SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITIES OF HYDROXYTRIAZENES AND THEIR COPPER (II) COMPLEXES

Kariuki, A.F. and Njagi, E.C.
Department of Physical Sciences, Chuka University, P. O. Box 109-60400, Chuka, Kenya
Email: echomba@chuka.ac.ke

ABSTRACT
Bacterial pathogens that are resistant to current antibacterial drugs pose serious clinical challenges including high treatment costs, increased mortalities and opportunistic infections during surgical operations and in immunocompromised patients. There is therefore an urgent need to develop novel antibiotics to counter threats posed by resistant bacterial pathogens. In this study, hydroxytriazene ligands and their copper (II) complexes were synthesized by the coupling of aryl hydroxylamines and diazonium salts of aromatic amines. The synthesized compounds were characterized by micro-elemental analysis, Fourier Transform Infrared (FT-IR) spectroscopy, Ultraviolet-Visible (UV-Vis) spectroscopy, Proton-Nuclear Magnetic Resonance (^{1}H-NMR) spectroscopy and molar conductivity measurements. The target hydroxytriazene ligands and their copper (II) complexes were obtained in high yields and purity. The synthesized complexes were non-electrolytes and exhibited a 1:2 metal to ligand stoichiometry. The synthesized compounds were all inactive against *Pseudomonas putida*, *Salmonella enteriditis*, *Klebsiella pneumoniae*, enteropathogenic *Escherichia coli*, enteroaggregative *Escherichia coli*, and enterohaemorrhagic *Escherichia coli*. Ligands (**L_1-L_5**) were active against *Vibrio cholera* (25-50 μg/ml) and *Proteus mirabilis* (12.5-25 μg/ml). Complexes **Cu-L_1** and **Cu-L_2** were active against *Vibrio cholerae* (50 μg/ml) while complexes **Cu-L_1** to **Cu-L_4** were active against *Proteus mirabilis* (12.5-25 μg/ml). The synthesized compounds are promising antibacterial agents for both Gram-positive and Gram-negative bacterial strains.
Keywords: Hydroxytriazenes, Bacterial resistance, Novel drugs, Antibacterial drugs

INTRODUCTION

Bacterial pathogens are increasingly becoming resistant to antibacterial drugs currently in use. Clinical cases of methicillin-resistant *Staphyloccus aureus*, vancomycin-resistant *enterococci* and multidrug resistant *Pseudomonas aeruginosa* have been reported worldwide (Nordmann *et al*., 2007; El′Garch *et al*., 2007). *Klebsiella pneumoniae*, the causative agent of several diseases (e.g. pneumonia, urinary tract infections, and bacteremia), has developed resistance to many classes of antibacterial drugs including aminoglycosides, cephalosporins, fluoroquinolones, tetracyclines, chloramphenicol and co-trimoxazole (Sikarwar and Batra, 2011; Nathisuwan *et al.*, 2001). Antimicrobial resistance by strains of *Escherichia coli* to first-line drugs such as fluoroquinolones and β-lactam antibiotics is causing huge clinical challenges (Fanning *et al*., 2011; Shaikh *et al*., 2014).

Vibrio cholerae, the causative agent of cholera, is increasingly becoming resistant to common antibiotics including sulfamethoxazole, co-trimoxazole, trimethoprim, chloramphenicol, ampicillin, tetracycline, nalidixic acid and gentamicin (Rahmani *et al*., 2012; Okoh and Igbinosa, 2010). Antibiotic resistance by *Shigella spp.* has been reported across several classes of antibacterial agents including ampicillin, nalidixic acid, tetracycline, co-trimoxazole (Mardanesh *et al*., 2013; Singhania *et al*., 2012). Moreover, antimicrobial resistance to cephalosporins and fluoroquinolones by *Proteus mirabilis* is increasingly being reported worldwide (Wu *et al*., 2008; Saito *et al*., 2007).

The emergence of multidrug resistant bacterial pathogens is creating serious clinical challenges including diagnostic uncertainties, increased treatment costs and high rates of morbidities and mortalities (Santajit and Indrawattana, 2016). Medical operations (e.g. surgeries) which are dependent on antibiotics for treatment of opportunistic infections are threatened by emergence of multi-drug resistant bacterial pathogens. Moreover, multidrug resistant bacterial strains are complicating treatment of opportunistic infections in immunocompromised patients. There is therefore an urgent need to develop novel antibiotics to counter threats posed by multidrug resistant bacterial pathogens (Penchovsky and Traykovska, 2015).

Hydroxytriazenes and their transition metal complexes are increasingly being explored for potential use as antimicrobial agents. These compounds have exhibited antibacterial (Kodli *et al*, 2014; Chundawat *et al*., 2014; Singh *et al*., 2014), antifungal (Joshi *et al*, 2010), anti-inflammatory (Jain *et al*, 2010) and insecticidal activities (Jodha *et al*, 2014; Ombaka, 2011). The biological activities of hydroxytriazenes can be tuned through variation of substituents on aryl groups and

complexation to optimize their electronic properties and hydrophobicity (Kumar *et al*., 2014; El-Sawaf, 2016; Hasi *et al*., 2016). In this study, select hydroxytriazenes and their Cu (II) complexes were synthesized, characterized and their antimicrobial activities evaluated.

MATERIALS AND METHODS

Chemicals and solvents used in this study were of analytical grade. Nitrobenzene (98%), 3-nitrotoluene (98%) , 4-aminobenzoic acid (99%), 3-aminobenzoic acid (98%), 4-toluidine (99%), absolute methanol (99%), acetone (98%), 1,4-dioxane (98%), Mueller Hinton Agar (99%), Luria Bertania broth medium (99%) and sodium chloride (98%) were obtained from Sigma Aldrich. Copper (II) acetate monohydrate (98%), sodium nitrite (98%) and bacto-tryptone (98%) were sourced from Merck. Zinc dust (99%) and dimethyl sulfoxide (98%) were purchased from Fluka and Finar, respectively. Chemicals and solvents were used as purchased without any further purification.

Synthesis of Hydroxytriazene Ligands

The hydroxytriazene ligands were synthesized following a standard method reported in the literature (Khan *et al*, 2013). In a typical synthetic protocol, a nitro aryl compound (0.1 mol) was reduced to aryl hydroxylamine with 20 g of Zn dust in the presence of 7.5 g of ammonium chloride (NH_4Cl) in a water-alcohol medium at 50-60°C. Then, a primary aromatic amine (0.1 mol) was diazotized with 0.1 mol sodium nitrite ($NaNO_2$) in an acidic solution containing 25 mL of HCl and 25 mL of distilled water. The aryl hydroxylamine and the diazonium salt were subsequently coupled at 0-5ºC in an acetate buffer (pH 5-6) medium to yield the hydroxytriazene ligands. The synthesized ligands were designated **L_1-L_5**.

Synthesis of Copper (II) Complexes

Copper (II) complexes were synthesized following a method described in the literature (Aliyu and Mohammad, 2012). Briefly, 0.002 mol of copper (II) acetate monohydrate was dissolved in 15 mL of double distilled water and the pH of the resultant solution adjusted to ca. 5 with an acetic acid-sodium acetate buffer. In a separate beaker, 0.004 mol of the ligand was dissolved in a minimum amount of hot ethanol and the pH of the solution adjusted to ~ 5 with an acetic acid-sodium acetate buffer. The metal solution was then added dropwise to the warm solution (40-50 ºC) of the ligand with continuous stirring.

The mixture was left to boil for 1 h with continuous stirring. The resultant precipitate was filtered under reduced pressure, washed thoroughly with distilled water and hot ethanol and dried in an oven at 70ºC. The synthesized compounds were designated **Cu-L_1** to **Cu-L_5** with **L_1-L_5** indicating the ligand used to synthesize the respective copper (II) complex.

Characterization of the Synthesized Compounds

The melting points of the synthesized compounds were determined with open glass capillaries using a MPA-12 Melting Point Apparatus. The elemental composition (C, H, N) of the ligands was determined using a Vario EL III Elementar CHNS analyzer. The amount of oxygen in the ligands was determined by difference. The concentration of copper in the complexes was determined by Flame Atomic Absorption (FAAS) spectroscopy using a PGI 990 Atomic Absorption Spectrophotometer. The functional groups present in the ligands and complexes were determined by Fourier Transform Infrared (FT-IR) spectroscopy using the KBr method (Shimadzu IRAffinity-1S FT-IR spectrophotometer).The chemical environment of protons in the ligands was studied by Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy. Spectra were recorded on a Bruker Ultra-shield 400 MHz spectrometer using deuterated DMSO-d_6 as the solvent and tetramethylsilane (TMS) as the internal standard. The electronic properties of the ligands and the complexes were determined by Ultraviolet-Visible (UV-Vis) spectroscopy using a Shimadzu UV-1800 double-beam spectrophotometer. The molar conductivities of the complexes were determined in dimethyl sulfoxide (DMSO) using a HANNA Instruments EC 215 Conductivity meter.

Antibacterial Assays

In vitro screening tests were done to investigate the antibacterial efficacy of the synthesized compounds. The bacterial strains tested included enteropathogenic *Escherichia coli* (EPEC), enteroaggregative *Escherichia coli* (EAEC), enterohaemorrhagic *Escherichia coli* (EHEC), *Pseudomonas putida*, *Proteus mirabilis*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Vibrio cholerae*, *Klebsiella pneumoniae* and *Salmonella enteritidis*. The strains used in the tests were clinical isolates obtained from Kenya Medical Research Institute. Antibacterial testing was done by disk diffusion method (Jorgensen and Ferraro, 2009) and tube dilution method (CLSI, 2012). Bacterial strains were first sub-cultured from freezer stocks onto Mueller Hinton agar plates and incubated at 37°C overnight.

In disk diffusion tests, 2000 μg/ml stock solutions of the ligands and the complexes were prepared in DMSO. The stock solutions were then diluted to prepare solutions of 1000, 500, 250, 125 and 62.5 μg/ml concentrations. Sterile filter paper disks (6 mm diameter) were soaked in the dilutions, then removed

and allowed to dry. The test bacteria were inoculated on Mueller Hinton Agar (MHA) by streaking with the aid of a spreader. The dry paper disks were then placed at equidistant positions on the inoculated MHA. A paper disk impregnated with DMSO was placed in each plate to serve as solvent control. The plates were incubated at 37°C for 24 h. The diameters of inhibition zones (in mm) of triplicate sets were measured and recorded at the elapse of the incubation period.

The minimum inhibitory concentration (MIC) of the synthesized compounds was carried out by tube dilution method in two-fold serial dilutions. Stock solutions (1000 μg/ml) of the test compounds were prepared in DMSO, then serial diluted to give solutions of 100, 50, 25 and 12.5 μg/ml concentrations. The inoculum was prepared by growing the bacteria at 37°C in Mueller Hinton Agar. Discrete colonies were picked with a loop and emulsified in 0.45% (w/v) sterile aqueous normal saline. The suspension optical density was standardized to a McFarland density of 0.5 (equivalent to 10^8 CFU/ml) with the aid of a DensiChekTM densitometer (bioMerieux, USA) apparatus. Then 1 ml of this adjusted inoculum was added to tubes containing Luria Bertania (LB) broth and different concentrations of the test compounds.

An un-inoculated tube of LB was incubated to serve as a negative growth control. In a different tube about 2 ml of DMSO was added and the tube was inoculated with test bacteria to serve as solvent control. The tubes were incubated at 37ºC for 24 h. At the elapse of the incubation period, all the tubes were examined for turbidity. The presence of turbidity was an indication of bacterial growth while a clear solution signaled inhibition of the test microbes. The lowest concentration of compounds that inhibited growth of the microbes was designated the minimum inhibitory concentration. All the tests were done in triplicates.

Physical Properties of the Synthesized Compounds

Compound **L_1** was synthesized by the coupling of the aryl hydroxylamine derived from nitrobenzene and the diazonium salt derived from 4-aminobenzoic acid. The target ligand was 3-Hydroxy-3-phenyl-*p*-carboxyphenyl triazene. Yellow crystals were obtained with a yield of 66% and a melting point of 169°C. Compound **L_2** was synthesized by the coupling of the aryl hydroxylamine derived from nitrobenzene and the diazonium salt derived from 3-aminobenzoic acid. The target ligand was 3-Hydroxy-3-phenyl-*m*-carboxyphenyl triazene. Brown crystals were obtained with a yield of 61% and a melting point of 160°C. Compound **L_3** was synthesized by the coupling of the aryl hydroxylamine derived from 3-nitrotoluene and the diazonium salt derived from 3-aminobenzoic acid. The target ligand was 3-Hydroxy-3-*m*-tolyl-1-*m*-carboxyphenyl triazene.

Yellow crystals were obtained with a yield of 64% and a melting point of 158°C. Compound **L_4** was synthesized by the coupling of the aryl hydroxylamine derived from 3-nitrotoluene and the diazonium salt derived from 4-aminobenzoic acid. The target ligand was 3-Hydroxy-3-*m*-tolyl-1-*p*-carboxyphenyl triazene. Yellow crystals were obtained with a yield of 74% and a melting point of 176 °C. Compound **L_5** was synthesized by the coupling of the aryl hydroxylamine derived from 3-nitrotoluene and the diazonium salt derived from 4-toluidine. The target ligand was 3-Hydroxy-3-*m*-tolyl-1-*p*-carboxyphenyl triazene. Yellow crystals were obtained with a yield of 56% and a melting point of 122°C.

Micro-Elemental Analysis

The synthesized ligands (**L_1-L_5**) were composed of carbon, hydrogen, nitrogen and oxygen. The physical properties and mass percentages of elements in each ligand are summarized in Table 1.

RESULTS

Table 1: Physical properties and Elemental Composition of the Synthesized Ligands

Ligand	Color	M.P. (°C)	Yield (%)	Percent Mass (%) C	H	N	O	Molecular formula
L_1	Yellow	169	66	60.70 (61.14)	4.32 (4.32)	16.33 (17.67)	18.65 (16.87)	$C_{13}H_{11}N_3O_3$
L_2	Brown	160	61	60.70 (59.31)	4.32 (4.50)	16.33 (17.66)	18.65 (18.53)	$C_{13}H_{11}N_3O_3$
L_3	Yellow	158	64	61.99 (61.45)	4.83 (4.94)	15.49 (16.39)	17.69 (17.22)	$C_{14}H_{13}N_3O_3$
L_4	Yellow	176	74	61.99 (61.25)	4.83 (4.95)	15.49 (16.39)	17.69 (17.41)	$C_{14}H_{13}N_3O_3$
L_5	Yellow	122	56	69.69 (69.29)	6.27 (6.26)	17.41 (16.70)	6.63 (7.75)	$C_{14}H_{15}N_3O$

Theoretical mass percent values are given in bracket, M.P = Melting Point

Table 2: Physical Properties and Elemental Composition of the Synthesized Copper (II) complexes

Complex	Molecular formula	MW	Percent mass (%)			
			C	H	N	Cu
Cu-L_1	$[Cu(C_{13}H_9N_3O_3)_2]$	574.38	55.67(54.37)	3.46(3.16)	14.68(14.63)	11.56(11.06)
Cu-L_2	$[Cu(C_{13}H_9N_3O_3)_2]$	574.38	55.41(54.37)	3.81(3.16)	14.73(14.63)	11.67(11.06)
Cu-L_3	$[Cu(C_{14}H_{11}N_3O_3)_2]$	602.55	56.09(55.81)	4.16(3.68)	14.24(13.95)	10.71(10.55)
Cu-L_4	$[Cu(C_{14}H_{11}N_3O_3)_2]$	602.55	54.30(55.81)	3.90(3.68)	13.82(13.95)	10.89(10.55)
Cu-L_5	$[Cu(C_{14}H_{13}N_3O)_2]$	542.46	61.75(61.99)	5.32(4.83)	15.76(15.49)	11.74(11.71)

Theoretical mass percent values are given in bracket, MW = Molecular weight

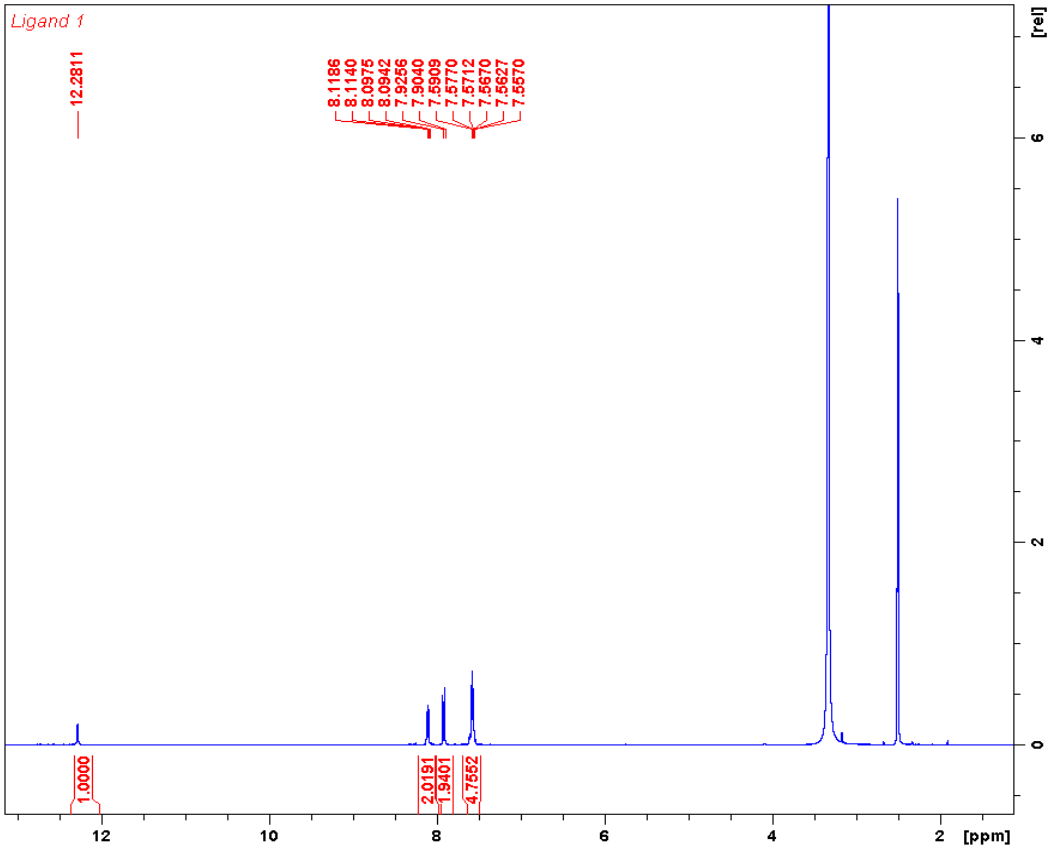


Figure 1: ^{1}H NMR Spectrum of the L_1 Compound

The synthesized copper (II) complexes (**Cu-L_1** to **Cu-L_5**) were composed of copper, carbon, hydrogen, nitrogen and oxygen. These complexes were green-yellowish with melting points >300 °C. The compounds **Cu-L_1** to **Cu-L_5** were all obtained in high yields of 97.5, 98.4, 96.3, 95.4 and 96.8%, respectively. The molar conductance of **Cu-L_1** to **Cu-L_5** were 2.5, 4.9, 2.2, 1.2 and 0.8 S cm^2 mol^{-1}, respectively. The physical properties and elemental composition of complexes are summarized in Table 2.

Spectroscopic Analyses

Figure 1 shows a representative ^{1}H NMR spectrum of the synthesized compounds. The chemical shifts (δ, ppm) and multiplicities (singlet, s; multiplet, m) of signals in the ^{1}H NMR spectra of compounds **L_1-L_5** were: **L_1**: 7.55-8.11 ppm (m) and 12.28 ppm (s); **L_2**: 7.44- 8.07 ppm (m) and 12.15 ppm (s); **L_3**: 7.34-8.03 ppm (m) and 12.12 ppm (s); **L_4**: 2.43 ppm (s), 7.35-7.92 ppm (m) and 12.25 ppm (s); **L_5**: 2.26 ppm (s), 7.13-7.88 ppm (m) and 11.87 (s).

Figure 2 shows a representative FT-IR spectrum of the synthesized compounds. The vibrational frequencies (cm^{-1}) of the major bands in the FT-IR spectra of **L_1-L_5** compounds are as follows: **L_1**: 1220, 1288, 1314, 1426, 1524, 1678, and 3209 cm^{-1}: **L_2**: 1210, 1275, 1337, 1434, 1508, 1678, and 3206 cm^{-1}. **L_3**: 1219, 1277, 1334, 1420, 1507, 1689 and 3201cm^{-1}. **L_4**:1206, 1289, 1311, 1431, 1523, 1683 and 3181cm^{-1}. **L_5**: 1217, 1289, 1304, 1304, 1455, 1521 and 3137 cm^{-1}. The vibrational frequencies of the **Cu-L_1 to Cu-L_5** compounds were:

Cu-L_1: 484, 545, 1172, 1293, 1317, 1411 and 1690. **Cu-L_2**: 497, 541, 1161, 1287, 1345, 1403, 1689. **Cu-L_3**: 495, 566, 1188, 1283, 1337, 1413, 1688. **Cu-L_4**: 506, 554, 1171, 1291, 1311, 1402 and 1693. **Cu-L_5**: 489, 514, 1175, 1296, 1317 and 1401.

Figure 3 shows a representative UV-Vis absorption spectrum of the synthesized materials. The spectra of all ligands exhibited an intense broad band in the 355.00-363.60 nm range. In addition, the spectra showed a weak absorption band in the 258.00-271.20 nm range. Similarly, the spectra of the copper (II) complexes exhibited a weak broad band at 370.00-383.40 nm and a high intensity band at 273.80-276.88 nm. However, the λ_{max} of the two peaks were shifted to higher wavelengths relative to those of constituent ligands. The UV-Vis results of the synthesized compounds are summarized in Table 3.

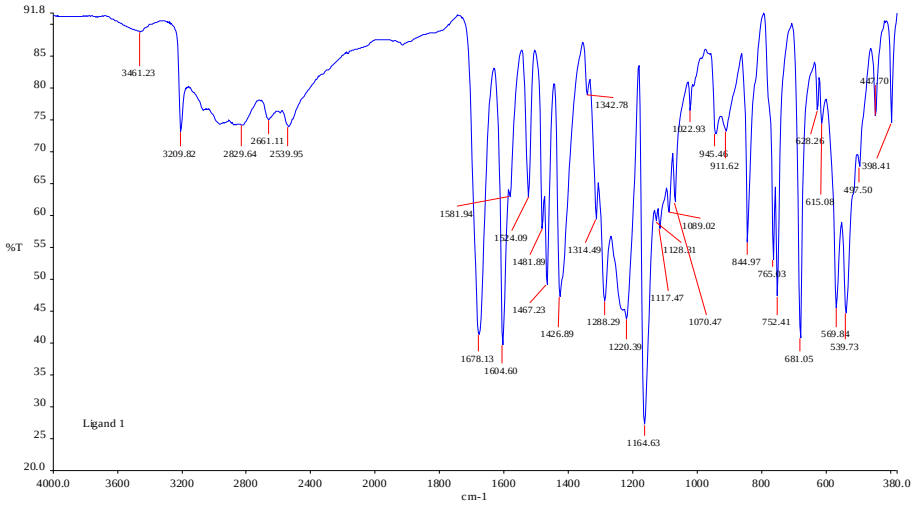


Figure 2: FT-IR Spectrum of the L_1 Compound

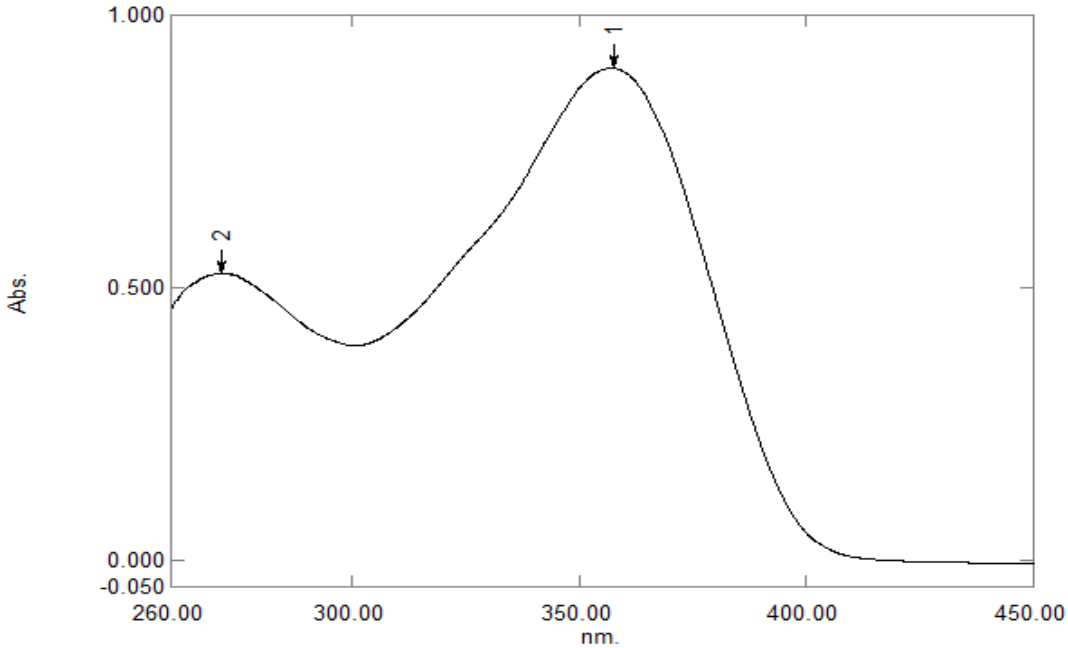


Figure 3: The UV-Vis Spectrum of the L_1 Compound (2.5 x 10^{-5} M in DMSO)

Antibacterial Studies

With the disk diffusion method, there were no observable zones of inhibition for all concentrations used against the bacterial strains presumably due to the poor diffusibility of test compounds in the agar medium. The antibacterial activities of the test

compounds were further investigated by tube dilution method (12.5-100 μg/ml). Antibacterial data obtained by the tube dilution method is tabulated in Table 4.

The synthesized compounds were all inactive against *Pseudomonas putida*, *Salmonella enteriditis*, *Klebsiella pneumoniae*, enteropathogenic *Escherichia coli*, enteroaggregative *Escherichia coli*, and enterohaemorrhagic *Escherichia coli*. The ligands (**L_1-L_5**) were active against *Vibrio cholera* (25-50 μg/ml) and *Proteus mirabilis* (12.5-25 μg/ml). Ligands **L_1-L_4** were active against *Shigella dysenteriae* (25 μg/ml) and *Staphylococcus aureus* (50 μg/ml). The copper (II) complexes were all inactive against *Shigella dysenteriae* and *Staphylococcus aureus*. However, complexes **Cu-L_1** and **Cu-L_2** were active against *Vibrio cholerae* (50 μg/ml) while complexes **Cu-L_1** to **Cu-L_4** were active against *Proteus mirabilis* (12.5-25 μg/ml).

Table 3: The UV-Vis Absorption Maxima of the Synthesized Compounds

λ_{max} (nm)	L_1	Cu-L_1	L_2	Cu-L_2	L_3	Cu-L_3	L_4	Cu-L_4	L_5	Cu-L_5
λ_{max1}	362.20	381.20	357.60	378.00	357.20	378.20	363.60	383.40	355.00	370.00
λ_{max2}	269.00	274.60	271.20	274.00	258.00	274.00	269.60	273.80	263.80	276.88

Table 4: Minimum inhibitory concentrations (MIC) of the synthesized compounds

Test compounds	Bacterial strains Minimum Inhibitory Concentration (MIC) (μg/ml)									
	EPEC	**Pp**	**Se**	**Vc**	**EAEC**	**EHEC**	**Kp**	**Pm**	**Sd**	**Sa**
L_1	ND	ND	ND	50	ND	ND	ND	25	25	50
L_2	ND	ND	ND	50	ND	ND	ND	25	25	50
L_3	ND	ND	ND	25	ND	ND	ND	12.5	25	50
L_4	ND	ND	ND	25	ND	ND	ND	12.5	25	50
L_5	ND	ND	ND	50	ND	ND	ND	25	ND	ND
Cu-L_1	ND	ND	ND	50	ND	ND	ND	25	ND	ND
Cu-L_2	ND	ND	ND	50	ND	ND	ND	12.5	ND	ND
Cu-L_3	ND	ND	ND	ND	ND	ND	ND	25	ND	ND
Cu-L_4	ND	ND	ND	ND	ND	ND	ND	25	ND	ND
Cu-L_5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
DMSO	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Pp - *Pseudomonas putida* Kp - *Klebsiella pneumoniae* Sa - *Staphylococcus aureus*
Se - *Salmonella enteriditis* Pm - *Proteus mirabilis* Sd - *Shigella dysenteriae*
Vc - *Vibrio cholera* ND - No MIC detected EPEC- *Escherichia coli*
EAEC- enteroaggregative *Escherichia coli* EHEC- enterohaemorrhagic *Escherichia coli*

DISCUSSION

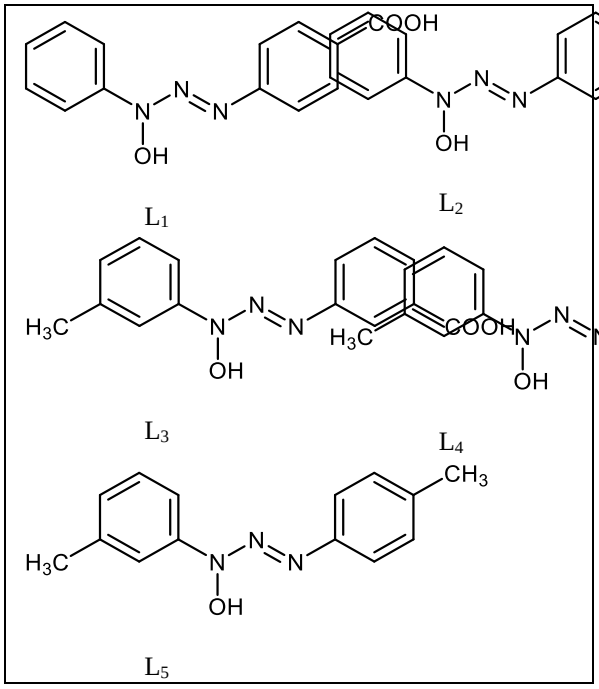
The calculated mass percentages of elements in the synthesized compounds are in good agreement with theoretical values of the target compounds. The calculated molecular formulas of the ligands are as follows: **L_1** (3-Hydroxy-3-phenyl-p-carboxyphenyl triazene): $C_{13}H_{11}N_3O_3$; **L_2** (3-Hydroxy-3-phenyl-*m*-carboxyphenyl triazene): $C_{13}H_{11}N_3O_3$; **L_3** (3-Hydroxy-3-*m*-tolyl-1-*m*-carboxyphenyl triazene): $C_{14}H_{13}N_3O_3$; **L_4** (3-Hydroxy-3-*m*-tolyl-1-*p*-carboxyphenyl triazene): $C_{14}H_{13}N_3O_3$; and **L_5** (3-Hydroxy-3-*m*-tolyl-1-*p*-carboxyphenyl triazene): $C_{14}H_{13}N_3O_3$. The molecular formulas of copper (II) complexes were: **Cu-L_1**: $Cu(C_{13}H_9N_3O_3)_2$; **Cu-L_2**: $Cu(C_{13}H_9N_3O_3)_2$; **Cu-L_3**: $Cu(C_{14}H_{11}N_3O_3)_2$; **Cu-L_4**: $Cu(C_{14}H_{11}N_3O_3)_2$; and **Cu-L_5**: $Cu(C_{14}H_{13}N_3O)_2$. The metal to ligand ratio in the copper (II) complexes was 1:2. The low molar conductivities of the complexes indicate that they are non-electrolytes.

The spectra of the five ligands showed a singlet signal at δ 11.87-12.28 ppm attributed to the proton of the OH group (Soni *et al*., 2016). Multiplet signals observed at δ 7.13-8.11 ppm are due to the presence of aromatic protons in the hydroxytriazene ligands (Soni *et al*., 2016). Ligands **L_3**, **L_4** and **L_5** also displayed signals at δ 2.26- 2.43 ppm, which were assigned to the protons of the methyl groups (Agrawal *et al*., 2016).

The FT-IR absorption frequencies of the synthesized compounds are comparable to those of hydroxytriazene ligands reported in the literature (Tomar *et al*., 2014). The spectra of the ligands and those of corresponding copper (II) complexes showed significant differences. The band appearing at 3137- 3209 cm^{-1}, attributed to the N-H stretching mode, is absent in the spectra of copper (II) complexes. The disappearance of this band strongly suggests the participation of the N-atom in

formation of the complexes through deprotonation of NH group (Oliveria *et al*., 2009). Moreover, the band at 1507-1524 cm^{-1}, attributed to N-H bending mode, is also absent due to deprotonation of the N-H group.

The new bands appearing in the spectra of copper (II) complexes at 484- 506 cm^{-1} can be assigned to the Cu-N vibrational modes (Muthukumar *et al*., 2016; Thakar *et al*., A., 2011). Moreover, the $\nu_{sy}N\rightarrow O$ bands for the tautomeric triazene 1-oxide [-NH-N=N(→O)-] exhibited greater intensity for the complexes compared to their ligands suggesting the strengthening of the N-O bond. The strengthening of this bond suggested the formation a new fragment (*i.e.* -N-O-Cu) incorporating the Cu-O bond in the metal complexes (Sajila and Mohabey, 2016). The bands at 514-566 cm^{-1} can been assigned to new Cu-O bond formed on complexation (Rajasekar and Ramachandramoorthy, 2013).



Scheme 1: Proposed Structures of the Synthesized Hydroxytriazene Ligands

The broad absorption band occurring at 355.00 - 363.60 nm in the UV-Vis spectra of the ligands is attributed to $n \rightarrow \pi^*$ intra-ligand transitions arising from a union of bands of the chromophore groups -N=N- for compounds **L_1- L_5** and -C=O for compounds **L_1** – **L_4** plus the auxochrome group -OH present in the all ligands (Bersch *et al*., 2014). The band appearing at 258.00 - 271.20 nm is attributed to $\pi \rightarrow \pi^*$ transitions within the aromatic rings (Domingues *et al*., 2010).

The band occurring at 370.00-383.40 nm in the spectra of the synthesized copper (II) complexes was attributed to N → Cu ligand-metal charge-transfer transitions (Manoj *et al*., 2009). The intense band at 273.80-276.88 nm is attributed to intra-ligand charge transfer transitions within the organic moiety of the complexes (Nkungli *et al*., 2015). The λ_{max} of **Cu-L_1** to **Cu-L_5** compounds were shifted to higher wavelengths relative to those of constituent ligands suggesting complexation of the metal ions and the ligands (Salman, 2015). Scheme 1 shows the structures of the ligands (**L_1- L_5**) proposed on the basis of micro-elemental and spectroscopic results obtained in this study and the substrates used to synthesize the compounds.

The biological activity of a compound is influenced by several factors including its lipophilicity and electronic properties (Patrick, 1995). Lipophilicity can tuned by incorporation of hydrophobic or hydrophilic substituents. Introduction of methyl substituents increases lipophilicity while carboxyl substituents increases hydrophilicity (Bazzini and Wermuth, 2008; Hassan *et al*., 2011). Electronic properties of hydroxytriazenes can be tuned by incorporation of electron donating or electron withdrawing substituents on the aryl rings (Kumar *et al*., 2014). The activity can be tuned further through complexation with a suitable metal (El-Sawaf, 2016; Hasi *et al*., 2016).

The activity of ligands L_1- L_4 was more than of some hydroxytriazenes reported in the literature (Baroliya *et al*., 2014). Ligands **L_1-L_4** were active against *Staphylococcus aureus* and *Shigella dysenteriae* while **L_5** had no observable activity. This suggests that the carboxyl group is vital to the activity of the synthesized hydroxytriazenes against these bacterial strains. However, complexation deactivated **L_1-L_4** against *Staphylococcus aureus* and *Shigella dysenteriae*.

The minimum inhibitory concentrations of **L_1** and **L_2** against *Proteus mirabilis* were twice those of **L_3** and **L_4**. This suggests that incorporation of a methyl substituent on the aryl ring enhanced the activity of the synthesized compounds against this bacterial strain. Complexation enhanced the activity of L_2 but decreased the activity of L_3, L_4 and L_5 against *Proteus mirabilis.* These results suggests that optimization of activities of the synthesized compounds with respect to a given bacterial strain should be performed independently.

CONCLUSION

Hydroxytriazene ligands having electron donating and electron withdrawing substituents and their copper (II) complexes were successfully synthesized using facile methods. The structures of the target compounds were

determined by elemental analysis and spectroscopic studies. Some of the synthesized compounds exhibited promising activities against both Gram-positive and Gram-negative bacterial strains and are therefore worthy optimizing for potential use as alternative antibacterial agents.

RECOMMENDATION

Hydroxytriazenes and their complexes are promising antibacterial agents for both Gram-positive and Gram-negative bacterial strains. Further studies should be undertaken to optimize the lipophilicity and electronic properties of these compounds through a systematic variation of substituent groups. The effects of complexation on aforementioned properties should also be established through electron paramagnetic resonance studies.

ACKNOWLEDGEMENT

The authors wish to thank Chuka University for providing financial support. We also wish to thank the Kenya Medical Research Institute for facilitating our microbiological analyses at their laboratories.

REFERENCES

Agrawal, S., Jain, S., Manwani, S., Bhalothia, M. and Goswami, A. K. 2016. Synthesis of biologically active 3-hydroxy-3-phenyl-1-(5-chloro-2-methyl) phenyl triazene and its application in the spectrophotometric study of iron complex. Journal of Applicable Chemistry, 5: 148-154.

Aliyu, H.N. and Mohammad, U. 2012. Studies on potentially therapeutic *cis*-and *trans*- bis(3-phenyl-o-carboxyphenyl-3-oxidato) nickel complexes. Global Advanced Research Journal of Microbiology, 1: 47-51.

Baroliya, P.K., Regar, M., Chauhan, R.S. and Goswami, A.K. 2014. Synthesis, characterization and antimicrobial activities of hydroxytriazenes and their Cobalt (II) complexes. Afinidad LXXI, 568: 305- 310.

Bazzini, P. and Wermuth, C.G. 2008. Substituent Groups. pp. 432. *In*: C.G. Wermuth (Ed.). *The Practice of Medicinal Chemistry*. Academic Press, London, UK.

Bersch, P., de Oliveira, H.P.M., Santos, A., Paraginski, G.L. and Hörner, M. 2014. Triazene 1-oxide compounds: Synthesis, characterization and evaluation as fluorescence sensor for biological applications. Journal of Molecular Structure, 1060:264-271.

Chundawat, N.S., Goswami, A. K., Singh, G.P. and Chauhan, R.S. 2014. Hydroxytriazenes and their cobalt (II) complexes as bioactive agents. International Journal for Pharmaceutical Research Scholars, 5: 3661-3664.

Clinical and Laboratory Standards Institute (CLSI). 2012. Performance standards for antimicrobial susceptibility testing; twenty second informational supplement, 32: M100-S22. 2012.

Domingues, V.O., Hörner, R., Reetz, L.G.B., Kuhn, F., Coser, V.M., Rodrigues, J.N., Bauchspiess, R., Pereira, W V., Paraginski, G.L., Locatelli, A., Fank, J.O., Giglio, V.F. and Hörner, M. 2010. *In vitro* evaluation of triazenes: DNA cleavage, antibacterial activity and cytotoxicity against acute myeloid leukemia cells. Journal of the Brazilian Chemical Society, 21:106-110.

El′Garch, F., Jeannot, K., Hocquet, D., Llanes-Barakat, C. and Plesiat, P. 2007. Cumulative effects of several non-enzymatic mechanisms on the resistance of *Pseudomonas aeruginosa* to aminoglycosides. Antimicrobial Agents and Chemotherapy, 51:1016-1021.

El-Sawaf, A.K. 2016. Synthesis, spectroscopic and biological studies of copper (II) complexes of benzoyl-3-methylphenyl-2-pyrazolin-5-one N(4) phenyl thiosemicarbazone. International Journal of Recent Scientific Research, 7:9164-9176.

Fanning, S., Karczmarczyk, M., Martins, M., Quinn, T. and Leonard, N. 2011. Mechanisms of fluoroquinolone resistance in *Escherichia coli* isolates from food-producing animals. Applied and Environmental Microbiology, 77:7113-7120.

Hasi, Q.M., Fan, Y., Yao, X.Q., Hu, D.C., Liu, J.C. 2016. Synthesis, characterization, antioxidant and antimicrobial activities of a bidentate Schiff base ligand and its metal complexes. Polyhedron, 109:75-80.

Hassan, M.Z., Javed, S.A., Amir, M. 2011. Synthesis and antimicrobial activity of pyrazolinones and pyrazoles having benzothiazole moiety. Medicinal Chemistry Research, 21:1691-1694.

Jain, C.P., Chauhan, L.S., Chauhan, R.S. and Goswami, A.K. 2010. Anti-inflammatory activity of some substituted hydroxytriazenes. Journal of Global Pharma Technology, 2:197-111.

Jodha, J.S., Khanam, R., Dashora, R. and Goswami, A. K. 2014. Synthesis and insecticidal activity of some hydroxytriazene derivatives and their ternary complexes with vanadium (V) and thiourea. Journal of Global Pharma Technology, 6:1-3.

Jorgensen, J.H. and Ferraro, M.J. 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clinical Infectious Diseases, 49:1749-1755.

Joshi, P., Pareek, N., Upadhyay, D., Khanam, R., Bhandari, A., Chauhan, R.S. and Goswami, A. K. 2010. Polarographic determination and antifungal activity of copper (II) complex with 3-hydroxy-3-m-tolyl-1-p-(sulphonamido) phenyltriazene.

International Journal of Pharmaceutical Sciences and Drug Research, 2: 278-280.

Khan, S., Khanam, R., Dashora, R., Chauhan, R. S. and Goswami, A. K. 2013. Analytical application of 3-hydroxy-3-isopropyl-1-(4-sulphonamidophenyl) triazene in the spectro-photometric determination of nickel (II). International Journal of Pharmaceutical, Chemical and Biological Sciences, 3:704-707.

Kodli, K.K., Joshi, P. and Goswami, A.K. 2014. Biological activities of Hydroxytriazenes and their nickel complexes. International Journal for Pharmaceutical Research Scholars, 3:785-788.

Kumar, N., Singh, G.P., Kumar, A., Goswami, A. K., Chauhan, R.S. 2014. Biological activities of hydroxytriazenes and their copper complexes. International Journal for Pharmaceutical Research Scholars, 3: 153-155.

Manoj, E., Kurup, M. R.P. and Punnoose, A. 2009. Preparation, magnetic and EPR spectral studies of copper (II) complexes of an anticancer drug analogue. Spectrochimica Acta Part A, 72:474-483.

Mardanesh, J., Poor, S.A. and Afrugh, P. 2013. Prevalence of *Shigella* species and antimicrobial resistance patterns of isolated strains from infected pediatrics in Tehran. International Journal of Enteric Pathogens, 1: 28-31.

Muthukumar, C., Sabastiyan, A., Valarselvan, S., Subramanian, M. and Shanmugavadivel, M. 2016. Synthesis, characterization and antimicrobial activity of manganese (II) and nickel (II) complexes of 7-diethylaminobenzyl-8-hydroxyquinoline. Journal of Advances in Chemistry, 12: 4324-4332.

Nathisuwan, S., Burgess, D.S. and Lewis, J.S. 2001. Extended-spectrum β-lactamases: Epidemiology, detection and treatment. Pharmacotherapy, 21: 920-928.

Nkungli, N. K., Ghogomu, J.N., Nogheu, L.N. and Godre, S.R. 2015. DFT and TD-DFT study of bis[2-(5-amino-[1,3,4]-oxadiazol-2-yl)phenol](diaqua) M(II) complexes [M = Cu, Ni and Zn]: Electronic structures, properties and analyses. Computational Chemistry, 3: 29-44.

Nordmann, P., Naas, T., Fortineau, N. and Poirel, L. 2007. Superbags in the new decades; multidrug resistance and prospects for treatment of *Staphylococcus aureus, Enterococcus spp*. and *Pseudonomas aeruginosa* in 2010. Current Opinion in Microbiology, 10: 436-440.

Okoh, A. and Igbinosa, E. 2010. Antibiotic susceptibility profiles of some *Vibrio* strains isolated from wastewater final effluents in rural community of the Eastern Cape Province of South Africa. BMC Microbiology, 10:143.

Oliveria, G.M., Hörner, M., Machado, A., Villetti, M. A., Back, D.F. and Iglesias, B.A. 2009. Stabilization of triazene oxides by lanthanides chelation: Synthesis, TGA evaluation and X-ray structural features of [M^{III} {O_2NPhNNN(O)Ph}](Et_2.NH).H_2O)(M = La^{3+}, Dy^{3+}; Et = C_2H_5). Journal of Molecular Structure, 928:85- 88.

Ombaka, O. and Gichumbi, J.M. 2011. Synthesis and insecticidal activities of some selected hydroxytriazenes. Journal of Environmental Chemistry and Ecotoxicology. 3:286-289.

Patrick, G.L. 1995. *An introduction to Medicinal Chemistry*. Oxford University Press, Oxford, UK.

Penchovsky, R. and Traykovska, M. 2015. Designing drugs that overcome antibacterial resistance: where do we stand and what should we do? Expert Opinion on Drug Discovery, 10: 631-650.

Rahmani, F., Fooladi, A.A., Marashi, S.M. and Nourani, M.R. 2012. Drug resistance in *Vibrio cholerae* strains isolated from clinical specimens. Acta Microbiologica et Immunologica Hungarica, 59:77-84.

Rajasekar, K. and Ramachandramoorthy, T. 2013. Synthesis, spectral characterization and biological activities of Cr (III), Co (II), Ni (II) and Cd (II) complexes with 4-aminoantipyrine and thiocyanate ion as ligands. International Journal of Pharma and Biosciences, 4: 271-276.

Saito, R., Okugawa, S., Kumita, W., Sato, K., Chida, T., Okamura, N., Moriya, K. and Koike, K. 2007. Clinical epidemiology of ciprofloxacin resistant *Proteus mirabilis* isolated from urine samples of hospitalized patients. Clinical Microbiology and Infection, 13:1204-1207.

Sajila, H. and Mohabey, H. 2016. IR spectra, magnetic and thermal studies of Cu (II) of N-Hydroxy-N-(4-chloro)phenyl N′-(4-fluoro)phenylbenzamidine hydrochloride. Material Science Research India, 11: 63-65.

Salman, A. B. 2015. Synthesis and characterization of 1-benzylbenzimidazole complexes with some transition metal salts. Australian Journal of Basic and Applied Sciences, 9:251-255.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.D. and Kamal, M.A. 2014. Antibiotic resistance and extended-spectrum beta-lactamases: Types, epidemiology and treatment. Saudi Journal of Biological Sciences, 22:90-101.

Santajit, S and Indrawattana, N. 2016. Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed Research International, 2475067: 1-9.

Sikarwar, A.S. and Batra, H.V. 2011. Prevalence of antimicrobial drug resistance of *Klebsiella pneumoniae* in India. International Journal of

Bioscience, Biochemistry and Bioinformatics, 1: 211-215.

Singh, G.P., Chauhan R.S., Chundawat, N.S. and Goswami, A. K. 2014. Synthesis and antimicrobial studies of some sulfonato (sodium salt) based hydroxytriazenes and their cobalt (II) complexes. World Journal of Science and Pharmaceutical Science, 3:1361-1366.

Singhania, M., Bharadwaj, A.P., Ghosh, A.R., Roy, S., Bhattacharya, D., Sugunan, A. P., Bhattacharjee, H., Thamizhmani, R., Sayi, D.S., Thanasekaran, K. and Manimunda, S.P. 2012. Antimicrobial resistance in *Shigella*-rapid increase and widening of spectrum in Andaman Islands, India. Indian Journal of Medical Research, 135:365-370.

Soni, A., Sharma, P., Dashora, R. and Goswami, A.K. 2016. Corrosion inhibition of brass in 0.5 N HNO_3 by 3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonato (sodium salt) phenyl triazene (HCST): Adsorption and thermodynamic study. Journal of Applicable Chemistry, 5:281-290.

Thakar, A., Joshi, K., Pandya, K. and Pancholi, A. 2011. Coordination modes of a Schiff base derived from substituted 2-aminothiazole with chromium (III), manganese (II), iron (II), cobalt (II), nickel (II) and copper (II) metal ions: Synthesis, spectroscopic and antimicrobial studies. E-Journal of Chemistry, 8:1750-1764.

Tomar, R., Singh, G.P., Chauhan, R.S. and Goswami, A.K. 2014. Characterization and prediction of some hydroxytriazenes by PASS. World Journal of Pharmacy and Pharmaceutical Sciences, 3:1304-1309.

Wu, J.J., Chen, H.M., Ko, W.C., Wu, H.M., Tsai, S.H. and Yan, J.J. 2008. Prevalence of extended-spectrum beta-lactamases in *Proteus mirabilis* in a Taiwanese University hospital, 1999 to 2005: Identification of a novel CTX-M enzyme (CXT-M-66). Diagnostic Microbiology and Infectious Disease, 60:167-175.