BIOLOGICAL EVALUATION OF NEWLY SYNTHESIZED AZO DYES OF SALICYLIC ACID

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ABSTRACT

The present work series of eight azo dyes were synthesized by the diazotization process of eight aromatic amines using NaNO2 and HCl, and coupling of these diazonium salts with Salicyclic acid. Newly synthesized azo dyes have been confirmed by FT-IR and H1 NMR spectral data. The synthesized compounds have been tested in vitro against human pathogens to assess their antimicrobial activities using the disk diffusion method. The compound analyzed for its antimicrobial activity were found to have effective antimicrobial activity at both concentrations against all the tested pathogenic culture.

Keywords: *Azo dyes, Salicylic acid, antimicrobial activity.*

Introduction

Azo compounds are the most fundamental class of commercial dyes and are well colored that have been used as dyes and pigments[1, 2]. Azo compounds are known to be involved in a number of biological reactions such as inhibition of DNA, RNA and protein synthesis, carcinogenesis, and nitrogen fixation[3, 4], also known for their use as antibacterial, antifungal antineoplastics, antidiabetics, antiseptics, anticancer, anti-inflammatory and other useful chemotherapeutic agents [5-8]. Azo dyes have been most widely used in dying textile fibres[9,10], biomedical studies[11,12], advanced applications in organic synthesis and high technology areas like lasers, liquid crystalline displays, electro-optical devices, and inkjet printer[13,14] as well as shows a variety of interesting biological activities including antibacterial and pesticidal activities[15,16] and also used in food.

The azo dyes possess antiseptic and antiprotozoal properties and some are useful as a chemotherapeutic agent. The cationic dyes are more active in acidic medium preferably attack on gram-positive bacteria as compared to anionic dyes. The most common azo dyes used as antiseptics are scarlet red and diamazon. The industrial use as a colorant and biological activities of azo compounds promotes us to synthesis of azo compounds.

In the present study, Salicyclic acid is coupled with diazonium salt of eight aromatic amines VIZ: Aniline, o-Nitro aniline, p-Toluedine, α-Naphthylamine, Sulphanilic acid, m-Nitro aniline, p-aminobenzoic acid, and m-aminobenzoic acid.

Methods and Materials

All the chemicals used in these experiments were of analytical grade. All the melting points were determined by the open capillary method and are uncorrected. The products were confirmed by 1H NMR (Burker avernce II 400 NMR Spectrometer) and IR technique (Shimatzu). The biological activity was evaluated against two kinds of bacteria gram-positive and gram-negative. The products were recrystallized by ethanol as a solvent.

General procedure for synthesis of azo compounds[17-19]

Substituted aromatic amines (0.01mole) were mixed with 2.5 ml conc. HCl and 2.5 ml (4N) cold solution of $NaNO_2$ was added with the stirring. The temperature of the reaction was maintained up to $0\text{-}5^0$ C. Diazonium salt solution prepared above was added drop wise to the alkaline solution of Salicylic acid. The reaction mixture stirred for 10 – 20 minutes maintaining the temperature $5\text{-}10^0$ C. The colored product so obtained is filtered washed with water and recrystallised from 80% ethanol. The general Scheme for the synthesis of azo dyes of Salicyclic acid is shown in figure (I).

Figure I: General Scheme for the synthesis of azo dyes of Salicylic acid

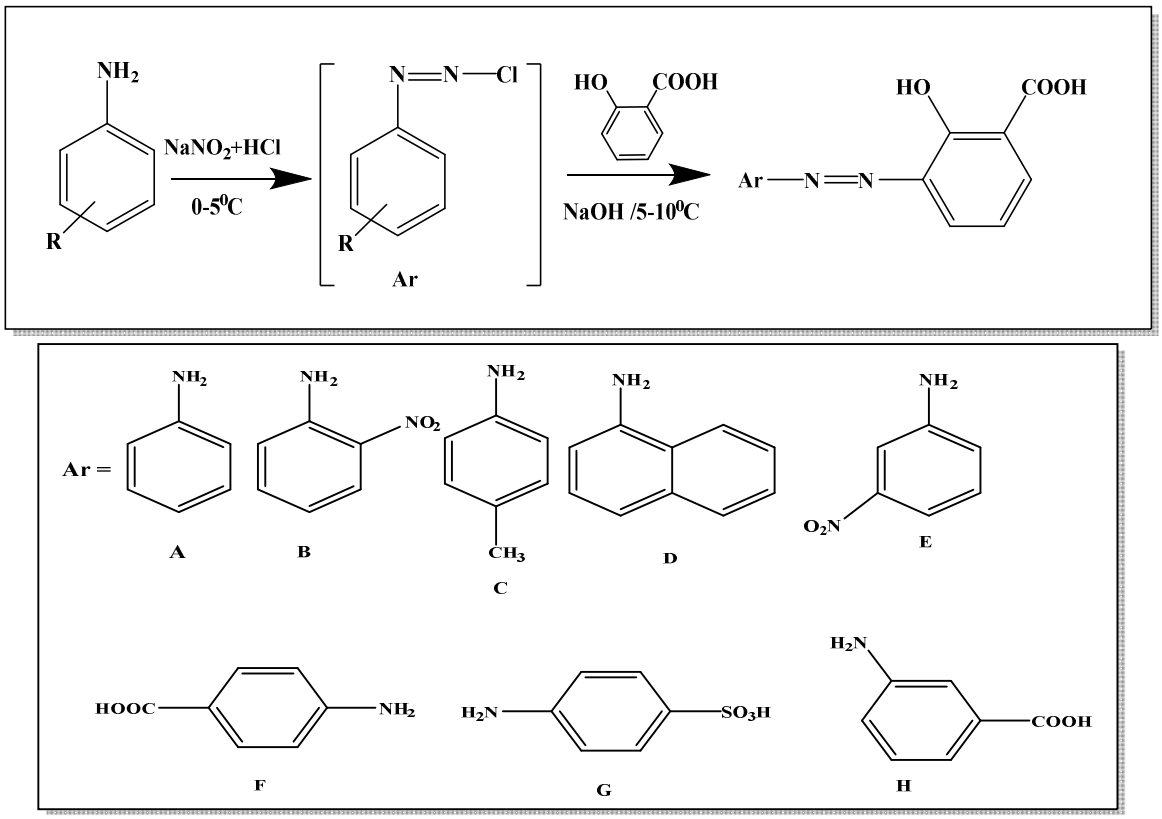


Table (I): The code, compound name, molecular formula, molecular weight, melting point and percentage yield of synthesized compounds of Salicyclic acid.

Code	Structure	Molecular Formulae	Molecular Weight	Melting Point (^{0}C)	Yield
1A	2-hydroxy-3-(phenyldiazenyl)benzoic acid	$C_{13}H_{10}N_2O_3$	242.23	165	62
1B	2-hydroxy-3-((2-nitrophenyl)diazenyl)benzoic acid	$C_{13}H_9N_3O_5$	287.23	155	61%
1C	2-hydroxy-3-((p-tolydiazenyl)diazenyl)benzoic acid	$C_{14}H_{12}N_2O_3$	256.26	162	53%
1D	2-hydroxy-3-(napthalen-1-yldiazenyl)benzoic acid	$C_{17}H_{12}N_2O_3$	292.29	156	52%
1E	2-hydroxy-3-((3-nitrophenyl)diazenyl)benzoic acid	$C_{13}H_9N_3O_5$	287.23	162	59%
1F	2-((4-carboxyphenyl)diazenyl)-2-hydroxybenzoic acid	$C_{14}H_{10}N_2O_5$	286.24	165	55%
1G	2-hydroxy-3-((4-sulfophenyl)diazenyl)benzoic acid	$C_{13}H_{10}N_2O_6S$	322.29	155	59%
1H	3-((3-carboxyphenyl)diazenyl)-2-hydroxybenzoic acid	$C_{14}H_{10}N_2O_5$	286.24	151	60%

Antimicrobial Activity

The newly synthesized azo compounds 1A-1H were analyzed for their antimicrobial activity against four gram positive and gram negative bacteria viz. *Escherichia coli, Staphylococcus aureus, Pseudomanas aeroginosa and Salmonella typhi* by using agar well diffusion method. These compounds were mixed in Ethanol to form the solution of concentration 1mg/ml. sterile disc were dipped in the solutions, dried it and placed on the nutrient agar medium spreaded with the bacteria. The plates were further incubated for 24 to 48 hours at 37^0 C and the diameter of zones of inhibition was measured in millimeter.

Result and Discussion

The azo dyes synthesized in the present study were characterized by IR and NMR spectroscopic methods. IR and ^{1}H-NMR spectra showed the expected signals which correspond to various groups present in each compounds. The IR and ^{1}H-NMR spectral values for different synthesis dyes are shown in table II.

Table (II): FTIR AND ^{1}H NMR data of azo compounds of Salicyclic acid.

Compound	Spectra	Spectroscopic Data
SDI 1A	IR (KBr. cm-1)	3389 cm^{-1} (Phenolic –OH stretch), 3063 cm^{-1} (Carboxylic acid O-H stretch), 1481 cm^{-1} (C=C Ring stretch), 1581 cm^{-1} (N=N stretch),1283 cm^{-1}(C-N stretch) 1687 cm^{-1} (C=O stretch of -COOH)
	NMR (δ ppm)	6.79 (s 1H of –OH), 12.41 (s 1H of –COOH), 7.44-8.31(m 8H of Ar-H).
SDI 1B	IR (KBr. cm^{-1})	3429 cm^{-1} (Phenolic –OH stretch), 3105 cm^{-1}(Carboxylic acid O-H stretch), 3078cm^{-1} (N-H stretch), 1493 cm^{-1}(C=C Ring stretch), 1579 cm^{-1} (N=N stretch), 1285 cm^{-1} (C-N stretch), 1523 cm^{-1} (-NO_2 stretch (N-O Asym), 1345 cm^{-1} (-NO_2 stretch (N-O sym),1660 cm^{-1} (C=O stretch of -COOH)
	NMR (δ ppm)	6.78 (s 1H of –OH), 12.01 (s 1H of –COOH), 7.61-8.26(m 8H of Ar-H).
SDI 1C	IR (KBr. cm^{-1})	3405 cm^{-1} (Phenolic –OH stretch), 3125 cm^{-1}(Carboxylic acid O-H stretch), 1482 cm^{-1}(C=C Ring stretch), 1600 cm^{-1} (N=N stretch), 1284 cm^{-1} (C-N stretch), 1651 cm^{-1} (C=O stretch of -COOH), 2915 cm^{-1} ($^{C-H}$ stretch of -CH_3)
	NMR (δ ppm)	6.73 (s 1H of –OH), 12.40 (s 1H of –COOH), 2.50 (s 3H of –CH_3), 7.12-8.25 (m 7H of Ar-H).
SDI 1D	IR (KBr. cm^{-1})	3391 cm^{-1} (Phenolic –OH stretch), 3046 cm^{-1}(Carboxylic acid O-H stretch), 1455 cm^{-1}(C=C Ring stretch), 1578 cm^{-1} (N=N stretch), 1285 cm^{-1} (C-N stretch)
	NMR (δ ppm)	6.88 (s 1H of –OH), 12.82(s 1H of –COOH), 7.84-8.48 (m 10H of Ar-H).
SDI 1E	IR (KBr. cm^{-1})	3471 cm^{-1} (Phenolic –OH stretch), 3286 cm^{-1}(Carboxylic acid O-H stretch), 3095cm^{-1} (N-H stretch), 1490 cm^{-1}(C=C Ring stretch), 1584 cm^{-1} (N=N stretch), 1288 cm^{-1} (C-N stretch), 1521 cm^{-1} (-NO_2 stretch (N-O Asym), 1348 cm^{-1} (-NO_2 stretch (N-O sym)
	NMR (δ ppm)	6.79 (s 1H of –OH), 11.90 (s 1H of –COOH), 7.33-8.32 (m 7H of Ar-H).
SDI 1F	IR (KBr. cm^{-1})	3438 cm^{-1} (Phenolic –OH stretch), 3001 cm^{-1} (Carboxylic acid O-H stretch), 1434 cm^{-1}(C=C Ring stretch), 1579 cm^{-1} (N=N stretch), 1235 cm^{-1} (C-N stretch)
	NMR (δ ppm)	6.81 (s 1H of –OH), 12.30 (s 2H of –COOH), 7.21-8.85 (m 7H of Ar-H).
SDI 1G	IR (KBr. cm^{-1})	3449 cm^{-1} (Phenolic –OH stretch), 2828 cm^{-1} (Carboxylic acid O-H stretch), 3070 cm^{-1} (N-H stretch), 1583 cm^{-1}(C=C Ring stretch), 1600 cm^{-1} (N=N stretch), 1287 cm^{-1} (C-N stretch), 1178 cm^{-1} (Sulphonic acid stretch)
	NMR (δ ppm)	6.68 (s 1H of –OH), 11.80 (s 2H of –COOH), 7.96 (s 1H of –SO_3H), 6.92-7.94 (m 7H of Ar-H).
SDI 1H	IR (KBr. cm^{-1})	3390 cm^{-1} (Phenolic –OH stretch), 3040 cm^{-1} (Carboxylic acid O-H stretch), 1488 cm^{-1}(C=C Ring stretch), 1586 cm^{-1} (N=N stretch), 1338 cm^{-1} (C-N stretch)
	NMR (δ ppm)	6.76 (s 1H of –OH), 12.40 (s 2H of –COOH), 7.14-8.30 (m 7H of Ar-H).

Antimicrobial Activity

Eight azo compounds of Salicylic acid have been synthesized, recrystaliesed and used separately for its study of antimicrobial activity against four gram positive and gram negative bacteria viz. *Escherichia coli, Staphylococcus aureus, Pseudomanas aeroginosa and Salmonella typhi*. The data of antimicrobial activity of these newly synthesized azo dyes of Salicyclic acid 1A-1H against four pathogens are presented in the tables 1-4.

Antibacterial properties of the synthesized azo compounds of Salicylic acid viz 1A – 1H [Zone of inhibition (mm)]

Table (1): Effect of azo compounds of Salicylic acid viz. 1A – 1H on the growth response of *Escherichia coli.*

Conc.(mg/ml)	1A	1B	1C	1D	1E	1F	1G	1H
0.5	I (12)	I (10)	I (10)	I (10)	I (13)	NI	I (10)	NI
1.0	I (10)	I (11)	I (10)	I (10)	I (11)	I (10)	NI	I (10)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition

Table (2): Effect of azo compounds of Salicylic acid viz. 1A – 1Hon the growth response of *S. aureus*.

Conc.(mg/ml)	1A	1B	1C	1D	1E	1F	1G	1H
0.5	I (9)	I (10)	I (10)	I (11)	I (10)	I (11)	I (10)	I (10)
1.0	I (10)	NI	I (10)	I (15)	NI	I (12)	I (12)	I (10)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition

Table (3): Effect of azo compounds of Salicylic acid viz. 1A – 1H on the growth response of *P. aeroginosa*.

Conc.(mg/ml)	1A	1B	1C	1D	1E	1F	1G	1H
0.5	I (9)	I (11)	I (8)	I (11)	I (10)	I (9)	I (10)	I (10)
1.0	I (10)	I (14)	I (16)	I (10)	I (14)	I (11)	I (10)	I (11)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition

Table (4): Effect of azo compounds of Salicylic acidviz. 1A – 1Hon the growth response of *Salmonella typhi.*

Conc.(mg/ml)	1A	1B	1C	1D	1E	1F	1G	1H
0.5	I (11)	I (9)	I (10)	I (9)	I (10)	I (10)	I (10)	I(8)
1.0	I (12)	I (11)	I (12)	I (10)	I (12)	I (13)	I (10)	I(10)

I = Inhibition, values of inhibition are given in parenthesis, NI = No inhibition

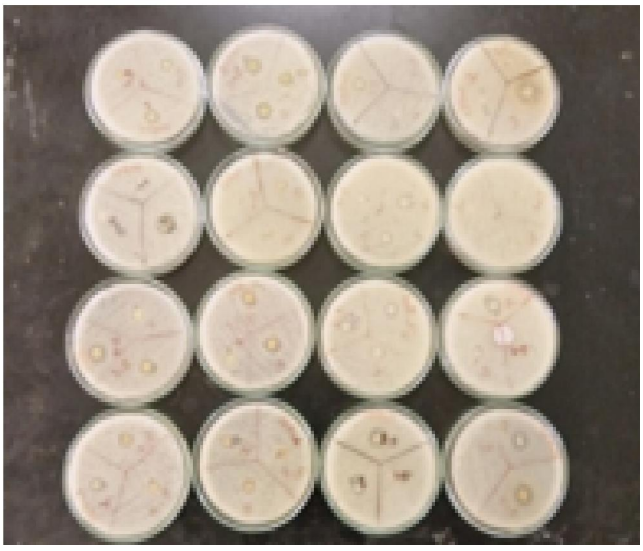


Figure 1: Effect of azo compounds of Salicylic acid 1A – 1Hon the growth of *E.Coli.*

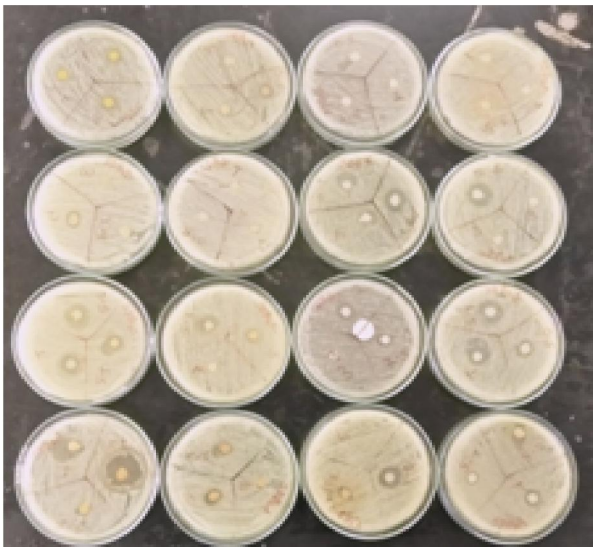


Figure 2: Effect of azo compounds of Salicylic acid1A – 1Hon the growth of *Staphylococcus aureusus.*

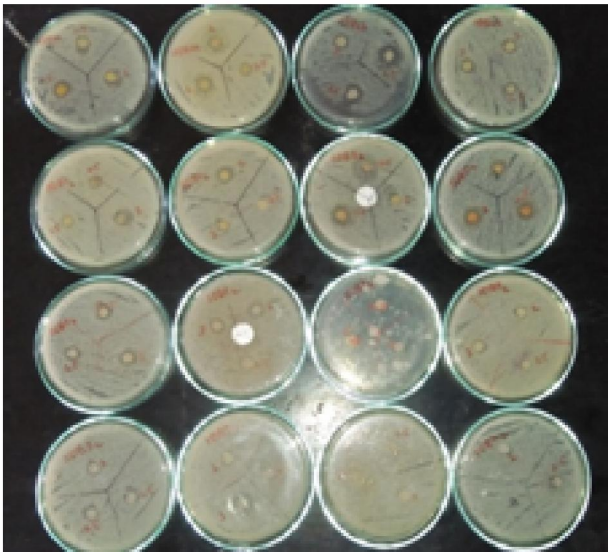


Figure 3: Effect of azo compounds of Salicylic acid1A – 1Hon the growth of *Pseudomonas aeroginosa.*

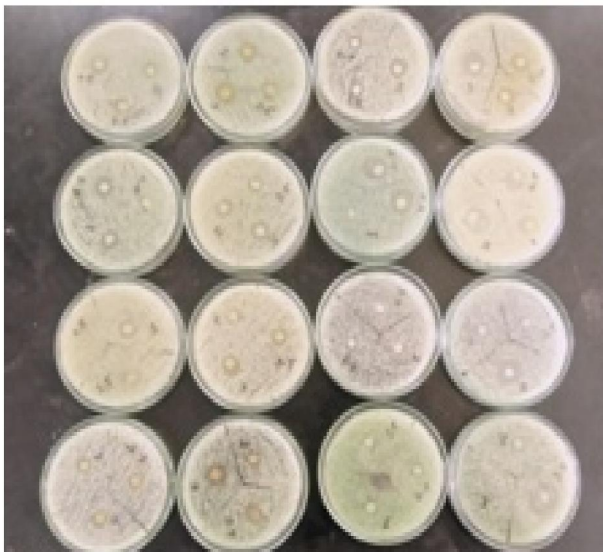
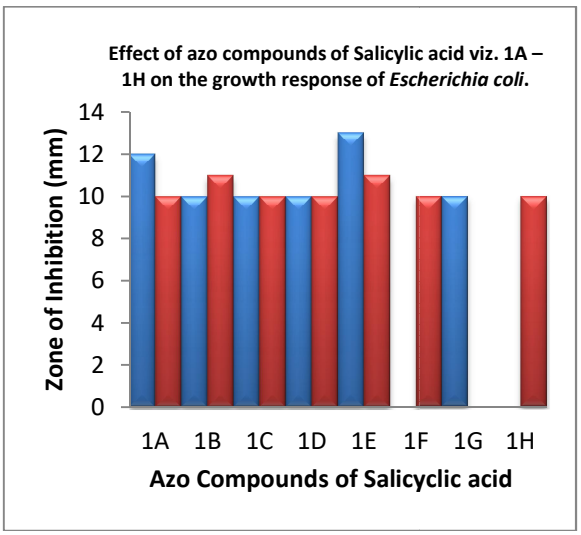
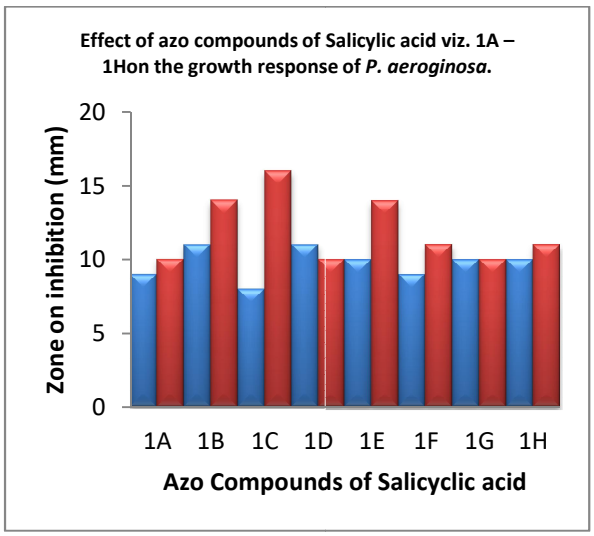
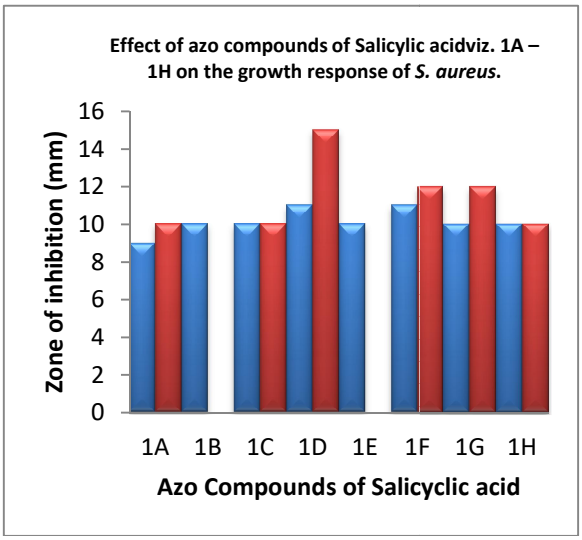
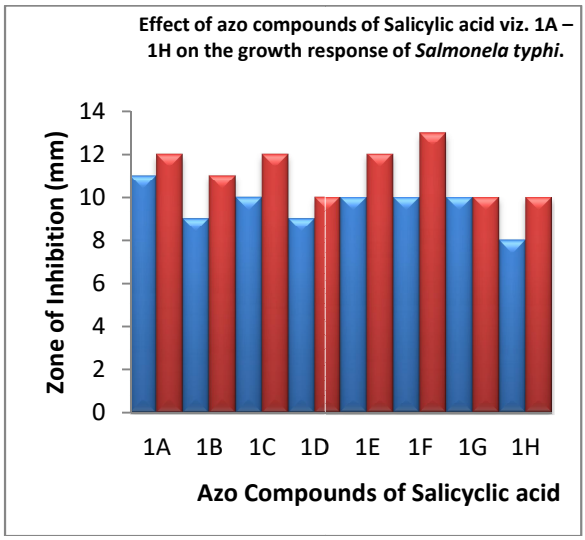


Figure 4: Effect of azo compounds of Salicylic acid1A – 1Hon the growth of *Salmonella typhi.*









Conclusion

All the azo compounds 1A-1H containing Salicyclic acid moiety were successfully synthesized in good yield and their structures were confirmed using FTIR, & 1HNMR spectroscopy. The results on antimicrobial activity tells that all the eight newly synthesized compounds viz 1A-1H found to have good antibacterial effect against *E.Coli*, *S. aureus, Pseudomonas aeroginosa,* and *Salmonella typhi* nearly at all the concentrations analysed. The results shown, the broad spectrum potential of all the compounds in inhibiting the growth of human pathogens, and this finding shows the possible help in drug discovery.

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