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SYNTHESIS OF OPEN-CHAIN SUGAR DERIVATIVES AS ANTICANCER AND ANTIMICROBIAL AGENTS

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ABSTRACT. In the present study, three sugar residues including mannose, galactose and lactose were modified with organic substituents via reductive amination reaction in order to get strong anticancer carbohydrate derivatives for A549 cell line and antibacterial agents targeting *E.coli* and *S.epidermidis*. The findings showed that carbohydrate residue along with substituted group play the major role for the observed activity of the carbohydrate ligands.

1. INTRODUCTION

Carbohydrates, the most common molecular group, are composed of simple sugars and complex polymeric structures [1]. Bioactive and bio-degradable sugars are of great interest in biochemistry due to their essential roles in molecular recognition [2]. The essential roles of carbohydrates are related to their interaction with lectins, which are proteins specific for carbohydrate recognition [3]. This is the reason behind the use carbohydrates as possible drugs and drug-precursors for different applications including carbohydrate-based antibiotic development [4]. For example, variety of monosaccharides and disaccharides have been developed and tested as antimicrobial and anticancer agents [5,6]. During the modification of carbohydrates, the groups attached to sugar residue bring tremendous difference for selectivity and sensitivity of the sugars for the lectins [7,8]. The observed sensitivity and selectivity between the sugars and lectins are similar to the outstanding selectivity observed for antigen-antibody interactions [4,9,10].

During cancer development, lectin composition of cell surface undergoes alteration; for example, new lectin groups can arise that result in the alteration of carbohydrate affinities towards the surface lectins. For instance, cancerous lung cells increase the number of galactose-binding lectins on their surfaces [11]. Similarly, mannose binding capacity of lung cells show increasing trend upon pathology development [12,13]. Particularly lactose-amine specific galectin-3 is increased during initial stages of lung cancer [14] while that takes place in metastasis of other cancer to lung [15]. Therefore, in this study mannose, galactose and lactose residues were used to develop carbohydrate ligands.

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2. MATERIAL AND METHOD

2.1. Materials

The following chemicals were purchased from Sigma-Aldrich (Ankara, Turkey), including Nutrient Broth, Nutrient Agar, NaCl, α-D-Mannose, α-D-Galactose, β-D-Lactose, 5-aminosalicylic acid, p-aminosalicylic acid, 4,4'-oxydianiline, 4-mercaptoanyline, acetic acid, borane dimethyl amine complex. 18.2 MΩ pure water was produced in our labs (Human Power 2 pure-water system).

2.2. Synthesis of Carbohydrate Derivatives

FIGURE 1: Synthesis of carbohydrate derivatives. R_1, R_2, R_3, R4 and R_4 can be –H, COOH, -OH and –SH while R group on the sugar residue was either –H or a monosaccharide.

Synthesis of sugar derivatives, as illustrated in Figure 1, were performed in 1:1 acetic acid/water mixture, for which purification was not needed to isolate imin from the media, where reducing agent boran dimethyl amine (DMA) was directly added to the mixture after fully completition of the imin formation [16]. Hydrophilic liquid interaction based (HILIC) approach was used to monitor reaction steps on thin layer chromatography.

2.3. Characterization of the carbohydrate-derivatives

^{1}H NMR and ESI-MS/MS based characterization techniques were used to characterize the synthesized sugar derivatives.

2.3.1 ^{1}H NMR Characterization

Each carbohydrate-derivative was dissolved in D_2O solvent at 5 mg/mL, followed by run in 400 MHz Jeol NMR. The results were analyzed in Delta NMR Software and Topsin 4.06 Software.

2.3.2. ESI-MS/MS Characterization

Molecular weight and fragmentation pattern of the sugar derivatives were characterized via Shimadzu LC-ESI-MS/MS-8030 Plus instruments.

2.4. Anticancer performance of the sugar ligands

Lung adenocarcinoma A549 cell line was used as target cell line while rat intestine epithelial non-cancerous immortalized IEC-6 cell line was used as control cell line. Cell culture studies for both cell lines were performed using Eagle modified medium (EMEM) supported with 4 mM L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 unit/ml bovine insulin, 10 % (v/v) fetal bovine serum and 4 mL/L of essential amino acids. The incubation was at 37 °C in 5% CO_2 incubator. Fresh 10^4 cells/mL in 100 µL were inoculated into 96-well plate, followed by 24 h incubation to provide cell attachment to the surface. Then after, sugar derivatives at 0.1 and 1 mM in 10 µL were added to the cells, where pure-water was used as the control. Cells were exposed to the carbohydrate derivatives for 24 h, followed by viability of the remaining cells were measured using Alamar-blue based fluorescent assay in a Microplate reader.

2.5. Antimicrobial performance of the sugar ligands

To characterize antibacterial activity of the carbohydrate derivatives, gram negative *Escherichia coli* and gram positive *S. epidermidis* were used as model organisms. Both cells were grown in Nutrient-Broth at 37 °C for overnight, followed by centrifugation at 3000 g. The collected cells were suspended in fresh Nutrient Broth. 1 mL samples of *E.coli* and *S.epidermidis* at 10^3 cfu/mL concentration were placed in 1.5 mL sterile polypropylene plastic tubes. Sugar derivatives at 1 mM in 50 µL were added to the bacteria samples to investigate the antimicrobial activity, where 16-h incubation at 37 °C was used as the minimum treatment period. After the incubation, turbidity of the medium was measured at 595 nm in PG Instruments T60 Spectrometer.

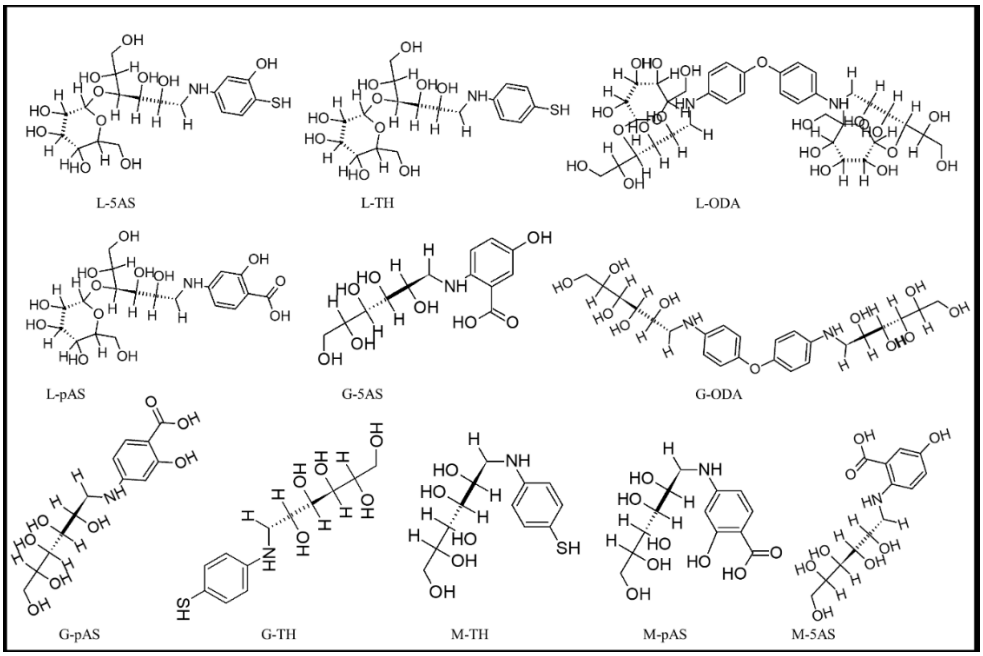

FIGURE 2: The carbohydrate derivatives synthesized in the study. Abbreviations: L-5AS: Lactose-5-aminosalicylic acid; L-pAS: Lactose-4-aminosalicylic acid; L-TH: Lactose-4-mercaptoaniline; L-ODA: Lactose-4,4'-oksidianiline; G-pAS: Galactose-4-aminosalicylic acid; G-5AS: Galactose-5-aminosalicylic acid; G-TH: Galactose-4-mercaptoaniline; G-ODA: Galactose-4,4'-oksidianiline; M-pAS: Mannose-4-aminosalicylic acid; M-TH: Mannose-4-mercaptoaniline; M-5AS: Mannose-5-aminosalicylic acid.

3. RESULTS AND DISCUSSION

3.1 Synthesis and Characterization of the Sugar Derivatives

The straight forward synthesis of open chain sugar derivatives allows cheap, fast and high yield production of novel sugar derivatives that can possess unique properties, which advance sensitivity and selectivity of the inherent carbohydrate molecule towards the target lectin [17]. Purification of the carbohydrate products was performed via liquid-liquid separation technique thanks to the elevated hydrophilicity of carbohydrate derivatives that does not allow them to be dissolved in hydrophobic solvents including acetone, tetrahydrofuran and relatively less hydrophilic ethanol. During the reaction, organic

substituents were used 20 % more than that of used for sugar residue in order to increase the effectiveness of the liquid-liquid extraction. All of the synthesized sugar derivatives are shown in Figure 2. Purified products were then characterized with ^{1}H NMR and ESI-MS/MS, where only the results belong to characteristic carbohydrate derivatives are given with original spectrums.

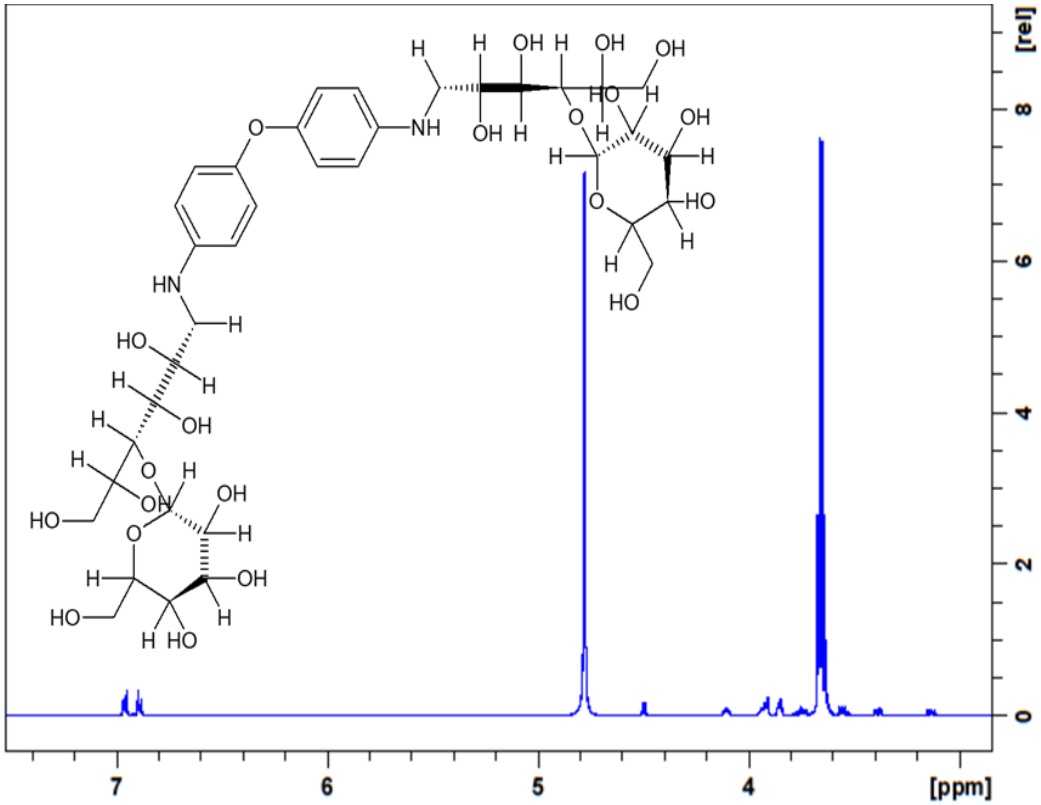

FIGURE 3a: ^{1}H NMR spectrum of Lactose-4,4'-oxydianiline.

ODA bound to lactose residue has only two characteristic microenvironment, so there are only two peaks at aromatic region (6.90 and 6.97 ppm) (Figure 3a). The lactose residue lost its shift at ~ 5.2 ppm related to α/β conversion of C_1-H, which revealed that the synthesis and purification was completed.

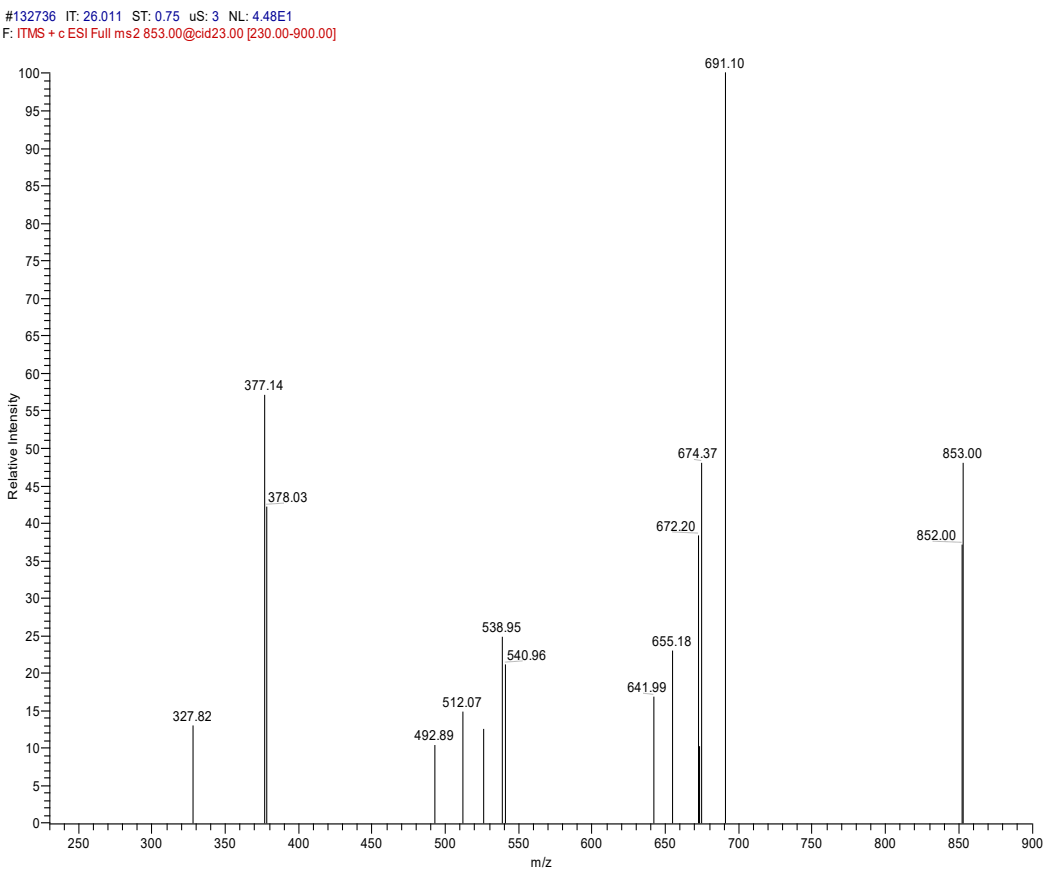

FIGURE 3b: MS/MS ms^2 pattern of Lactose-4,4'-oxydianiline.

As seen in Figure 3b, L-ODA gave 853 m/z; L-ODA originally has 848 Da, and +1 comes from positive EIS mode while the +4 comes from protonation of two amin groups within the molecule. The fragmentation pattern gave 691 m/z that resulted from loss of one deoxy-glucose from lactose while 674 m/z and 655 m/z values were resulted from loss of water molecules of the sugar residue.

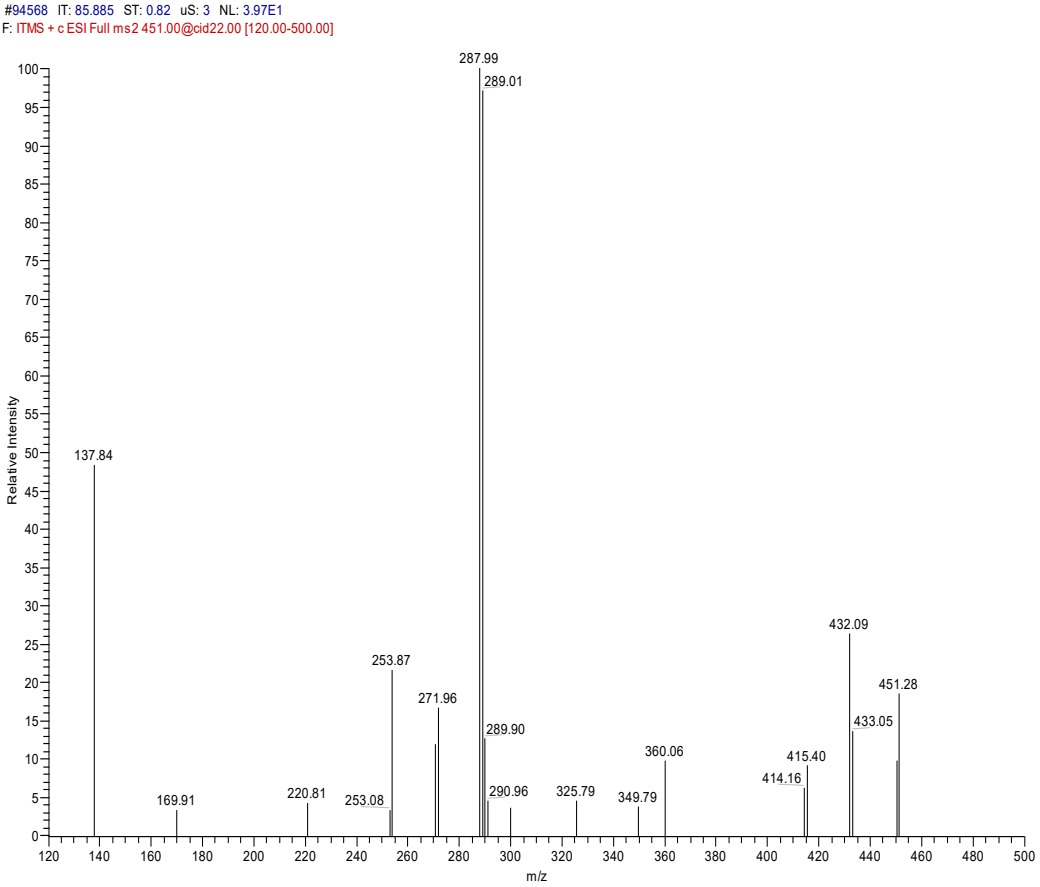

FIGURE 3c: MS/MS ms^2 pattern of Lactose-*p*-mercaptoanilin (L-TH).

As seen in Figure 3c, L-TH gave 451 m/z; L-TH originally has 449 Da, and +1 comes from positive EIS mode while the +1 comes from protonation of amin groups within the molecule. Amine groups found on different sugar resides tend to gain different proton because of the geometry of the molecule. 433 and 415 m/z values were resulted sequential elimination of water molecules from sugar residues of the L-TH. As observed for L-ODA, glucose residue of lactose within L-TH molecule ditched during fragmentation and 290 m/z value arose.

3.2. Anticancer Activity

Either anticancer activity of carbohydrate-based drugs is resulted from carbohydrate-lectin interaction, which can intervene signaling cascades responsible in gene expression controlling expression of such genes including apoptosis related genes, or the carbohydrates can interact with enzymes. Besides, a more complex mechanism can determine the carbohydrate mediated intervention of cellular mechanisms. As shown in Figure 4a, the carbohydrate derivatives showed dose-dependent toxicity on A549 cancerous cell line. Sugar residues substituted with same organic groups revealed dramatic toxicity differences, where the sugar residue played key role for the observed anticancer activity. For example, in the case of 4-mercaptoanilin modification, mannose derivative gave by far the most toxic activity for A549 cells while L-TH showed nearly no toxicity. Besides, G-TH derivative at 1 mM concentration gave lower toxicity than 0.1 mM of M-TH. Similarly, 4-aminosalicylic modified mannose revealed relatively higher toxicity than that of observed for lactose and galactose derivatives. Another important observation was that 5-aminosalicylic acid modified derivative of galactose gave higher toxicity than its 4-aminosalicyclic acid modified version, which situation was opposite for mannose and lactose derivatives. Therefore, it can be speculated that alteration in the position of functional group located on the substituent shows its potency depending on the sugar residue.

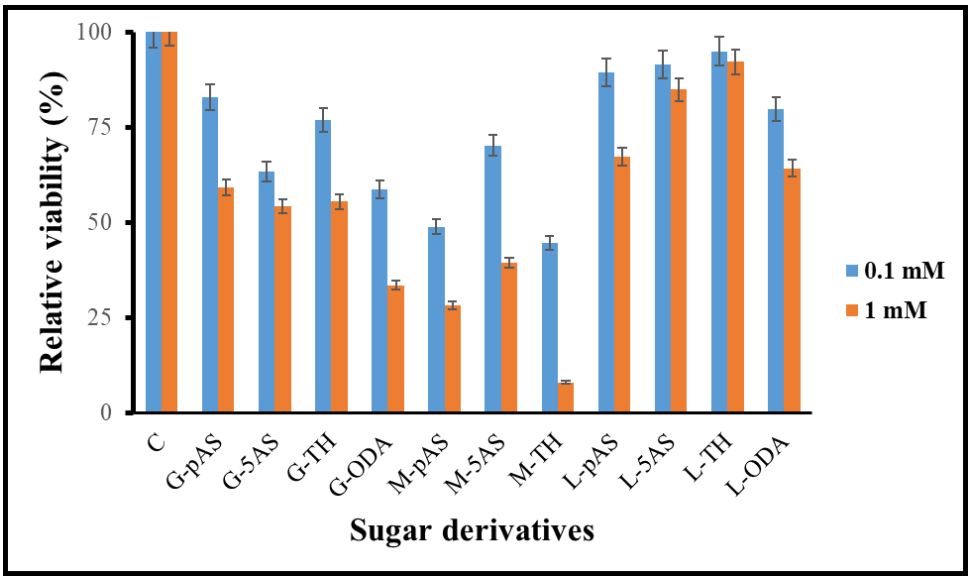

FIGURE 4a: Cytotoxicity of the carbohydrates on A549 cell line at 0.1 and 1 mM concentration.

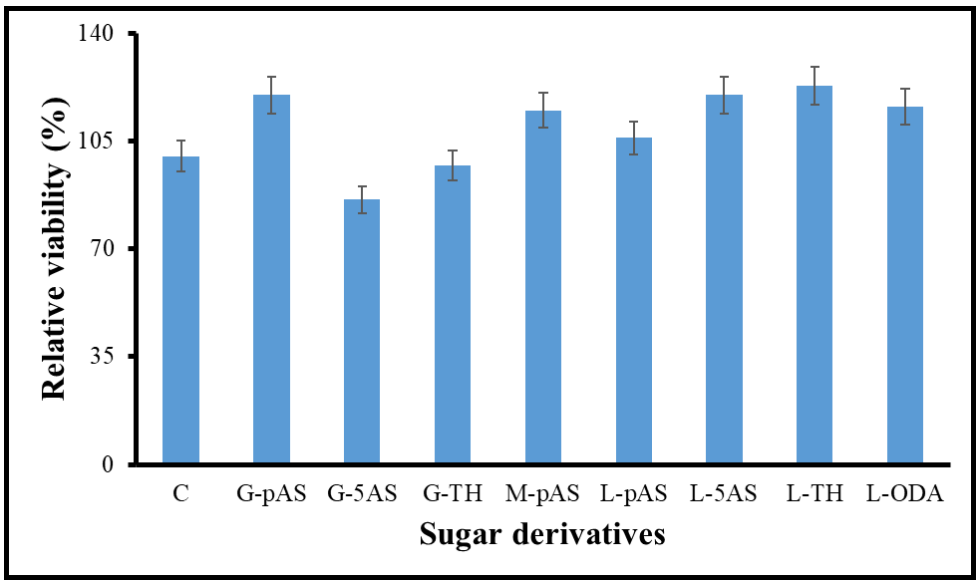

FIGURE 4b: Cytotoxicity of the carbohydrates on IEC-6 cell line at 1 mM concentration.

The sugar derivatives were then applied to non-cancerous IEC-6 cells to show their selectivity for the cancerous cell line. As seen Figure 4b, only G-5AS showed discernible toxicity (14%) while most of the derivatives behaved as either nutrient or signaling agent to enhance cellular growth in comparison to the control.

In carbohydrate-based drug development studies, the carbohydrates are modified in order to improve the interaction between the sugars and the carbohydrate-recognition domain of the lectin along with facilitating CRD's surrounding involvement to the recognition [3,9]. Another important phenomenon is that homo- and/or hetero-disaccharides in comparison to their monosaccharide versions can show similar or even higher affinities for the same lectin [17]. Therefore, the obtained differences can result from the differences in CRD regions along with its surroundings.

3.3. Antibacterial Results

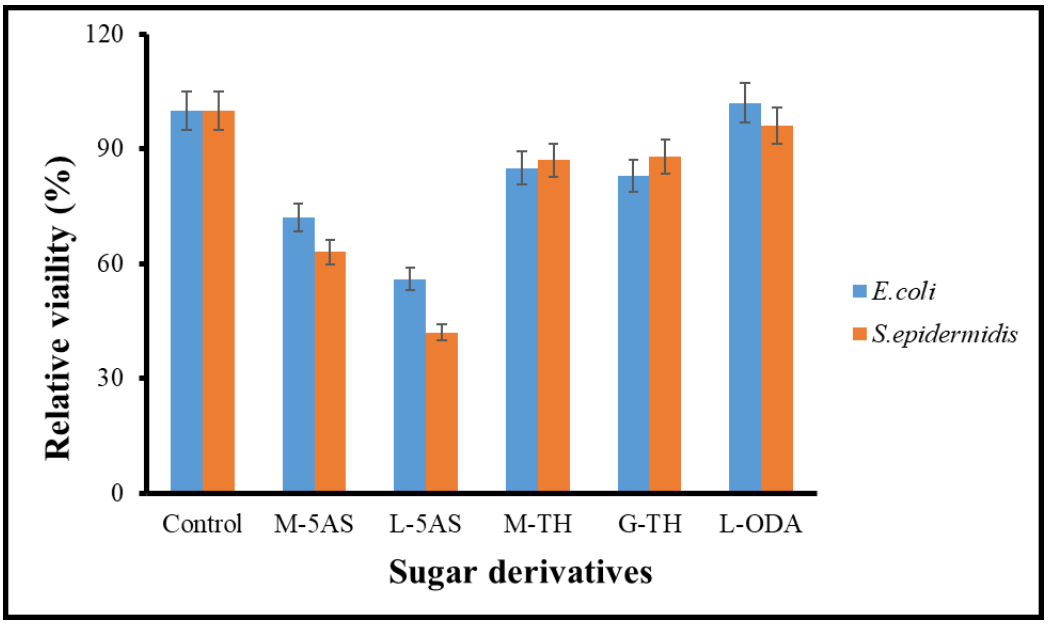

FIGURE 5: Antibacterial activity of selected carbohydrate derivatives.

As seen in Figure 5, L-5AS showed the highest toxicity towards *E.coli* and *S.epidermidis* while L-ODA did not show any toxicity for the tested bacteria. When the antibacterial capability of the sugar derivatices was compared with the anticancer capabilities, the carbohydrates can provide a good candidates as anticancer agents.

4. CONCLUSION

Carbohydrates play key roles in regulation of cell metabolism and cell-cell interaction that make them unique molecules, whose regulations strictly controlled by genes. Their modifications introduce novel properties for their sensitivity and selectivity towards the target mammalian and microbial cells. Therefore, in this study, mannose, galactose and lactose were modified with a variety of organic groups to advance the sensitivity towards cancerous A549 cell line and gram negative E.coli and gram positive S.epidermidis. The findings revealed that substituted organic group brought anticancer and antibacterial activity along with sugar residue selection. The findings can call new research on to understand how all these modified carbohydrates showed their unprecedented capabilities.

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