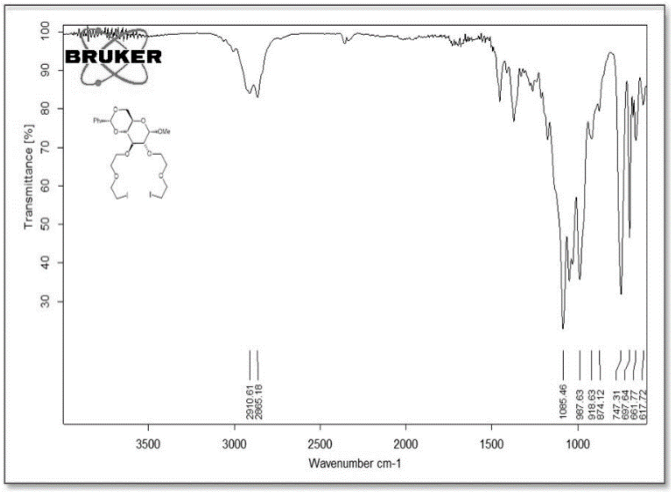


Fig 5. FTIR spectrum of compound ***4***

The ^{1}H-NMR spectrum of compound 4 showed there were signals in the ranges of (3.80–3.63) ppm for (OCH_2, CH_2I), 5.45 ppm (s, 1H) benzylidene proton and showed there was signals in the range of (7.42–7.32) ppm for Haromatic of benzylidene group (Fig.6).

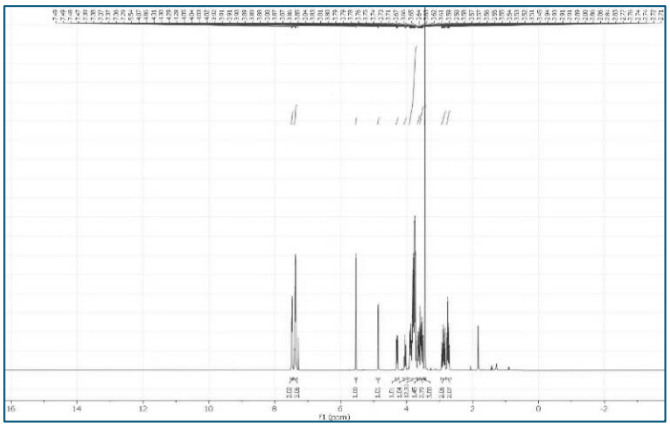


Fig 6. ^{1}H-NMR spectrum of compound ***4***

Besides, the ^{13}CNMR spectrum of compound ***4*** showed a new carbon signals at (72.44 - 66.11) ppm for (**C**H_2O), (4.84 & 4.81) ppm for (2 **C**H_2-I), (103.7) ppm for benzylidene carbon and (137.2, 129.02, 128.2, 126) ppm of C-aromatic for benzylidene group (Fig. 7).

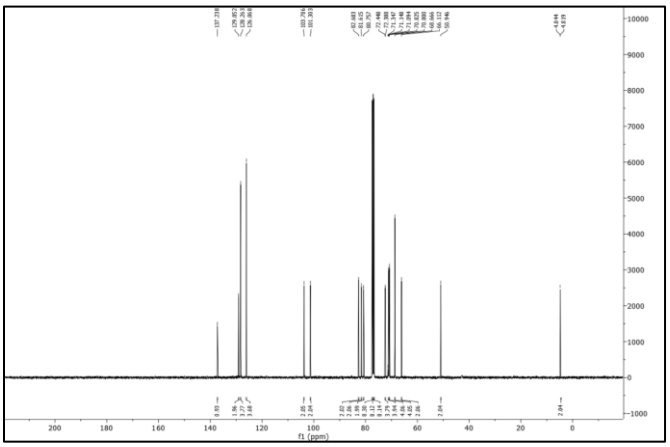


Fig7. ^{13}CNMR spectrum of compound ***4***

Anti-bacterial activities of synthesized compounds

The anti-bacterial study was performed on the *E. coli* and *S. aureus*, due to their spread in almost every environment. As a part of testing new materials to overcome the resistance to commercially available antibiotics, which is developed by the two bacteria strains. However, the new derivatives that only differ in the type of halogens on the same position gave promising candidate for overwhelmed the resistance towards other antibiotics. Indeed, the sugar moiety of the derivatives is compatible with bacterial components of carbohydrates which could be assisted the interaction with cell membrane and as a result can penetrated to the inside environments. From table 1 the derivatives (B1(***3***) and B2(***4***) have a broad-spectrum activity in that it inhibited growth of both Gram positive and Gram-negative bacteria when comparing with standard antibiotic. The inhibition zones increased with increasing the concentration of these compounds.

Indeed, the sugar moiety of the derivatives is compatible with bacterial components of carbohydrates which could be assisted the interaction with cell membrane and as a result can penetrated to the inside environments. From table 1 the derivatives (B1(***3***) and B2(***4***) have a broad-spectrum activity in that it inhibited growth of both Gram positive and Gram-negative bacteria when comparing with standard antibiotic (Figs 8 and 9). The inhibition zones increased with increasing the concentration of these compounds.

Table1. The inhibition zone (mm) by B1(*3*) and B2 (*4*) against bacteria.

Compound	Concentration (µg/ml)	Inhibition Zone (mm)	
		S. aureus	*E. coli*
(***3***)	125	0	0
(***3***)	250	0	9
(***3***)	500	12	13
(***3***)	1000	17	18
Ampicillin	1000	16	14
(***4***)	125	0	0
(***4***)	250	0	0
(***4***)	500	11	12
(***4***)	1000	16	18
Ampicillin	1000	15	13

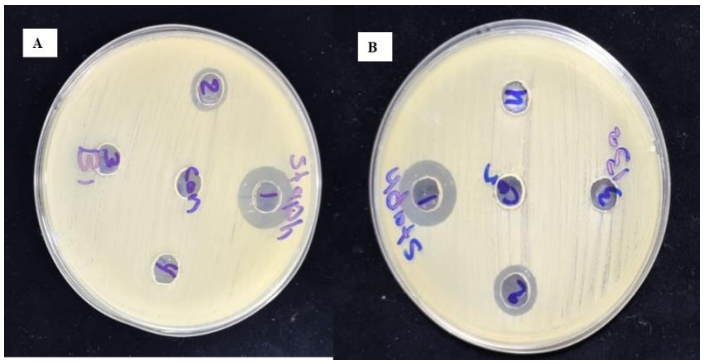


Fig 8. Effect of synthesized compounds on *S. aureus*, A: B1 (***3***) and B, B2 (***4***).

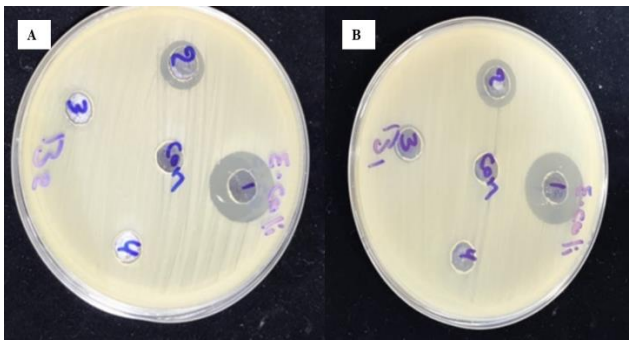


Fig 9. Effect of synthesized compounds on *E. coli*, A: B1 (***3***) and B, B2 (***4***).

Conclusion

In conclusion, the synthesis of derivatives on glucose with two halogen atoms have been achieved in highly yields. The simple strategy of protecting group enable the synthesis in specific positions, i.e. **2** and **3** on the pyranoside ring. The chloro-derivative ***3*** show a reasonable activity aginst. In general, the derivatives ***3*** and ***4*** have high ability to inhibiting the growth of bacteria compared with Ampicillin at same concentrations. Therefore, these products are promising to be gentle biological active materials due to the presence of glucose molecule inside their scaffold, so the toxicity and incompatibility can be omitted.

Acknowledgements

The author thanks the advisor Dr. Kareem Alabidi for his continuous guidance and advices during this work.

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