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Synthesis and Evaluation of *in vitro* Antibacterial Properties of Secoisolariciresinol Diglucoside

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JR, SK and SM designed the study, wrote the protocol and supervised the work. Author SM carried out all laboratories work, performed the statistical analysis, managed the analyses of the study and wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

In the present study, Secoisolariciresinol diglucoside (SDG) was synthesized from commercially available 3,4-dimethoxytoluene, butanediol and penta acetyl glucose. This includes bromination, alkylation, glycosylation, deacetylation and demethylation reactions to yield the interested title compound (SDG), further the assignment of the chemical structure of the synthetic SDG was performed with IR, MS-TOF, ^{1}H and ^{13}C NMR. The antibacterial property of the synthetic SDG was investigated against seven different Gram positive and Gram negative bacteria. The results of the present study proved that SDG can be obtained with good yield (56.2%) under this scheme. Moreover, it exerts an exceptional antibacterial activity where it showed maximum zone of inhibition with 30 and 27.3 mm against *E. coli* and minimum 7 and 6.1 mm against *S. pyogens* and *S. typhimurium* in agar well and disk diffusion respectively. It also showed maximum activity (18 mm at MIC 200 ppm) against *S. typhimurium*, while minimum inhibitory activity was 4 mm with

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MIC at 300 ppm against *S. pyogens*. The scheme of synthesis was found to be an efficient method and the synthetic SDG was also proved to possess a broad spectrum of activity against a panel of bacteria responsible for causing most common diseases.

Keywords: Synthetic secoisolariciresinol diglucoside; IR; MS-TOF; ^{1}H and ^{13}C NMR and anti-bacterial activity.

1. INTRODUCTION

With the development and clinical use of antibiotics in the nineteenth century, there has been a drastic reduction in the incidences of bacterial infections diseases, which were a leading cause of human mortality world-wide. But, there has been an alarming increase in bacterial resistance to the present chemotherapeutic agents largely due to their injudicious use and this has turned out to be an issue of global concern [1,2]. Emergence of multidrug-resistant pathogens threatened the clinical efficacy of many existing antibiotics [3], leading the scientists to increasingly turn their attention to finding out new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for the new infectious diseases [4]. Phytoestrogens have been found *via* several *in vivo* and *in vitro* studies to exert a potent biological activity besides their major estrogenic and anti-estrogenic activities due to their structural similarity with estrogens [5,6]. Lignans are second major class of phytoestrogens that have been proved to exert antioxidant, anti-inflammatory, anticancer, anti-diabetic and many other biological properties. Isoflavones and coumestans are the other classes of phytoestrogens [7]. Lignans are natural poly phenolic compounds which have long been recognized as challenging targets for organic synthesis due to their complex and diverse chemical structure as well as their important pharmacological properties. Epidemiological studies have reported the chemo preventive effects of lignans on tumors of colon, skin and mammary glands [8]. Lignans also exert antibacterial and cytotoxic activities [9]. Lignans also showed antimicrobial activity against several important pathogenic bacteria with different minimum inhibitory concentrations for each microorganism [10]. Additionally, there are not any reports on side effects and toxicity on lignan usage [11]. Flaxseed, the richest source of SDG, has gained importance in food industry as a component in designer food, functional food and in value added products because of its high content of lignans, which exert nutraceutical and therapeutic properties [12]. SDG from different cultivars of Indian flax seed have been found to exert potential anti-bacterial activity against microbial pathogens [13]. It is also found to possess potential properties such as both *in vivo* and *in vitro* antioxidant activities [14], anti-diabetic type I and II [15], anti-cancer effect on the breast [16], prostate [17], and anti-inflammatory agents [18].

It is believed that isolation of SDG from natural sources is a very laborious, sensitive and time consuming process. Additionally, isolation methods involve use of chemicals and reagents which may cause deleterious effects on animals including humans [19]. Apart from this, the yield of the product is less on isolation, purity will not be 100% and bioavailability of this SDG is low in animals [20]. Moreover, isolated and commercially available SDG is quite expensive. Lignan synthesis relies on limited number of integral reactions that are required for the construction of the basic 18-carbon skeleton whose modification (e.g. cyclization, reduction, oxidation and hydration) can generate the entire set of lignan compounds. Despite their attractive and proven biological properties, only few studies have approached the chemical synthesis of lignans, for instance alphahydroxylated lactone lignans [21], benzopyran [22], erythronordihydroguaiaretic acid [23] and olive type lignans [24]. Therefore, the objective of the present investigation was to design, synthesize and corroborate the antibacterial activity of SDG, a principal lignan of flaxseed against a panel of Gram positive and Gram negative bacteria responsible for causing the most prevalent infectious diseases.

2. MATERIALS AND METHODS

The chemicals used were purchased from Sigma Aldrich, USA and the general solvents used in the experiments were of analytical grade. Melting points were determined by using open capillary method with KOFLER Microscope and are uncorrected, IR spectra were obtained in (cm^{-1}) JASCO FT-IR spectrometer using KBr pellet technique. ^{1}H and ^{13}CNMR spectra were recorded (in ppm) on BRUKER AV 400MHz

spectrometer using deuterated methanol as solvent and TMS as internal standard. Mass Spectroscopy analysis was carried out by ESI-TOF MS. Elemental analysis was performed on a Perkin Elmer 2400 Elemental Analyser to confirm the structure of the synthetic compounds. Flash column chromatography was generally performed on silica gel (60 - 200 mesh) for purification of the compounds with the solvent mixture of chloroform/hexane. Precoated alumina with silica gel 60 F_{254} (E. Merck) plates were used for monitoring of reactions and purity determination. Spots were detected by UV (254 nm).

2.1 Chemistry

Synthesis of SDG was reported in this researcher's earlier study wherein the yield was less from the adopted scheme [25]. Therefore, herein a new scheme was designed for the synthesis of SDG.The chemical synthesis of SDG was accomplished through a novel five-step synthesis sequence (Fig. 1) starting from the bromination of the commercially available compound 3,4-dimethoxytoluene (**1**) with N-bromosuccinimide (NBS) in the presence of carbon tetrachloride to achieve 1,2-dimethoxy-4-bromomethylbenzene (**2**). 2,3-bis(3,4-dimethoxybenzyl) butane-1,4-diol (**3**) was afforded by stirring compound **2** with 1,4-butanediol in the presence of n-butyl lithium in 15 mL of DMF. Condensation of compound **3** with 2,3,4,6-tetra-*O*-acetyl-alpha-D-glucopyranosyl bromide in presence of catalytic amount of 2N hydrochloric acid and ethanol as solvent gave 2,3- bis (3,4-dimethoxybenzyl)butane-1,4-*O*-tetra acetyl glucopyranoside (**4**). Compound, 2,3-bis(3,4-dimethoxybenzyl)butane-1,4-*O*-glucose(**5**) was obtained in good yield by deacetylation of compound **4** with sodium hydroxide in presence of ethanol. Finally, the synthesis of the title compound (**6**), i.e. secoisolariciresinol diglucoside (SDG) was attained *via*regioselective partial demethylation of compound **5** using stannous chloride in presence of dichloromethane and 2N hydrochloric acid.

2.1.1 Synthesis of 1,2-dimethoxy-4-bromomethylbenzene (2)

The 3,4-dimethoxytoluene (**1**) was selectively brominated according to the regioselective method of Hong et al. [26]. Using NBS as brominating agent. NBS (5.83 g, 32.80 mmol) was added to a stirred solution of 3,4-dimethoxytoluene (5.00 g, 32.80 mmol) in 70 mL of CCl_4 and the reaction mixture was refluxed for 11 h. The reaction mixture was cooled; succinimide was filtered off and washed with CCl_4. The solvent was evaporated under reduced pressure to yield the crude product (**2**). Recrystallization from the mixture of hexane and diethyl ether (1:1) afforded white needles of compound **2**.

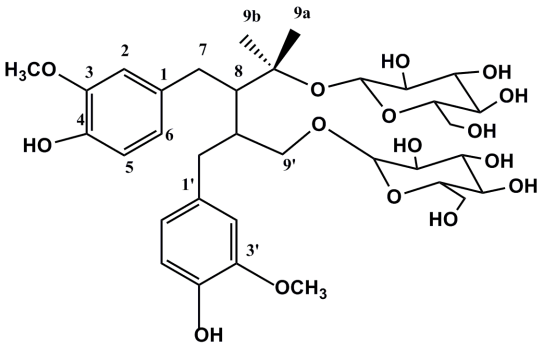


Fig. 1. Chemical structure of SDG
Chemical structure of SDG skeleton represents the position of the carbon and hydrogen atoms

Yield: 5.054 g (66.6%), mp 50.0 - 52.1℃. ^{1}H-NMR (CD_3OD) δ 6.90 (H, s, C_2-H $_{arom}$), 6.76 (H, d, J= 8.1 Hz, C_5-H $_{arom}$), 6.68 (H, d, J= 7.8 Hz, C_6-H $_{arom}$), 4.54 (2H, s, CH_2), 3.38 (6H, s, 2-OCH_3). ^{13}C-NMR (CD_3OD) 150.1 (C4), 150 (C3), 131(C1), 122.1 (C6), 114 (C2), 112.5 (C5), 33.6 (C7). MS: m/z 231.0874(M + 1). $C_9H_{11}BrO_2$ (231.09) Anal. Cal. C, 46.78; H, 4.80. Found: C, 46.72; H, 4.78.

2.1.2 Synthesis of 2,3-bis (3,4-dimethoxybenzyl)butane-1,4-diol (3)

The alkylation of compound **2** was carried out based on the modified procedure of Garonski and Garonska [25]. One equivalent of compound **2**(0.97 g, 10.67 mmol) was added to butanediol (5.00 g, 21.60 mmol) and n-butyl lithium (2.20 g, 16.00 mmol) in 15 mL of DMF. The mixture was stirred at (- 60℃ to 05℃) for 3 h and then the reaction mixture was stirred at 20℃ for 10 h. The reaction mixture was cooled to room temperature, extracted with hexane (3 × 30 mL), and the solvent was evaporated to yield compound **3** (Oil).

Yield: 3.50 g (83.8%), IR (nujol, cm^{-1}): 3430, 3320 (OH). ^{1}H NMR (CD_3OD) δ 6.83 (2H, d, J= 8.2 Hz, $C_{5,5'}$- H $_{arom}$), 6.78 (2H, s, $C_{2,2'}$ -H $_{arom}$), 6.70 (2H, dd, J= 1.9, 8.0 Hz, $C_{6,6'}$-H $_{arom}$), 3.85 (12H, s, 4-OCH_3), 3.64 (2H, dd, J= 7.6, 7.8 Hz, 2

-OH), 3.62 (2H, dd, J= 5.6, 9.5 Hz, $C_{9a,9a'}$-H *aliph*), 3.37 (2H, dd, J= 6.6, 9.8 Hz, $C_{9b,9b'}$-H *aliph*), 2.62 (2H, dd, J= 8.1, 13.6 Hz, $C_{7a,7a'}$-H *aliph*), 2.40 (2H, dd, J= 6.9, 13.7 Hz, $C_{7b,7b'}$-H *aliph*), 1.92 (2H, m, $C_{8,8'}$-H *aliph*). ^{13}C NMR (CD_3OD) 149.2 (C3,3'), 147 (C4,4'), 132 (C1,1'), 122.4 (C6,6'), 113.4 (C2,2'), 112.6 (C5,5'), 65.6 (C9,9'), 56.2 (C-OCH_3),42.8 (C8,8'), 35.3 (C7,7'). MS: m/z 390.4721(M+ 1). $C_{22}H_{30}O_6$ (390.47) Anal. Cal. C, 67.67; H, 7.74. Found: C, 67.51; H, 7.66.

2.1.3 Synthesis of 2,3-bis (3,4 -dimethoxybenzyl)butane-1,4-*O*-tetra acetyl glucose (4)

Compound **3** (3.50 g, 9.00 mmol) was added to a solution of 2,3,4,6 tetra-*O*-acetyl α-D glucopyranosyl bromide (7.12 g, 18.00 mmol) in 10 mL ethanol. The reaction mixture was stirred and refluxed for 19 h. After the completion of the reaction monitored by TLC, the reaction mixture was kept overnight to achieve crystalline product. Finally, the crystalline product was extracted with ether (3 × 30 mL), and the ether layer was passed through anhydrous sodium sulphate. The ether was evaporated and the product was recrystallized from the mixture of hexane and diethyl ether (1:1) to give compound **4** as white crystalline solid.

Yield: 5.07 g (52.9%), mp 65 - 66.2℃, IR (nujol, cm^{-1}): 1710 (C=O for COO). ^{1}H NMR (CD_3OD) δ 6.83 (2H, d, J= 8.0 Hz, $C_{5,5'}$-H *arom*), 6.78 (2H, s, $C_{2,2'}$-H *arom*), 6.74 (2H, dd, J= 1.8,8.1Hz, $C_{6,6'}$-H *arom*), 3.80 (12H, s, 4-OCH_3), 3.65 (2H, d, J= 7.1 Hz, $C_{9,9'}$-β-*O*-Gluanomeric), 3.62 (2H, dd, J= 5.4, 10 Hz, $C_{9a,9a'}$- H *aliph*), 3.38 (2H, dd, J= 6.5,10 Hz, $C_{9b,9b'}$-H *aliph*), 2.20 - 4.52 (38H, $C_{9,9'}$-*O*-Glu), 2.60 (2H, dd, J= 8.6,13.8 Hz, $C_{7a,7a'}$-H *aliph*), 2.41 (2H, dd, J= 8.2,13.4 Hz, $C_{7b,7b'}$-H *aliph*), 2.18 (2H, m, $C_{8,8'}$-H *aliph*).^{13}C NMR (CD_3OD) 149.2 (C3,3'), 147 (C4,4'), 132 (C1,1'), 122.6 (C6,6'), 113.2 (C2,2'), 112.6 (C5,5'), 72.7 (C9,9'), 59.8 (C9,9'-O-Glu anomeric), 56.1 (C-OCH3), 39.7 (C7,7'), 37.8 (C8,8'), 20.8 - 170.0 (C9,9'-O-Glu).MS: m/z 1051.0498(M + 1).$C_{50}H_{66}O_{24}$ (1051.04) Anal. Cal. C, 57.14; H, 6.33. Found: C, 56.42; H, 6.65.

2.1.4 Synthesis of 2,3-bis (3,4-dimethoxybenzyl) butane-1,4 -*O*-glucose (5)

Deacetylation of compound **4** (5.00 g, 4.76 mmol) was achieved by adding 0.75 g NaOH in 10 mL water and 15 mL ethanol. The reaction mixture was stirred and refluxed on water bath for 30 min. The reaction mixture was cooled to room temperature and the crude product was extracted with diethyl ether (3 × 30 mL), and the ether layer was passed through anhydrous sodium sulphate. The solvent was evaporated to yield compound **5** as a pasty mass.

Yield: 2.01 g (59.23%), IR (nujol, cm^{-1}): 3200, 3400 (OH). ^{1}H NMR (CD_3OD) δ 6.80 (2H, d, J= 8.2 Hz, $C_{5,5'}$-H *arom*), 6.78 (2H, s, $C_{2,2'}$-H *arom*), 6.76 (2H, dd, J= 2,8.0 Hz, $C_{6,6'}$-H *arom*), 4.2 (2H, d, J= 7.1, $C_{9,9'}$-β-*O*Gluanomeric), 3.83 (12H, S, 4-OCH_3), 3-3.8 (22H, m, $C_{9,9'}$-*O*-Glu), 3.44 (2H, dd, J= 5.6, Hz, $C_{9a,9a'}$-H *aliph*), 3.20 (2H, dd, J= 6.5, 10 Hz, $C_{9b,9b'}$-H *aliph*), 2.64 (2H, dd, J= 8.4, 13.8 Hz, $C_{7a,7a'}$-H *aliph*), 2.38 (2H, dd, J= 8.4, 13.6 Hz, $C_{7b,7b'}$-H *aliph*), 2.18 (2H, m, $C_{8,8'}$-H *aliph*).^{13}C NMR (CD_3OD) 149.1 (C3,3'), 147 (C4,4'), 132.4 (C1,1'), 122.6 (C6,6'), 113.3 (C2,2'), 112.4 (C5,5'), 104 (C9,9'-O-Glu anomeric), 71.8 (C9,9'), 64 - 79.5 (C9,9'-O-Glu), 56.2 (C-OCH3), 40.6 (C8,8'), 39.8 (C7,7'),. MS: m/z 714.7548(M+ 1). $C_{34}H_{50}O_{16}$ (714.75) Anal. Cal. C, 57.13; H, 7.05. Found: C, 58.50; H, 7.86.

2.1.5 Synthesis of secoisolariciresinol diglucoside (6)

Partial demethylation of compound **5** was carried out according to the regioselective method of Arvind et al. [27]. With minor modification in the reagent quantities. To a solution of compound **5** (1.90 g, 2.66 mmol) in 20 mL of dichloromethane, stannous chloride (1.00 g, 5.27 mmol) was added. The reaction mixture was stirred for 3 h at 40℃. Then 10 mL of 2 N HCl was added to the reaction mixture and stirring was continued for 10 min to obtain the crude demethylated compound **6**. The reaction mixture was washed with water (3 × 25 mL), the organic layer was dried over anhydrous sodium sulphate, and the solid product was chromatographed using silica gel column and hexane/chloroform (1:1) as eluent to obtain yellow crystal of title compound **6** with chemical structure represented in Scheme 1.

Yield: 1.30 g (56.2%) mp 119 - 121℃ (literature m.p118 - 120℃). IR (nujol, cm^{-1}): 3150, 3450 (OH). ^{1}H-NMR (CD_3OD) δ 6.6 (2H, s, $C_{2,2'}$-H *arom*), 6.64 (2H, d, J= 8.1 Hz, $C_{5,5'}$-H *arom*), 6.54 (2H, dd, J= 2, 8.1 Hz, $C_{6,6'}$-H *arom*), 4.22 (2H, d, J= 7.8 Hz, $C_{9,9'}$ –β-*O*Gluanomeric), 4.1 (2H, dd, J= 5.6, 10 Hz, $C_{9a,9a'}$-H *aliph*), 3.73 (6H, s, 2-OCH_3), 3.48 (2H, dd, J= 6.5, 9.9 Hz, $C_{9b,9b'}$-H *aliph*), 3.12 - 3.94 (22H, m, $C_{9,9'}$-*O*-Glu) 2.7 (2H, dd, J= 7, 13.8 Hz, $C_{7b,7b'}$-H *aliph*), 2.62 (2H, dd, J= 8.2, 13.8 Hz, $C_{7a,7a'}$-H *aliph*), 2.10 (2H, m, $C_{8,8'}$-H *aliph*).^{13}C-NMR (CD_3OD) δ 148.8 (C3/3'), 145.4 (C4/4'), 134 (C1/1'), 123 (C6/6'), 115.5 (C5/5'), 113.7 (C2/2'), 104.8 (C-*O*-Glu'.), 71.4 (C9/9'), 62.7 - 78.3

(C9/9'-*O*- Glu.), 56.4 (C-OCH3), 41.2 (C8/8'), 35.8 (C7/7'). MS: m/z: 686.7014(M + 1). $C_{32}H_{46}O_{16}$ (686.71) Anal. Cal. C, 55.97; H, 6.75. Found: C, 57.88; H, 7.80.

2.2 Determination of Antibacterial Effects

The antibacterial activity of the synthetic SDG was tested against *Salmonella typhimurium* (MTCC 98)*, Escherichia coli* (MTCC 40)*, Shigellaflexineri* (H57)*, Staphyllococcusaureus* (MTCC 96)*, Xanthomonas* (XL)*, Bacillus subtilis* (MTCC 619)*,* and *Streptococcus pyogens* (1925) which were obtained from the stock culture maintained at Department of Studies in Microbiology, University of Mysore, Mysore, India.

All the MTCC cultures employed in this current task were maintained on nutrient agar slants (Hi Media chemicals, India) and preserved at 4℃. Broth cultures of *S. typhimurium, E. coli, S. flexineri, S. aureus, Xanthomonas, B. subtilis,* and *S. pyogens* were prepared by inoculating the bacterial pathogens in nutrient broth (Hi-Media chemicals, India) and incubated at 28℃ for 24 h. Spread plate method was followed by inoculating 100 µL of broth cultures on a nutrient agar plate.

The agar medium was purchased from Hi-Media laboratories Ltd., Mumbai, India. Nutrient broth, subculture, base layer medium, agar medium and peptone water were prepared as per the standard procedure.

2.2.1 Agar–well diffusion assay

The antimicrobial activity was measured by agar well diffusion assay following the method of Bauer et al [28]. Sample dissolved in ethanol (2.5 mg/mL) was used for the assay. The wells were prepared in the plates with the help of a cork-borer (0.85 cm). About 50 µL of the test compound was introduced into the wells and allowed to diffuse for 2 h at 4℃. Plates were incubated at 37℃ for 48 h and the microbial growth was determined by measuring the diameter of inhibition zones. Methanol alone was used as a negative control and streptomycin as a positive control. The assay was carried out in triplicate and the mean values are presented.

2.2.2 Agar–disc diffusion method

Synthetic SDG was screened for antimicrobial activity by disc diffusion based on the method of Parekh et al [29]. Using Mueller-Hinton agar medium to study the preliminary antibacterial activity against *S. typhimurium, E. coli, S. flexineri, S. aureus, Xanthomonas*, *B. subtilis*, and *S. pyogens*. Synthetic SDG was dissolved in 5 mL of dimethyl sulfoxide (DMSO) (1000 ug/mL). 0.1 mL of this compound was introduced on the disc (0.7 cm) (Hi-Media) and allowed to dry. Then the disc was impregnated on the seeded agar plate. DMSO was used as a negative control, whereas, Streptomycin was used as a positive control. The plates were done in triplicates and were incubated. The antimicrobial activity was taken on the basis of the diameter of zone of inhibition.

2.2.3 Minimum Inhibitory Concentration (MIC)

The MIC was determined by the modified method of Dufouret al., and Gary et al. [30,31] Different concentrations (100 ppm to 300 ppm) of synthetic SDG and Chloramphenicol standard (positive control) and 100 µL of the bacterial suspension (10^5 CFU/mL) were placed aseptically in 10 mL of nutrient broth and incubated for 24 h at 37℃. The growth was observed both visually and by measuring O.D. at 600 nm at regular intervals followed by plating with nutrient agar. The lowest concentration of test sample showing no visible growth was recorded as the minimum inhibitory concentration. Sample tubes were maintained for each concentration of test sample and the readings were plotted against O.D at 600 nm as growth curves.

3. RESULTS AND DISCUSSION

3.1 Chemistry

The reaction sequence for title compound 6 was outlined in **Scheme 1**.This synthesis begins with the regeoselective bromination of the commercially available compound **1** (3,4-dimethoxy toluene) obtained from Sigma Aldrich Chemicals Co. (St. Louis, MO, USA). Compound **3** was achieved by the alkylation of compound **2** with butanediol. The methoxy groups in compound **3** played an important role in hindering the condensation reaction with pentaacetyl glucose which occurs at phenyl rings. Finally, compound **6** was achieved by the deacetylation and partial demethylation of compound **4** and **5** respectively. All the synthetic compounds were obtained in moderate yield and their structures were confirmed by IR, NMR and MS-TOF. The current synthesis scheme of SDG was found to be an efficient approach. The great advantage of this approach is that the compounds used are readily synthesized in quick

time with less quantity of chemicals. Moreover, a good yield of the titled compound was achieved in this approach for the first time in contrast to previous procedures. Furthermore, purification process needed less time, as all the starting materials can be removed easily by flash chromatography, and purification of the products can be carried out by column chromatography.

3.2 Antibacterial Activities

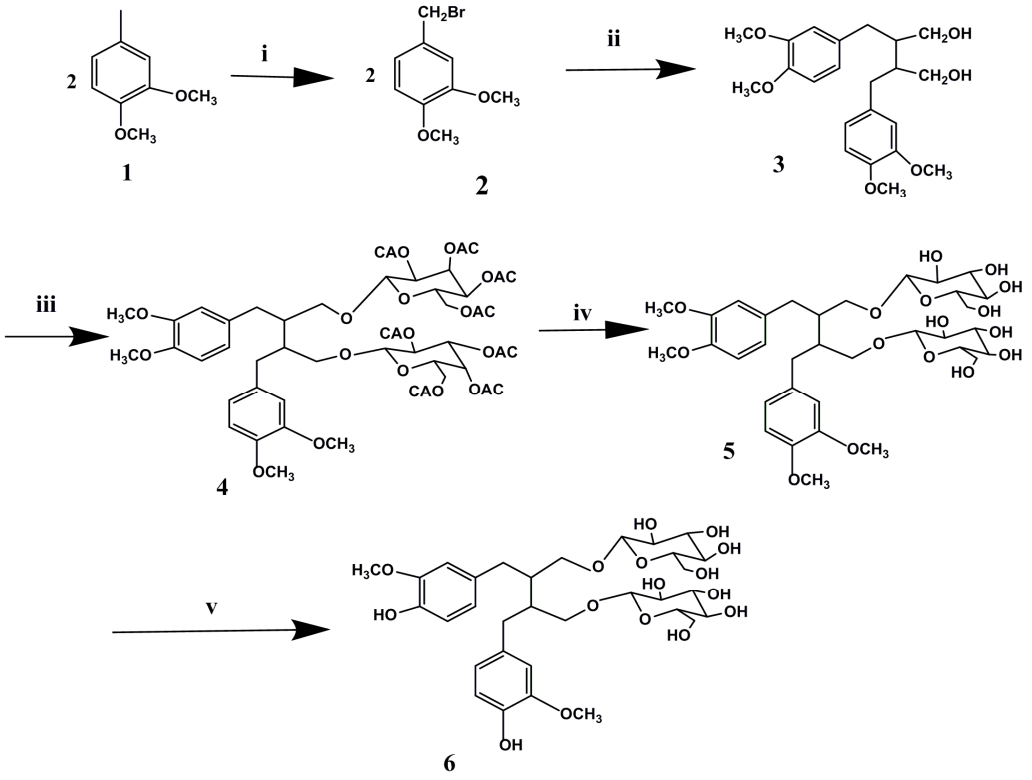
3.2.1 Agar-well diffusion assay

The antibacterial activity of synthetic SDG was evaluated *in vitro* by agar well diffusion method against seven bacterial species. It exhibited significant antibacterial activity against all tested bacterial strains with various degrees of inhibition. The maximum activity (30 mm) was against E. coli, whereas, it was minimum (7 mm) against *S. pyogens*. On the other hand the activity was moderate for the rest of the tested bacterial strains as shown in Table 1.

3.2.2 Agar-disc diffusion method

The evaluation of the antibacterial activity of synthetic SDG was also carried out using another *in vitro* method which is agar disc diffusion.

The synthetic SDG was found to exert high and most pronounced antibacterial activity with inhibition zone of 27.3 mm against *E. coli* and lesser activity with minimum inhibition zone of 6.1 mm against *S. typhimurium. S. flexineri, S. aureus, Xanthomonas, B. subtilis,* and *S. pyogens* were also found to be moderately inhibited by the synthetic SDG as displayed in the Table 2.



Scheme 1. Schematic representation of synthesis

Schematic representation for the Reagents and conditions used in the synthesis of SDG and various intermediates: (i) NBS, CCl_4, reflux, 11 h; (ii) 1,4- butanediol, n-But Li, DMF, mix together - 60℃ – 05℃, 13 h; (iii) 2,3,4,6 tetra-o-acetyl α-D glucopyranosyl bromide,HCl, ethanol, reflux, 19 h; (iv) NaOH, ethanol, diethylether; (v) dichloromethane, stannous chloride, stirred, 3 h, 40℃, 2 N HCl

Table 1. Antibacterial activity of synthetic SDG against some bacteria using agar-well diffusion method

Compound	Conc.	Microorganisms						
		Diameter of zone of inhibition (mm)						
		S. typhimurium	***E. coli***	***S. flexineri.***	***S. aureus.***	***Xanthomonas***	***B. subtilis***	***S. pyogens***
Streptomycin	30 µg/mL	25±0.12	34±0.09	34±0.16	26±0. 20	32±0.15	23±0.23	30±0.05
Syn. SDG	2.5 mg/mL	18±0.34	30±0.21	17±0.31	23±0.14	12±0.17	21.4±0.53	7±0.11
Methanol	50 µL	-	-	-	-	-	-	-

**Each value represents mean of three different observations ± S.D.(-) - no zone of inhibition observed, Streptomycin - as positive control, Methanol - as negative control*

Table 2. Antibacterial activity of synthetic SDG against some bacteria using agar-discdiffusion method

Compound	Conc.	Microorganisms						
		Diameter of zone of inhibition (mm)						
		S. typhimurium.	***E. coli***	***S. flexineri.***	***S. aureus.***	***Xanthomonas.***	***B. subtilis***	***S. pyogens***
Streptomycin	30 µg/mL	27.2±0.10	29.1±0.20	25.8±0.09	26.3±0.30	27.1±0.17	20.6±0.24	32.2±0.43
Syn. SDG	2.5 mg/mL	6.1±0.32	27.3±0.10	23±0.27	15.7± 0.54	21.2±0.22	19.3±0.19	10.2±0.51
DMSO	50 µL	-	-	-	-	-	-	-

**Each value represents mean of three different observations ± S.D. (-) - no zone of inhibition observed, Streptomycin - as positive control, DMSO- as negative control*

Table 3. MIC values for Chloramphenicol as well as synthetic SDG against several bacteria

Compound	Microorganisms MIC (ppm)						
	S. typhimurium	***E. coli***	***S. flexineri.***	***S. aureus.***	***Xanthomonas.***	***B. subtilis***	***S. pyogens.***
Chloramphenicol	100	150	200	200	250	150	100
Syn. SDG	200	100	300	150	100	200	200

**Chloramphenicol was used as positive control*

3.2.3 Minimum Inhibitory Concentration (MIC)

The agar dilution method was used to determine the MIC of the synthetic SDG. MIC was defined as the lowest concentration of the test agent that inhibited bacterial growth, as indicated by the absence of turbidity. MIC valuesrange from 100 ppm to 300 ppm as given in Table 3. The test compound was effective against *E. coli* and *Xanthomonas* with MIC of 100 ppm and also it inhibited the growth of *S. aureus* at 150 ppm. *S. typhimurium, B. subtillis*and *S. flexineri* were completely inhibited by synthetic SDG at 200, 200, and 300 ppm respectively.The minimum concentration of the synthetic SDG that inhibits the growth of the tested organism in comparison to the antibacterial standard chloramphenicol was determined and listed in the Table 3. All the concentrations of the synthetic SDG showed strong activity against all the test organisms on concentration dependent manner.

The assessment of antibacterial properties associated with synthetic SDG was accomplished against important pathogenic bacteria in comparison to some antibacterial standards. The synthetic SDG was found to be a worthy antibacterial agent as it showed higher activity against *E. coli* when compared with other microorganisms either in agar well or disk diffusion assay. It also showed high inhibitory activities at minimum concentration against E. coli as well as against *Xanthomonas*. The activities of synthetic SDG against bacteria may be indicative to the broad spectrum activities. Similarly, closely related structure matairesinol, the second major lignans with traces of pinoresinol, lariciresinol and isolariciresinol showed antibacterial activity against several important pathogenic bacteria [9,10]. The phenolic chemical structure of SDG helps justify its antibacterial activity against various bacterial strains. In other words, presence of electron donating groups, methoxy group at Meta position and hydroxyl group at Para position in our target compound (SDG) have revealed good antimicrobial activity against these sorts of bacteria.As reported earlier, phenolic compounds at low concentration affect enzyme activity, especially of those enzymes associated with energy production, while at greater concentrations they cause protein denaturation. Furthermore, Mundt et al. [32] reported that, the effect of phenolic compounds on microbial growth could be the result of the ability of these compounds to alter microbial cell permeability, permitting the loss of macro-molecules from the interior and these compounds could also interact with membrane proteins causing a deformation in their structure and functionality as well as affecting cellular activity.

4. CONCLUSION

From the present study it can be concluded that, an efficient route for the synthesis of SDG was achieved and also the characterization of these compounds was discussed. The preliminary antimicrobial activities of the synthetic SDG showed a broad spectrum of activity against clinically important pathogenic bacteria responsible for causing most common bacterial diseases. This antibacterial effect of the synthetic SDG against these panels of vital pathogenic bacteria can be a preferred supplement due to its known health benefits as an antibacterial agent. It would also assist for development of a new alternative medicine system and opens the possibility of finding new clinically effective antimicrobial compounds and production of new antibiotics. It is essential that the research should be continued to use this promising compound in experimental animals and carefully investigate and elucidate its mechanism of action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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