Medicinal Chemistry, **2024**, ***20***, **130-139**

RESEARCH ARTICLE

Antibacterial Evaluation of Gallic Acid and its Derivatives against a Panel of Multi-drug Resistant Bacteria

Mohamed Abdella[1], Chandrajit Lahiri[1,2], Iskandar Abdullah[3,*] and Ayaz Anwar[1,*]

[1]*Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Selangor, 47500, Malaysia;* [2]*Department of Biotechnology, Faculty of Science, Atmiya University, Rajkot, 360005, India;* [3]*Department of Chemistry, Faculty of Science, Universiti Malaya, Kuala Lumpur, 50603, Malaysia*

ARTICLE HISTORY

Received: March 08, 2023
Revised: July 05, 2023
Accepted: July 28, 2023

DOI:
10.2174/1573406419666230823104300

Abstract: ***Background*:** Infectious diseases are the second leading cause of deaths worldwide. Pathogenic bacteria have been developing tremendous resistance against antibiotics which has placed an additional burden on healthcare systems. Gallic acid belongs to a naturally occurring phenolic class of compounds and is known to possess a wide spectrum of antimicrobial activities.

***Aims & Objectives*:** In this study, we synthesized thirteen derivatives of gallic acid and evaluated their antibacterial potential against seven multi-drug resistant bacteria, as well as cytotoxic effects against human embryonic kidney cell line *in vitro*.

***Methods*:** 13 compounds were successfully synthesized with moderate to good yield and evaluated. Synthesized derivatives were characterized by using nuclear magnetic resonance spectroscopy, mass spectrometry, and Fourier transformation infrared spectroscopy. Antibacterial activity was determined using microdilution while cytotoxicyt was assessed using MTT assay.

***Results*:** The results of antibacterial assay showed that seven out of thirteen compounds exhibited antibacterial effects with compound **6** and **13** being most potent against *Staphylococcus aureus* (MIC 56 µg/mL) and *Salmonella enterica* (MIC 475 µg/mL) respectively. On the other hand, most of these compounds showed lower cytotoxicity against human embryonic kidney cells (HEK 293), with IC_{50} values ranging from over 700 µg/mL.

***Conclusion*:** Notably, compound **13** was found to be non-toxic at concentrations as high as 5000 µg/mL. These findings suggest that the present synthetic derivatives of gallic acid hold potential for further studies in the development of potent antibacterial agents.

Keywords: Gallic acid, antibacterial agents, multi-drug resistance, infectious diseases, cytotoxic effects, antibacterial assay.

1. INTRODUCTION

Infections caused by bacteria are significantly harmful and have a substantial impact on humans, animals, and plants [1-3]. Penicillin G was first used to treat infections caused by *Streptococci*, *Staphylococci*, and *Pseudomonas aeruginosa* [4-5]. For many years, antibiotics have been misused, and as a result, bacteria have evolved antimicrobial resistance (AMR). Moreover, the emergence of AMR among microorganisms has a high impact on the financial and mortality rate. According to World health organization (WHO), the most threatening microbes incudes *Salmonella enterica* (*S. enterica*), *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. [6]. Tannins, lignins, and flavonoids are polyphenols that act as antioxidants and metal chelators. Gallic acid (GA) is one of the phenolic compounds that contains 3 phenolic OH groups. GA has been known to possess an antibacterial action on bacteria [7]. It works against multi-drug resistant (MDR) *Staphylococcus aureus*, and MDR *Escherichia coli* germs, as well as MDR processes including efflux pumps in different bacteria. Moreover, GA exhibits antiallergic, antifungal, antiviral, anti-inflammatory, anti-mutagenic, and anticarcinogenic properties [7]. Previous research has demonstrated that GA can inhibit the biofilm formation of MDR *A. baumannii* isolates [8]. Moreover, GA has been found to be cytotoxic to cancer cells without harming normal cells [9]. Some of the pharmacological activities of GA and its various derivatives are shown in Table **1**, as reviewed in [10-12]

The aim of this study was to assess the antibacterial activity of thirteen synthetic derivatives of gallic acid against MDR strains, which have not been previously reported.

*Address correspondence to these authors at the Department of Chemistry, Faculty of Science, Universiti Malaya, Kuala Lumpur, 50603, Malaysia; E-mail: iskandar.a@um.edu.my
Department of Biological Sciences, School of Medical and Life Sciences, Sunway University, Selangor, 47500, Malaysia; Tel: 60-(0)3-7491-8622; Ext: 7174; Fax: 60-(0)3-5635-8630; E-mail: ayazanwarkk@yahoo.com

1875-6638/24 $65.00+.00

Table 1. The pharmacological activity of GA and its derivatives.

-	Diseases Category	Diseases	References
Gallic Acid and its Derivatives	Inflammatory diseases	LPS-induced inflammation, formalin-induced inflammation, arthritis	[11-12]
	Gastrointestinal diseases	Hepatotoxicity, IBD, Gastric ulcers	
	Metabolic diseases	Diabetes, Obesity, Dyslipidemia	
	Neuropsychological diseases	Neurotoxicity, Locomotor dysfunction, Memory deficits, Neuropathy	
	Urogenital diseases	Renal fibrosis, Nephrotoxicity	
	Dermal diseases	UV-induced skin damage, Melanoma	
	Respiratory diseases	Pulmonary fibrosis	
	Cardiovascular diseases	Myocardial injury, Cardiac fibrosis	
	Malignancies disease	Colorectal cancer, Leukemia, Skin carcinogenesis	
	Oral health	Bacteria-induced enamel caries	

2. MATERIALS AND METHODS

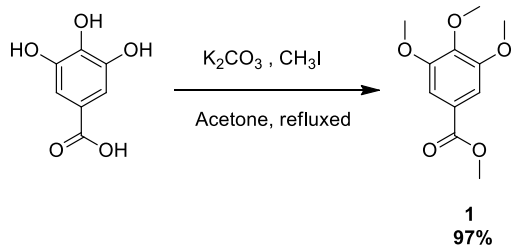
Microplate reader (Device: infinite 200Pro), adjusted to wavelength 600 nm (Neotec Scientific Instrumentation Ltd.), 96-well microplates (NEST_96 flat transparent_701001 96 Flat Bottom), Dimethyl sulfoxide (DMSO) (Sigma/ Germany), Mueller Hinton Broth (MHB) (Oxoid/ Germany), Libra S4 spectrophotometer adjusted to wavelength 600 nm (Biochrom /United Kingdom). Dulbecco's Modified Eagle Medium (DMEM) culture medium (Sigma-Aldrich/ Germany), (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (1x), at 5 mg/ml in phosphate buffered (MTT) (Goldbio/ United States), Human Embryonic Kidney cells (HEK-293), American Type Culture Collection, microplate reader (Device: infinite 200 Pro), adjusted at 570 nm and 630 nm as reference (Neotec Scientific Instrumentation Ltd). All chemicals and solvents were of analytical grade purchased from Sigma-Aldrich unless stated otherwise. All melting points were taken on a Stuart Melting Point SMP 30 melting point apparatus. Nuclear Magnetic Resonance (NMR) spectra were obtained from Bruker AVN 600 and JEOL JNM-ECX400II, GC-MS Shimadzu Model: GCMS-QP 2010 Plus was used for obtaining the mass spectrum and Perkin Elmer Spectrum Two was used to obtain the FTIR spectrum. ^{1}H, and ^{13}C analyses were used to characterize all the synthesized compounds. Topspin 4.1.3 (Bruker) and Delta 6.0.0 (Jeol) were used for viewing and analyzing the NMR spectra. Thin Layer Chromatography (TLC) was carried out by using aluminium sheets TLC silica 60 F_{254} purchased from Merck. Flash column chromatography using silica gel (40-60 μm) was purchased from Merck.

2.1. Synthesis of Gallic Acid Derivatives

A series of thirteen compounds were synthesized by utilizing different chemical reactions to afford the following derivatives. The schemes are given along with every compound, whereas Table **2** shows the names, molecular weight, and structure of the synthesized compounds. The structures of synthesized compounds were confirmed by NMR, MS, and FT-IR analysis which are presented as supplementary materials separately.

2.1.1. Synthesis of methyl-3,4,5-trimethoxybenzoate (1)

At ambient temperature, potassium carbonate (30.0 g, 0.217 mol) was added to a solution of Gallic acid (5.4 g, 0.0318 mol) in anhydrous acetone (150 mL) under nitrogen, followed by the addition of iodomethane (13.0 mL, 0.09 mol). The resulting mixture was then refluxed for 24 h until the completion of the reaction which was monitored by TLC analysis. The reaction mixture was cooled to room temperature and extracted with petroleum ether (50 mL x 4). The organic phase was then washed with water, brine, dried over Na_2SO_4, and concentrated to afford crude methyl-3,4,5-trimethoxybenzoate brownish yellow solid which was further used without any purification (Scheme **1**) [13, 14].



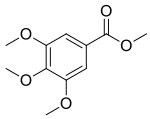
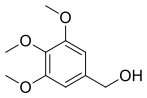
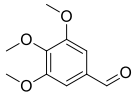
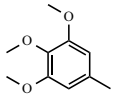
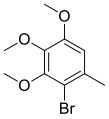
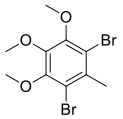
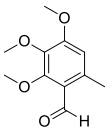
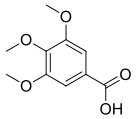
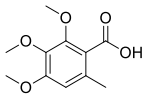
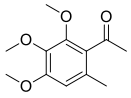
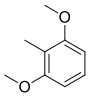
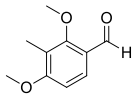
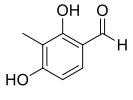
1
97%

Scheme 1. Synthesis of compound 1.

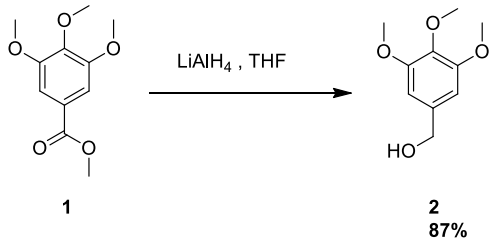
2.1.2. Synthesis of 3,4,5-trimethoxybenzyl alcohol (2)

In a round-bottomed flask, Lithium Aluminum Hydride ($LiAlH_4$) (0.45 g, 0.011 mol) was dissolved in 10 mL anhydrous tetrahydrofuran (THF) and cooled to 0°C under nitrogen gas. A solution of methyl-3,4,5-trimethoxybenzoate (**1**) (3.1 g, 0.014 mol) dissolved in 40 mL anhydrous THF was then added dropwise for 10 mins and left to stir for an additional 1 h and 30 mins to room temperature. After reaction completion judged by TLC analysis, the reaction mixture was cooled in an ice bath and quenched with 5.0 mL of distilled water followed by dropwise of 25.0 mL 10% HCl for

Table 2. Structures, molecular weights, and names of compounds synthesized in this study.

Compound No.	IUPAC Name	MW	Structural Formula	Reference for Antimicrobial Activity
1	Methyl-3,4,5-trimethoxybenzoate	226		N/A
2	3,4,5-Trimethoxybenzyl alcohol	198		N/A
3	3,4,5-Trimethoxybenzaldehyde	196		Antifungal against *Candida albicans* [12]
4	3,4,5-Trimethoxytoluene	182		N/A
5	2-Bromo-3,4,5-trimethoxy-1-methylbenzene	260		N/A
6	1,5-Dibromo-2,3,4-trimethoxy-6-methylbenzene	337		N/A
7	2,3,4-Trimethoxy-6-methylbenzaldehyde	210		N/A
8	3,4,5-Trimethoxybenzoic acid	212		N/A
9	2,3,4-Trimethoxy-6-methylbenzoic acid	226		N/A
10	1-(2,3,4-Trimethoxy-6-methylphenyl) ethanone	224		N/A
11	1,3-Dimethoxy-2-methylbenzene	152		N/A
12	2,4-Dimethoxy-3-methylbenzaldehyde	180		N/A
13	2,4-Dihydroxy-3-methylbenzaldehyde	152		N/A

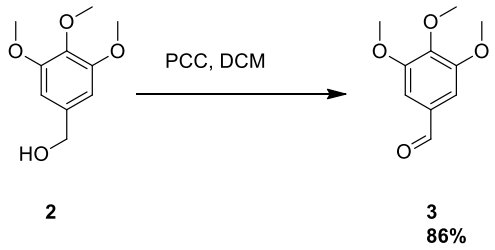
20 mins. The resulting mixture was extracted with EtOAc (50 mL x 2), washed with water, and brine followed by drying over Na_2SO_4 and concentrated under reduced pressure to give yellowish brown thick viscous liquid of crude 3,4,5-trimethoxybenzyl alcohol (Scheme **2**) [15].



Scheme 2. Synthesis of compound 2.

2.1.3. Synthesis of 3,4,5-trimethoxybenzaldehyde (3)

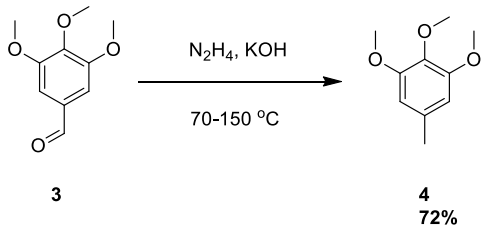
To a solution of 3,4,5-trimethoxybenzyl alcohol (2.0 g, 0.0105 mol) in 25 mL dichloromethane (DCM), Pyridinium chlorochromate (PCC) (4.5 g, 0.021 mol) was added and the reaction mixture was left to stir at room temperature for 3 h. The reaction completion was monitored using TLC analysis followed by quenching with 11.0 mL of methyl tert-butyl. The resulting mixture was left to stir for another 20 mins and filtered through silica gel. The organic phase was washed with distilled water (25 mL x 2), washed with water, brine, dried over Na_2SO_4, and filtered to remove chromate ester byproducts followed by concentration under reduced pressure. The crude residue was subjected to column chromatography (PE:EtOAc; 5:2) to afford 3,4,5-trimethoxybenzaldehyde as pale yellow solid (Scheme **3**) [16].



Scheme 3. Synthesis of compound 3.

2.1.4. Synthesis of 1,2,3-trimethoxy-5-methylbenzene (4)

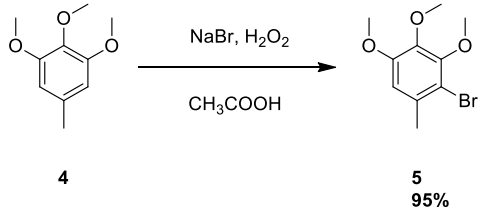
3,4,5-Trimethoxybenzaldehyde (8.0 g, 0.04 mol), hydrazine hydrate (6.2 g, 0.12 mol), and potassium hydroxide (1.0 g, 0.017 mol) were charged in a round-bottomed flask followed by the addition of 100 mL ethylene glycol. The reaction was conducted at three temperatures, 70°C for 3 h, 120 °C for 2 h, and 150°C for another 2 h. The reaction was monitored using TLC analysis. After completion of the reaction, the reaction mixture was cooled to room temperature. Petroleum ether was added to the reaction mixture and stirred for an additional 10 mins. The organic layer was separated and washed using water, dried over Na_2SO_4, and evaporated using a rotary evaporator to afford 3,4,5-trimethoxytoluene as white solid (Scheme **4**) [17].



Scheme 4. Synthesis of compound 4.

2.1.5. Synthesis of 1-bromo-2,3,4-trimethoxytoluene (5)

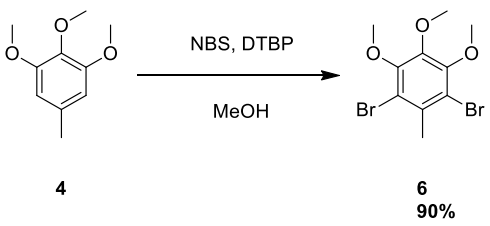
In a round-bottomed flask, 3,4,5-trimethoxytoluene (4.0 g, 0.021 mol), sodium bromide (2.2 g, 0.021 mol), and acetic acid (10 mL) was added at room temperature. 30% hydrogen peroxide, H_2O_2 (20 mL) was added dropwise to the reaction mixture at 25°C and was left to stir for 1.5 h. Upon reaction completion judged by TLC analysis, the reaction was quenched with water and petroleum ether was added. The organic layer was separated and treated with saturated $NaHCO_3$ to adjust the pH to 7. The organic layer was separated and washed using water, brine, dried over Na_2SO_4, and evaporated under reduced pressure [18]. The crude residue was subjected to column chromatography (PE:EtOAc; 6:2) to afford 1-bromo-2,3,4-trimethoxytoluene as red oil liquid (Scheme **5**).



Scheme 5. Synthesis of compound 5.

2.1.6. Synthesis of 1,3-dibromo-4,5,6-trimethoxy-2-methylbenzene (6)

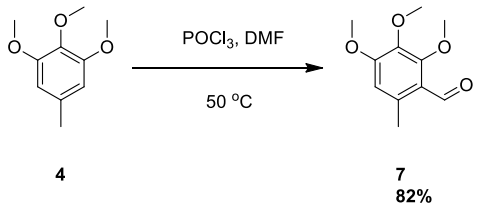
To a solution of 3,4,5-trimethoxytoluene (0.22 g, 0.0012 mol) in 5.0 mL MeOH, 0.1 mL of N-bromosuccinimide (NBS) was added at room temperature. The reaction mixture was cooled to 25°C and Di-tert-butyl peroxide (DTBP) (0.1 mL, 0.0005 mol) was added dropwise followed by stirring for 1 h. Upon reaction completion judged by TLC analysis, MeOH was removed *in-vacuo*. Extraction of the reaction was conducted by using EtOAc three times. The organic was washed using water, brine, dried over Na_2SO_4, and evaporated under reduced pressure to afford 1,3-dibromo-4,5,6-trimethoxy-2-methylbenzene as yellow-red oil liquid (Scheme **6**).



Scheme 6. Synthesis of compound 6.

2.1.7. Synthesis of 2,3,4-trimethoxy-6-methylbenzaldehyde (7)

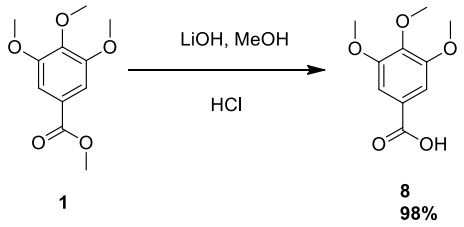
3,4,5-Trimethoxytoluene (2.0 g, 0.01 mol) was dissolved in 20 mL DMF and stirred at 50°C. 2.0 mL of phosphoryl chloride ($POCl_3$) was added dropwise to the stirring solution in 20 mins. Upon reaction completion based on TLC analysis, the reaction mixture was cooled to room temperature and the pH was adjusted to **7** using sodium hydroxide solution. EtOAc was added to the reaction mixture and stirred for 10 mins and the organic layer was collected, washed with water, dried over Na_2SO_4, and evaporated using a rotary evaporator to give 2,3,4-trimethoxy-6-methylbenylaldehyde as light brown crystals (Scheme **7**) [19].



Scheme 7. Synthesis of compound 7.

2.1.8. Synthesis of 3,4,5-trimethoxybenzoic Acid (8)

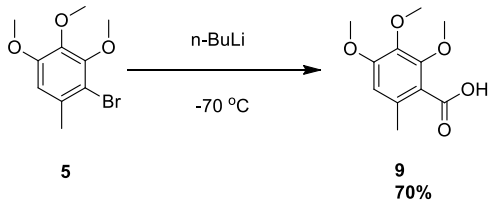
LiOH (0.5 g) was added to a stirred solution of methyl 3,4,5-trimethoxybenzoate (1.0 g, 0.004 mol) in MeOH: H_2O (1:1 v/v) for 3 h at room temperature. The reaction was monitored using TLC. After reaction completion, MeOH was removed using a rotary evaporator and the aqueous solution was treated with 2M HCl to pH 2. The crude mixture was extracted thrice with EtOAc, combined, washed with water, dried over Na_2SO_4, and evaporated using a rotary evaporator to give 3,4,5-trimethoxybenzoic acid as light-yellow solid (Scheme **8**) [20].



Scheme 8. Synthesis of compound 8.

2.1.9. Synthesis of 2,3,4-trimethoxy-6-methylbenzoic Acid (9)

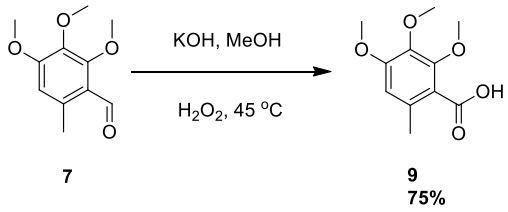
In a solution of dry ether (5.0 mL), 0.4 g of 2-bromo-3,4,5-trimethoxytoluene (0.0018 mol) was added and stirred at -78 °C under N_2. A solution of 0.1 ml of n-butyllithium 1.8M/Hexane was added dropwise to the reaction mixture and left to stir for another 40 mins followed by the addition of dry ice. The reaction progress was monitored by TLC analysis. Upon reaction completion, the reaction mixture was warmed up to room temperature, stirred for 30 mins, and quenched with water. Diluted HCl was added, and the reaction mixture was extracted with EtOAc three times. The combined organic layer was washed with water, brine, dried over Na_2SO_4, and concentrated under reduced pressure giving 2,3,4-trimethoxy-6-methylbenzoic acid as yellow crystals (Scheme **9a**) [21].



Scheme 9a. Synthesis of compound 9.

2.1.10. Synthesis of 2,3,4-trimethoxy-6-methyl-benzoic acid (9)

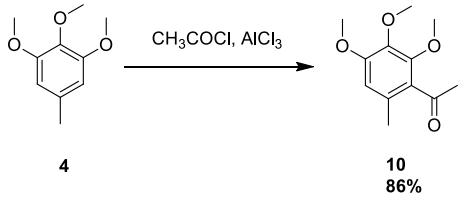
A round-bottomed flask was charged with 10 mL MeOH, 7.0 mL of 50% KOH solution, and 2,3,4-trimethoxy-6-methylbenzaldehyde (2.1 g, 0.01 mol). To the stirred reaction mixture, 10 mL of 30% H_2O_2 was added dropwise and was allowed to stir at 45°C for 1 h. After reaction completion based on TLC analysis, MeOH was removed *in-vacuo* and acidified with 12 M HCl to pH 2. The crude was extracted with EtOAc three times, combined, washed with water, dried over Na_2SO_4 and evaporated using a rotary evaporator giving 2,3,4-trimethoxy-6-methyl-benzoic acid as white solid (Scheme **9b**) [22].



Scheme 9b. Synthesis of compound 9.

2.1.11. Synthesis of 1-(2,3,4-trimethoxy-6-methylphenyl) Ethanone (10)

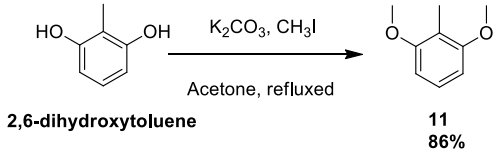
In an oven-dried round-bottomed flask, 3,4,5-trimethoxytoluene (1.0 g, 0.005 mol) and 20 mL of anhydrous benzene were added followed by the addition of $AlCl_3$ (1.0 g, 0.007 mol) under N_2. The resulting reaction mixture was allowed to stir for 10 mins with subsequent dropwise addition of acetyl chloride (0.5 mL, 0.002 mol) and was left to stir for another 3 h. After the reaction was completed judged by TLC analysis, the reaction mixture was quenched by pouring 20 mL HCl in 50.0 g of ice and further stirred for another 1 h. The crude was extracted with EtOAc three times, combined, washed with water, dried over Na_2SO_4, and evaporated using a rotary evaporator to furnish 1-(2,3,4-trimethoxy-6-methylphenyl) ethenone as brown viscous liquid (Scheme **10**) [23].



Scheme 10. Synthesis of compound 10.

2.1.12. Synthesis of 2,6-dimethoxytoluene (11)

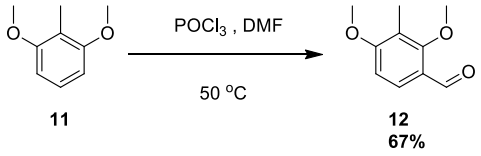
In a dried round-bottomed flask, 2,6-dihydroxytoluene (2.25 g, 0.018 mol), K_2CO_3 (12.0 g, 0.08 mol), and dry acetone (60 mL) was added followed by the addition of iodomethane (5.6 mL, 0.11 mol). The reaction mixture was stirred under reflux condition until the reaction completion and was monitored by TLC (~24 h). Solvents were removed *in-vacuo*. Subsequently, the reaction mixture was extracted with EtOAc, combined, washed with water, brine, dried over Na_2SO_4, and evaporated using a rotary evaporator. The crude residue was recrystallized with EtOH to afford 2,6-dimethoxytoluene as yellow solid (Scheme **11**) [13].



Scheme 11. Synthesis of compound 11.

2.1.13. Synthesis of 2,4-dimethoxy-3-methylbenzaldehyde (12)

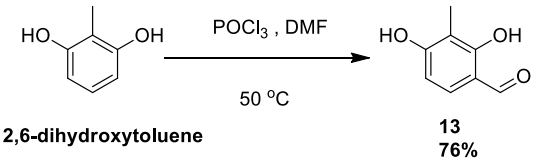
In a round bottom flask equipped with a magnetic stir bar, 2,6-dimethoxytoluene (1.0 g, 0.0065 mol) was dissolved in DMF (20 mL) and stirred at 50°C. $POCl_3$ (0.5 ml, 0.005 mol) was added dropwise to the stirring solution in 20 mins and was allowed to stir for another 1 h. Upon reaction completion, the reaction mixture was allowed to cool at room temperature and the pH was adjusted to 7.1 using 10% NaOH solution. The reaction mixture was extracted with EtOAc, combined, washed with water, and brine, dried over Na_2SO_4, and evaporated using a rotary evaporator to afford 2,4-dimethoxy-3-methylbenzaldehyde as yellow solid (Scheme **12**) [24].



Scheme 12. Synthesis of compound 12.

2.1.14. Synthesis of 2,4-dihydroxy-3-methylbenzaldehyde (13)

Compound **13**, using 2,6-dihydroxytoluene (2.0 g, 0.016 mol) as the starting material was prepared in a similar fashion as compound **12** and compound **7**. Compound **13** was obtained as pale-yellow solid (Scheme **13**) [24].



Scheme 13. Synthesis of compound 13.

The characterization data and spectra of all compounds is presented in supplementary information.

2.2. Bacteria used in Current Study

A list of bacterial strains used in this study is given below in Table **3** along with the reported resistance of these strains against antibiotics [26-30].

2.3. Inoculation of Media

The media were inoculated by making a standardized suspension of the organism to be tested in sterile MHB overnight at 37°C. 1 mL of the bacterial suspension was transferred to 15 mL sterile MHB and incubated at 37°C for 4 h, after the incubation period the growth was detected using a spectrophotometer at 600 nm and corrected to sterile MHB media. The bacterial suspension was adjusted to 10^8 CFU then diluted to give 10^4 CFU [31]. Organisms used are *S. enterica* ATCC 14028, *E. faecium* ATCC 19434, *S. aureus* MTCC 381128, *K. pneumoniae* ATCC 700603, *A. baumannii* ATCC 19606, *P. aeruginosa* ATCC 10145, and *E. aerogenes* ATCC 13048.

2.4. Testing the Antibacterial Activity of the Synthesized Compounds

Using 96-well microplates (TPP® – Techno Plastic Products, Trasadingen, Switzerland) containing a total volume of 100 µL of sterile MHB in each well, and the adjusted inocu-

Table 3. Names and resistance profiles of bacteria used in this study.

***E. faecium* ATCC 19434**	**Vancomycin [25]**
K. pneumoniae ATCC 700603,	Ceftazidime, oxyimino-β-lactams, cefotaxime, ceftazidime, and ceftriaxone) [26].
P. aeruginosa ATCC 10145	Carbapenems, β lactams, fluoroquinolones, and aminoglycoside [27].
S. enterica ATCC 14028	Ampicillin, streptomycin, sulfisoxazole, and tetracycline [28].
S. aureus MTCC 381128	Erythromycin, clindamycin, aminoglycosides, fluoroquinolones, co-trimoxazole, and rifampin
A. baumannii ATCC 19606	Sulfonamide, sulfamethoxazole, ampicillin, spectinomycin, and chloramphenicol [29].
E. aerogenes ATCC 13048	Imipenem, meropenem, cefpodoxime, ceftazidime, cefotaxime, ceftriaxone, cefepime and aztreonam [30].

lum of the organisms used was in a final concentration of 10^4 CFU/mL [31, 32].

2.5. Preparation of the Compounds' Stock Solutions

10000 µg/mL stock solutions were made for each compound by dissolving 10 mg in 1 mL of DMSO (10% solution) and using vortex followed by sonication to homogenize the solution.

2.6. Microdilution Assay Procedure

Each bacterial inoculate was prepared by incubation overnight in MHB. Thereafter, 1 mL of the culture was transferred to a fresh MHB media and incubated for 4 h. Following this, the bacterial culture was tested using the spectrophotometer at wavelength of 600 nm and the culture was diluted to give 0.2 OD to reflect 1.7 X 10^8 CFU/mL. Using dilution law, the bacterial culture was adjusted to 10^4 CFU/mL and 80 µL of this bacterial suspension was injected using an automatic micropipette to the 96-well microplates having three replicas for each bacterium (as three rows). This was followed by the addition of 20 µL of the dissolved synthetic compounds to each of the wells (resulting to 2000 µg/mL concentration for a total volume of 100 µL per well). Two types of negative controls were used; three replicas of the negative control were prepared in MHB with the bacterial inoculum without addition of the synthetic compounds. Additionally, three replicas of the negative control were prepared in MHB with the bacterial inoculum, the same concentration of the solvent used in dissolving the compounds and free of the antibacterial agents, while three replicas of the positive control were prepared in MHB having Gentamycin as an antibiotic. Following the addition of the compounds, the microplates were centrifuged for 3 mins to make sure that the bacterial culture with the compounds were mixed, and then the microplates were incubated at 37°C for 18 h. At the end of the incubation period, the plates were analyzed using the microplate reader. The growth was corrected to zero time and negative control. All tests were performed in triplicates. The results were then plotted and visualized.

2.7. Cytotoxicity Evaluation Using MTT Cell Viability Assay

MTT assay was used to detect the toxic effect of the tested compounds on human embryonic kidney cell HEK-293 [33]. Cells were seeded in 96-well plates with a seeding density of 5 x 10^4 cells per well into a volume of 100 µL DMEM culture medium. The cells were incubated for 24 h with the serially diluted compounds at the desired concentration under 5% CO_2 at 37°C. Following the incubation period, MTT was added to each well to the final concentration of 0.5 mg/mL. The plates were incubated in a humidified atmosphere for 2 - 4 h. Formation of purple formazan crystals by the activity of the living cells (whose metabolism converts MTT into formazan while dead cells do not affect MTT) was observed. Thereafter, the media was removed and 100 µL DMSO was added to each well to dissolve formazan crystals. The absorbance of the samples was measured using a microplate reader at 570 nm and using 630 nm as a reference wavelength. The treated absorbance was normalized against the control and the IC_{50} values were calculated for each of the compounds [33]. All tests were performed in triplicates with three technical repeats.

2.8. IC50

IC_{50} is the concentration that inhibits the growth to half of an inoculum. The results produced from the MIC testing were used to plot a scatter chart between the concentrations of the tested compound on the X-axis and the inhibition on the Y-axis, then a linear equation was made.

The slope-intercept formula

Equation 1 was used to determine the IC_{50} by making y = 50. M is calculated by Equation 2 [34].

Equation 1. General slope-intercept formula (m is the slope, b is the intercept).

$$y = mx + b \quad (1)$$

Equation 2 slope (x_1, y_1 are coordinates of the first point in the line, x_2, y_2 are coordinates of the second point in line)

$$m = \frac{y_2 - y_1}{x_2 - x_1} \quad (2)$$

3. RESULTS AND DISCUSSION

The morbidity, mortality, duration of hospital stays, and healthcare expenses all rise as a result of antimicrobial resistance [35]. Since the treatments of MDR pathogens are limited, current studies being conducted, have a similar focus [35]. The aim of this study was to find out the potential active compounds against MDR bacteria. In the procedures outlined in this report, chemical synthesis, antimicrobial testing, and cell viability assay were carried out. Gallic acid is regarded as a viable lead molecule for the development of novel drugs because it is clear that it and its derivatives play a significant role in various therapeutic activities [11]. Gallic acid derivatives were synthesized with good yield. The synthesised compounds were confirmed by FTIR, ^{13}C NMR, ^{1}H NMR and GC-MS spectroscopy (Supplementary information). The synthesized compounds were tested for their antibacterial activity against the MDR *S. enterica* and MDR ESKAPE pathogens using microdilution assay. The MICs of the tested compounds are summarized in Table **4** and show the following results.

The result showed that most of the compounds were active against the tested organisms, with MICs ranging from 31 µg/mL to more than 2000 µg/mL. In the case of compound 13, the MICs were 475 µg/mL, 1860 µg/mL, 1000 µg/mL, 897 µg/mL, 930 µg, 2000 µg and > 2000 µg/mL against *S. enterica* (ATCC 14028), *E. faecium* (ATCC 19434), *S. aureus* (MTCC 381128), *K. pneumoniae* (ATCC 700603), *A. baumannii* (ATCC 19606), *P. aeruginosa* (ATCC 10145) and *E. aerogenes* (ATCC 13048) respectively.

Compound **2** showed MIC of 1700 µg/mL against *A. baumannii* ATCC 19606. Notably, it is used to treat urinary tract infections, while it can also be used to treat any aerobic bacterial species that is susceptible to it. *Pneumocystis jiroveci* pneumonia is treated and prevented with it [36]. While in the case of compound **3**, it showed antimicrobial activity against *A. baumannii* ATCC 19606 with MIC of 1680 µg/mL. It is worth mentioning that compound **3** showed

Table 4. MIC of the synthesized compounds tested against all bacteria.

Compound	*S. enterica* ATCC 14028	*E. faecium* ATCC 19434	*S. aureus MTCC 381128*	*K. pneumoniae* ATCC 700603	*A. baumannii* ATCC 19606	*P. aeruginosa* ATCC 10145	*E. aerogenes* ATCC 13048
1	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL
2	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	1700 µg/mL	>2000 µg/mL	>2000 µg/mL
3	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	1680 µg/mL	>2000 µg/mL	>2000 µg/mL
4	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	1000 µg/mL	>2000 µg/mL	>2000 µg/mL
5	NI	>2000 µg/mL	1540 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL
6	>2000 µg/mL	>2000 µg/mL	56 µg/mL	>2000 µg/mL	800 µg/mL	964 µg	>2000 µg/mL
7	>2000 µg/mL	NI	>2000 µg/mL	NI	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL
8	NI	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	NI	>2000 µg/mL	>2000 µg/mL
9	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	NI	>2000 µg/mL	>2000 µg/mL	NI
10	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	NI	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL
11	NI	NI	>2000 µg/mL	>2000 µg/mL	2000 µg	>2000 µg/mL	NI
12	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL	>2000 µg/mL
13	475 µg/mL	1860 µg/mL	1000 µg/mL	897 µg/mL	930 µg/mL	2000 µg/mL	>2000 µg/mL

Note: No inhibition: NI

Table 5. Cytotoxicity assay results against HEK 293 and comparison with antibacterial activity against *S. aureus.*

Compound	Antibacterial MIC (µg/mL)	Toxic Conc. (µg/mL)	IC_{50} for Toxicity Testing (µg/mL)
Compound 2	1700	> 100	757.698
Compound 3	1680	> 100	1004.482
Compound 4	*1000*	> 100	775.436
Compound 5	1540	> 100	750.71
Compound 6	56- *964*	> 100	725.876
Compound 11	*2000*	> 100	783.905
Compound 13	*475* -1860	Not toxic for 5000 µg/mL	-

antifungal activity against *Candida albicans* [12]. Compound **4** showed activity against *A. baumannii* ATCC 19606 with MIC of 1000 µg/mL. Compound **11** showed MICs 2000 µg/mL against *A. baumannii* ATCC 19606. Moreover, compound **6** showed MICs of 56 µg/mL, 800 µg/mL, and 964 µg/mL against *S. aureus* MTCC 381128, *A. baumannii* ATCC 19606 and *P. aeruginosa* ATCC 10145, respectively. The activity of compound **6** might have resulted from the introduction of bromine group. Compound **5** showed MIC of 1540 µg/mL against *S. aureus* MTCC 381128. Compound containing bromo groups have been reported both naturally and synthetically with biological properties, including enzyme inhibition, antibacterial, anti-diabetic [37].

The cytotoxicity assay results (Table **5**) revealed that compound **13** was found to be non-toxic to human embryonic kidney cells (HEK 293), whereas compounds **2**, **3**, **4**, **5**, and **11** were toxic at concentration greater than 100 µg/mL, with toxicity IC50 of 757.698 µg/mL, 1004.482 µg/mL, 775.436, 750.71 µg/mL and 783.905 µg/mL, respectively and the antibacterial MIC was more than 1000 µg/mL, each suggesting that the active concentration is toxic to HEK 293. Meanwhile, the toxic concentration of compound **6** was more than 100 µg/mL with toxicity IC_{50} of 725.876 µg/mL while the antibacterial MIC of compound **6,** active against *S. aureus*, was 56 µg/mL, indicating that the active concentration was non-toxic to HEK 293. The current limitations of the study are that most compounds showed antibacterial effects at higher concentrations except for compound **6**. Furthermore, their mechanism to elicit the antibacterial activity is unclear. The *in-silico* evaluation and determination of the mode of action of active compounds against known targets is part of our future studies. Furthermore, conjugation of active compounds with nanoparticles has been shown to enhance the bioactivity of antibacterial agents [38] which can also be tried in future for these compounds.

CONCLUSION

We have successfully synthesized, characterized and evaluated antibacterial activity of a series of gallic acid derivatives. The antibacterial evaluation of the synthesized compounds (**1** to **13**) revealed that **7** of the **13** tested compounds showed high to moderate antibacterial activity. Compound **6** showed low MIC of 56 µg/mL with *S. aureus* and this concentration did not exhibit any toxicity against HEK 293 cells. Compound **13** was found to be the most potent compound among the tested compounds. It showed antibacterial activity against *Salmonella enterica, Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa.* Moreover, compound **13** did not exhibit cytotoxicity against HEK 293 cells *in vitro* suggesting its potential for further inhibitory studies as drug against pathogenic bacteria.

LIST OF ABBREVIATIONS

WHO	=	World health organization
TLC	=	Thin Layer Chromatography
NMR	=	Nuclear Magnetic Resonance
HEK-293	=	Human Embryonic Kidney cells
DMEM	=	Dulbecco's Modified Eagle Medium
MHB	=	Mueller Hinton Broth
DMSO	=	Dimethyl sulfoxide
DTBP	=	Di-tert-butyl peroxide

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information is available within the article.

FUNDING

This work was supported by the FRGS project number FRGS/1/2018/STG05/SYUC/02/1 and Sunway University Grant GRTIN-IGS(02)-DBS-10-2022.

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

ACKNOWLEDGEMENTS

This research work was supported by Sunway University, Malaysia, and Drug Design and Research Development Group (DDDRG), Universiti Malaya, Malaysia.

REFERENCES

[1] Contini, C.; Rotondo, J.C.; Magagnoli, F.; Maritati, M.; Seraceni, S.; Graziano, A.; Poggi, A.; Capucci, R.; Vesce, F.; Tognon, M.; Martini, F. Investigation on silent bacterial infections in specimens from pregnant women affected by spontaneous miscarriage. *J. Cell. Physiol.,* **2019**, *234*(1), 100-107. http://dx.doi.org/10.1002/jcp.26952 PMID: 30078192

[2] Pfeilmeier, S.; Caly, D.L.; Malone, J.G. Bacterial pathogenesis of plants: future challenges from a microbial perspective. *Mol. Plant Pathol.,* **2016**, *17*(8), 1298-1313. http://dx.doi.org/10.1111/mpp.12427 PMID: 27170435

[3] Uzal, F.A.; Arroyo, L.G.; Navarro, M.A.; Gomez, D.E.; Asín, J.; Henderson, E. Bacterial and viral enterocolitis in horses: A review. *J. Vet. Diagn. Invest.,* **2022**, *34*(3), 354-375. http://dx.doi.org/10.1177/10406387211057469 PMID: 34763560

[4] Cuddy, M.L.S. Centers for Disease Control and Prevention. *Antibiotic resistance.,* **2005**. https://www.cdc.gov/drugresistance/about.html

[5] Wright, A.J. The Penicillins. *Mayo Clin. Proc.,* **1999**, *74*(3), 290-307. http://dx.doi.org/10.4065/74.3.290 PMID: 10090000

[6] World Health Organization. **2020**. https://www.who.int/health-topics/antimicrobial-resistance

[7] Rajamanickam, K.; Yang, J.; Sakharkar, M.K. Gallic acid potentiates the antimicrobial activity of tulathromycin against two key bovine respiratory disease (BRD) causing-pathogens. *Front. Pharmacol.,* **2019**, *9*, 1486. http://dx.doi.org/10.3389/fphar.2018.01486 PMID: 30662404

[8] Sherif, M.M.; Elkhatib, W.F.; Khalaf, W.S.; Elleboudy, N.S.; Abdelaziz, N.A. Multidrug resistant *Acinetobacter baumannii* biofilms: Evaluation of phenotypic–genotypic association and susceptibility to cinnamic and gallic acids. *Front. Microbiol.,* **2021**, *12*(September), 716627. http://dx.doi.org/10.3389/fmicb.2021.716627 PMID: 34650528

[9] Zhao, B.; Hu, M. Gallic acid reduces cell viability, proliferation, invasion and angiogenesis in human cervical cancer cells. *Oncol. Lett.,* **2013**, *6*(6), 1749-1755. http://dx.doi.org/10.3892/ol.2013.1632 PMID: 24843386

[10] Kahkeshani, N.; Farzaei, F.; Fotouhi, M.; Alavi, S.S.; Bahramsoltani, R.; Naseri, R.; Momtaz, S.; Abbasabadi, Z.; Rahimi, R.; Farzaei, M.H.; Bishayee, A. Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iran. J. Basic Med. Sci.,* **2019**, *22*(3), 225-237. http://dx.doi.org/10.22038/ijbms.2019.32806.7897 PMID: 31156781

[11] Nayeem, N.; Smb, A. Gallic acid: A promising lead molecule for drug development. *J. Appl. Pharm.,* **2016**, *8*(2), 8-11. http://dx.doi.org/10.4172/1920-4159.1000213

[12] Rajput, S.B.; Shinde, R.B.; Routh, M.M.; Karuppayil, S.M. Anti-Candida properties of asaronaldehyde of *Acorus gramineus* rhizome and three structural isomers. *Chin. Med.,* **2013**, *8*(1), 18. http://dx.doi.org/10.1186/1749-8546-8-18 PMID: 24010893

[13] Dos Santos, E.D.A.; Prado, P.C.; De Carvalho, W.R.; De Lima, R.V.; Beatriz, A.; De Lima, D.P.; Hamel, E.; Dyba, M.A.; Albuquerque, S. Synthesis and biological activity of sulfur compounds showing structural analogy with combretastatin A-4. *Quimica Nova.,* 2013, *36*(2), 279-283. http://dx.doi.org/10.1590/S0100-40422013000200014

[14] Kolb, M. Synthetic communications: An international journal for rapid communication of synthetic organic chemistry. *Synth. Commun.,* **1993**, *23*(1), 7. http://dx.doi.org/10.1080/00397919308020392

[15] Oshimizu, S.; Takeshi, S.; Sato, T.; Yamakado, R.; Tatewaki, Y.; Okada, S. Synthesis and solid-state polymerization of 5-(pyren-1-yl)penta-2,4- diyn-1-ol derivatives with an n-phenylurethane or n-benzylurethane group. *Cryst. Growth Des.,* **2020**, *20*(10), 6356-6365. http://dx.doi.org/10.1021/acs.cgd.0c00438

[16] Prakasham, A.P.; Saxena, A.K.; Luqman, S.; Chanda, D.; Kaur, T.; Gupta, A.; Yadav, D.K.; Chanotiya, C.S.; Shanker, K.; Khan, F.; Negi, A.S. Synthesis and anticancer activity of 2-benzylidene indanones through inhibiting tubulin polymerization. *Bioorg. Med. Chem.,* **2012**, *20*(9), 3049-3057. http://dx.doi.org/10.1016/j.bmc.2012.02.057 PMID: 22472045

[17] Wang, J.; Xia, F.; Jin, W.B.; Guan, J.Y.; Zhao, H. Efficient synthesis and antioxidant activities of N-heterocyclyl substituted Coenzyme Q analogues. *Bioorg. Chem.,* **2016**, *68*, 214-218. http://dx.doi.org/10.1016/j.bioorg.2016.08.008 PMID: 27561631

[18] Nomura, K.; Terwilliger, P. Simple and convenient two step synthesis of 5-bromo-2,3-dimethoxy-6-methyl-1,4-benzoquinone. *Spec. Matrices,* **2019**, *7*(1), 1-19.

http://dx.doi.org/10.1515/spma-2019-0001

[19] Greene, L.E.; Godin, R.; Cosa, G. Fluorogenic ubiquinone analogue for monitoring chemical and biological redox processes. *J. Am. Chem. Soc.,* **2016**, *138*(35), 11327-11334. http://dx.doi.org/10.1021/jacs.6b06899 PMID: 27508986

[20] Theodorou, V.; Skobridis, K.; Tzakos, A.G.; Ragoussis, V. A simple method for the alkaline hydrolysis of esters. *Tetrahedron Lett.,* **2007**, *48*(46), 8230-8233. http://dx.doi.org/10.1016/j.tetlet.2007.09.074

[21] Risch, P.; Pfeifer, T.; Segrestaa, J.; Fretz, H.; Pothier, J. Verification of the major metabolic oxidation path for the naphthoyl group in chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTh2) antagonist 2-(2-(1-naphthoyl)-8-fluoro-3,4-dihydro-1 H -pyrido[4,3- b]indol-5(2 H)-yl)acetic Acid. *J. Med. Chem.,* **2015**, *58*(20), 8011-8035. http://dx.doi.org/10.1021/acs.jmedchem.5b00824 PMID: 26398218

[22] Cong, Z.Q.; Wang, C.I.; Chen, T.; Yin, B.Z. Efficient and rapid method for the oxidation of electron-rich aromatic aldehydes to carboxylic acids using improved basic hydrogen peroxide. *Synth. Commun.,* **2006**, *36*(5), 679-683. http://dx.doi.org/10.1080/00397910500408456

[23] Yamaura, M.; Suzuki, K.; Tsuruga, T.; Hiranuma, K. Synthesis of 7,8-Dihydroxy-5-hydroxymethyl-2-phenyl-chroman-4-one; the aglycon of actinoflavoside. *Synlett,* **2004**, *1*(1), 116-118. http://dx.doi.org/10.1055/s-2003-43365

[24] LibreTexts. Ashenhurst, J. Oxidation by PCC (pyridinium chlorochromate) **2020**. https://chem.libretexts.org/@go/page/15407

[25] Ozbak, H.A. A novel high-resolution melting analysis approach for rapid detection of vancomycin-resistant enterococci. *Ann. Saudi Med.,* **2018**, *38*(3), 200-207. http://dx.doi.org/10.5144/0256-4947.2018.200 PMID: 29848938

[26] Rasheed, J.K.; Anderson, G.J.; Yigit, H.; Queenan, A.M.; Doménech-Sánchez, A.; Swenson, J.M.; Biddle, J.W.; Ferraro, M.J.; Jacoby, G.A.; Tenover, F.C. Characterization of the extended-spectrum β-lactamase reference strain, *Klebsiella pneumoniae* K6 (ATCC 700603), which produces the novel enzyme SHV-18. *Antimicrob. Agents Chemother.,* **2000**, *44*(9), 2382-2388. http://dx.doi.org/10.1128/AAC.44.9.2382-2388.2000 PMID: 10952583

[27] Pachori, P.; Gothalwal, R.; Gandhi, P. Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes Dis.,* **2019**, *6*(2), 109-119. http://dx.doi.org/10.1016/j.gendis.2019.04.001 PMID: 31194018

[28] V T Nair, D.; Venkitanarayanan, K.; Kollanoor, J.A. Antibiotic-resistant *Salmonella* in the food supply and the potential role of antibiotic alternatives for control. *Foods,* **2018**, *7*(10), 167. http://dx.doi.org/10.3390/foods7100167 PMID: 30314348

[29] Hamidian, M.; Blasco, L.; Tillman, L.N.; To, J.; Tomas, M.; Myers, G.S.A. Analysis of complete genome sequence of *Acinetobacter baumannii* strain ATCC 19606 reveals novel mobile Genetic elements and novel prophage. *Microorganisms,* **2020**, *8*(12), 1851. http://dx.doi.org/10.3390/microorganisms8121851 PMID: 33255319

[30] Yigit, H.; Anderson, G.J.; Biddle, J.W.; Steward, C.D.; Rasheed, J.K.; Valera, L.L.; McGowan, J.E., Jr; Tenover, F.C. Carbapenem resistance in a clinical isolate of *Enterobacter aerogenes* is associated with decreased expression of OmpF and OmpC porin analogs. *Antimicrob. Agents Chemother.,* **2002**, *46*(12), 3817-3822. http://dx.doi.org/10.1128/AAC.46.12.3817-3822.2002 PMID: 12435682

[31] Mezger, A.; Gullberg, E.; Göransson, J.; Zorzet, A.; Herthnek, D.; Tano, E.; Nilsson, M.; Andersson, D.I. A general method for rapid determination of antibiotic susceptibility and species in bacterial infections. *J. Clin. Microbiol.,* **2015**, *53*(2), 425-432. http://dx.doi.org/10.1128/JCM.02434-14 PMID: 25411178

[32] Silva, A.A.; Santos, I.F.; Anjos, M.M.; Pascoli, I.C.; Ruiz, S.P.; Graton M.J.M.; Machinski, M., Junior; Ueda N.T.; Nakamura, C.V.; Abreu F.B.A. Evaluation of the antibacterial and sporicidal activity of the essential oils of *Copaifera multijuga* and *Thymus vulgaris* against *Alicyclobacillus acidoterrestris. Int. Food Res. J.,* **2019**, *26*(4), 1327-1337.

[33] Mitra, I.; Mukherjee, S.; Reddy B, V.P.; Dasgupta, S.; Bose K, J.C.; Mukherjee, S.; Linert, W.; Moi, S.C. Benzimidazole based Pt(II) complexes with better normal cell viability than cisplatin: synthesis, substitution behavior, cytotoxicity, DNA binding and DFT study. *RSC Advances,* **2016**, *6*(80), 76600-76613. http://dx.doi.org/10.1039/C6RA17788C

[34] Cell Biology Protocols. *How to calculate IC50.,* https://www.sciencegateway.org/protocols/cellbio/drug/hcic50.htm

[35] Medina, E.; Pieper, D.H. Tackling threats and future problems of multidrug-resistant bacteria. In: *Curr Top Microbiol Immunol*; , **2016**; 398, pp. 3-33. http://dx.doi.org/10.1007/82_2016_492

[36] Otto Chemie pvt ltd.. 3,4,5-Trimethoxybenzyl alcohol, 98% https://www.ottokemi.com/alcohols-compounds/345-trimethoxybenzyl-alcohol-98.aspx

[37] Longkumer, N.; Richa, K.; Karmaker, R.; Kuotsu, V.; Supong, A.; Jamir, L.; Bharali, P.; Bora S.U. Green synthesis of bromo organic molecules and investigations on their antibacterial properties: An experimental and computational approach. *Acta Chim. Slov.,* **2019**, *66*(2), 276-283. http://dx.doi.org/10.17344/acsi.2018.4580 PMID: 33855504

[38] Ahmed, D.; Anwar, A.; Khan, A.K.; Ahmed, A.; Shah, M.R.; Khan, N.A. Size selectivity in antibiofilm activity of 3-(diphenylphosphino)propanoic acid coated gold nanomaterials against Gram-positive *Staphylococcus aureus* and *Streptococcus mutans. AMB Express,* **2017**, *7*(1), 210. http://dx.doi.org/10.1186/s13568-017-0515-x PMID: 29164404