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Synthesis, Characterization and Antimicrobial screening of some Azo compounds derived from Ethyl vanillin

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Abstract

Azo compounds were synthesized in excellent yield by diazotization of some substituted aromatic amines using $NaNO_2$ and concentrated HCl followed by coupling with ethyl vanillin in alkaline medium. These azo compounds were characterized by FTIR and H^1 NMR spectroscopic technique and have been tested against the growth of five gram positive and negative microorganisms in order to assess their antimicrobial activity.

Keywords: Azo compounds, ethyl vanillin, diazotization, antimicrobial activity.

Introduction

Azo compounds are mostly used as dyes due to its various applications in the fields such as textile fibres, colouring of different materials, biomedical studies and organic synthesis[1-2]. The azo dyes containing azo linkages have advanced applications in high technology areas like lasers[3], LCD color filters[4]. In addition to this, azo dyes were reported to have variety of biological applications like antineoplastics, antidiabetics, antiseptics, anti-inflammatory and other useful chemotherapeutic agents[5-8]. Scarlet red and diamazon are the most commonly used azo dyes which are antiseptics. Several azo compounds derived from thymol[9], aspirin[10], paracetamol[11], m-cresol[12], resorcinol[13] and vanillin[14] moieties have been frequently reported and exhibit excellent biological properties. In the present work, we have synthesized four azo compounds derived from ethyl vanillin and characterized by FTIR and H^1 NMR spectral technique. The antimicrobial potential of synthesized azo compounds of ethyl vanillin has been tested against the growth of five gram positive and negative microorganisms using agar well diffusion method.

Material and Methods

In the present synthesis, chemicals and reagents used were of analytical grade, Merck and Alfa Aesar Company Ltd. The azo compounds were characterized by FTIR and H^1 NMR spectroscopic techniques. The Perkin-Elmer spectrum One FTIR instrument was used for characterization of IR spectra in the form of KBr pallet. Bruker Avance II 400 MHz NMR spectrometer was used for characterization of H^1 NMR spectra of azo compounds using $CDCl_3$ as a solvent and TMS as an internal standard. The purity of all the compounds was checked by thin layer chromatography and were recrystalised from hot ethanol. The melting points were measured by open capillary method and they are uncorrected.

Procedure for the synthesis of azo compounds[9-10]: Aromatic amines (0.01 mole) were mixed with 2.5 ml of concentrated HCl. To this resultant solution, 25 gm of crushed ice and 2.5 ml of $NaNO_2$ (4N) was added with constant stirring. The temperature of the reaction mixture was kept constant up to 0-5^{0}C. The diazonium salt solution prepared was added to the alkaline solution of ethyl vanillin drop by drop with constant stirring for 10-25 minutes at constant temperature of 5-10^{0}C. The colored precipitate given out was filtered and washed with water number of times. The colored product was recrystallised from hot ethanol. The general reaction scheme for synthesis of azo compounds of ethyl vanillin is shown in figure-1.

Antimicrobial Activity: The azo compounds 1a-d were analysed for their antibacterial activity against five gram positive and negative pathogens viz. *Escherichia coli, Pseudomonas aeroginosa, Salmonella typhi, Bacillus subtilis and Staphylococcus aureus* by using agar well diffusion method[15-16]. These compounds were mixed in DMSO to form solutions of concentration 1mg/ml. Sterile discs were dipped in this solutions, dried it and placed on nutrient agar plates spreaded with the bacteria. The plates were further incubated for 24 to 48 hours at 37^{0}C and the diameters of zones of inhibition were measured in millimeter.

Results and Discussion

The data of synthesized azo compounds of ethyl vanillin (i.e. symbols, compounds name, molecular formulae, molecular weights, melting points and percentage yield) are given in table-1. The FTIR and H^1 NMR spectroscopic data of synthesized azo compounds are illustrated in table-2 and are shown in figure-6 to 13. A total four azo compounds of ethyl vanillin have been synthesized, recrystalised and used separately to study its antimicrobial activity against five gram positive and negative microorganism's viz. *Escherichia coli, Pseudomonas aeroginosa, Salmonella typhi, Bacillus subtilis and*

Staphylococcus aureus. The data on antimicrobial activity of azo compounds of ethyl vanillin 1a-d against five pathogens are presented in table-3. From the results it was observed that the azo compounds of ethyl vanillin have showed miraculous antibacterial potential against all five pathogens. The compound 1a showed 10.4 and 9.6 mm zones of inhibition against the test pathogens *Escherichia coli* and *Staphylococcus aureus* respectively as shown in figure-2 and do not showed any inhibitory action against *Salmonella typhi*, *Pseudomonas aeroginosa and Bacillus subtilis.* The azo compound 1b showed 10.6, 12.7, 11.8 and 9.8 mm zones of inhibition against the test pathogens *Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus* respectively shown in figure-3 but do not showed any inhibitory action against *Pseudomonas aeroginosa.* The azo compound 1c showed 10.3, 13.1, 13.5 and 12.8 mm zones of inhibition against the pathogens *Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Staphylococcus aureus* respectively as shown in figure-4 but do not showed any inhibitory action against *Pseudomonas aeroginosa.* The azo compound 1d showed 13.6, 12.8, 15.6 and 14.4 mm zones of inhibition against the test pathogens *Escherichia coli, Salmonella typhi, Pseudomonas aeroginosa* and *Staphylococcus aureus* as shown in figure-5. Compound 1d showed 11.3 mm zone of inhibition against the pathogen *Bacillus subtilis.*

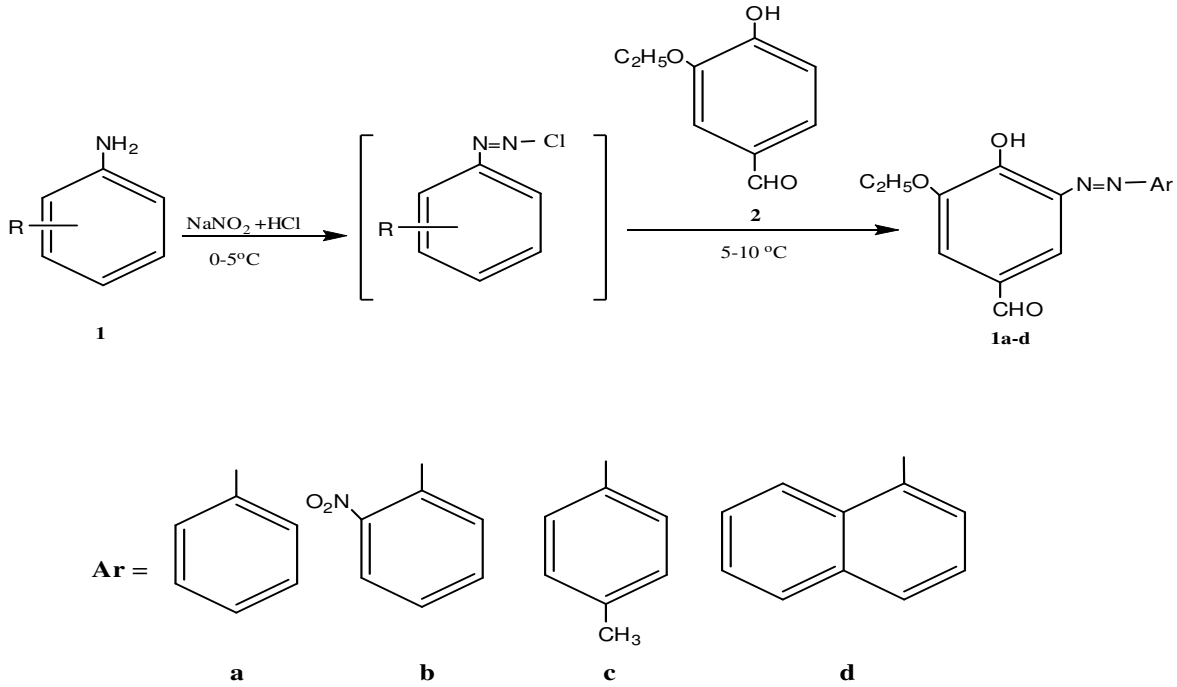


Figure-1
The general reaction scheme for synthesis of azo compounds of ethyl vanillin

Table-1
The symbols, compounds name, molecular formulae, molecular weights, melting points and percentage yield of synthesized azo compounds of ethyl vanillin

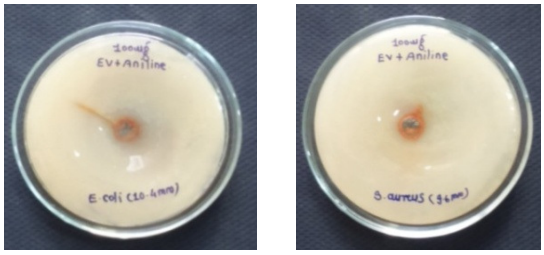
Symbols for antimicrobial activity	Symbols for FTIR and H^1 NMR	Compounds Name	Molecular Formulae	Molecular Weights	Melting points (^{0}C)	Yield (%)
1a	4P	3-ethoxy-4-hydroxy-5-(phenyldiazenyl)benzaldehyde	$C_{15}H_{14}N_2O_3$	270.28	67	67
1b	4Q	3-ethoxy-4-hydroxy-5-((2-nitrophenyl)diazenyl)benzaldehyde	$C_{15}H_{13}N_3O_5$	315.28	128-130	65
1c	4R	3-ethoxy-4-hydroxy-5-(p-tolyldiazenyl)benzaldehyde	$C_{16}H_{16}N_2O_3$	284.31	64	72
1d	4S	3-ethoxy-4-hydroxy-5-(napthalen-1-yldiazenyl)benzaldehyde	$C_{19}H_{16}N_2O_3$	320.34	127-129	80

Table-2
FTIR and H^1 NMR data of synthesized azo compounds of ethyl vanillin

Symbols for compounds	Types of spectra	FTIR and H^1 NMR spectral data
4P	FTIR (KBr,cm^{-1})	3362 (OH of Phenol), 2829 (C-H of H-C=O), 1686 (C=O), 1516 (C=C), 1255 (C-O), 1280 (C-N), 1579 (N=N).
	H^1 NMR (δ ppm)	1.4 (t, 3H of -CH_3), 4.2 (q, 2H of -OCH_2), 6.2 (s, 1H of Phenolic -OH), 9.8 (s, 1H of -CHO), 7.0 to 7.5 (m, 7H of Aromatic-H).
4Q	FTIR (KBr,cm^{-1})	3398 (OH of Phenol), 2882 (C-H of H-C=O), 1673 (C=O), 1519 (C=C), 1262 (C-O), 1232 (C-N), 1590 (N=N).
	H^1 NMR (δ ppm)	1.5 (t, 3H of -CH_3), 4.4 (q, 2H of -OCH_2), 4.2 (s, 1H of Phenolic -OH), 9.8 (s, 1H of -CHO), 7.0 to 8.5 (m, 6H of Aromatic-H), 9.9 due to moisture.
4R	FTIR (KBr,cm^{-1})	3363 (OH of Phenol), 2828 (C-H of H-C=O), 1681 (C=O), 1514 (C=C), 1281 (C-O), 1168 (C-N), 1580 (N=N).
	H^1 NMR (δ ppm)	1.5 (t, 3H of -CH_3), 4.3 (q, 2H of -OCH_2), 6.3 (s, 1H of Phenolic -OH), 9.8 (s, 1H of -CHO), 7.0 to 7.4 (m, 6H of Aromatic-H).
4S	FTIR (KBr,cm^{-1})	3333 (OH of Phenol), 2842 (C-H of H-C=O), 1682 (C=O), 1515 (C=C), 1261 (C-O), 1231 (C-N), 1592 (N=N).
	H^1 NMR (δ ppm)	1.5 (t, 3H of -CH_3), 4.1 (q, 2H of -OCH_2), 4.3 (s, 1H of Phenolic -OH), 9.8 (s, 1H of -CHO), 7.0 to 8.5 (m, 9H of Aromatic-H).

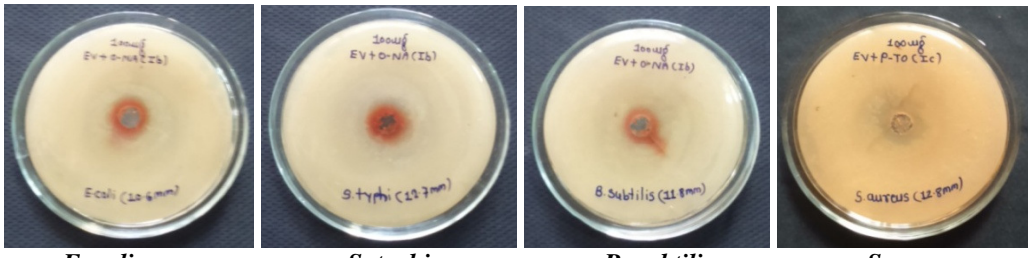
Table-3
Antimicrobial activities of the synthesized azo compounds of ethyl vanillin

Symbols for compounds	Microbial species and diameter of zone of inhibition in mm				
	Escherichia coli	***Salmonella typhi***	***Pseudomonas aeroginosa***	***Bacillus subtilis***	***Staphylococcus aureus***
1a	10.4	No inhibition	No inhibition	No inhibition	9.6
1b	10.6	12.7	No inhibition	11.8	9.8
1c	10.3	13.1	No inhibition	13.5	12.8
1d	13.6	12.8	15.6	11.3	14.4



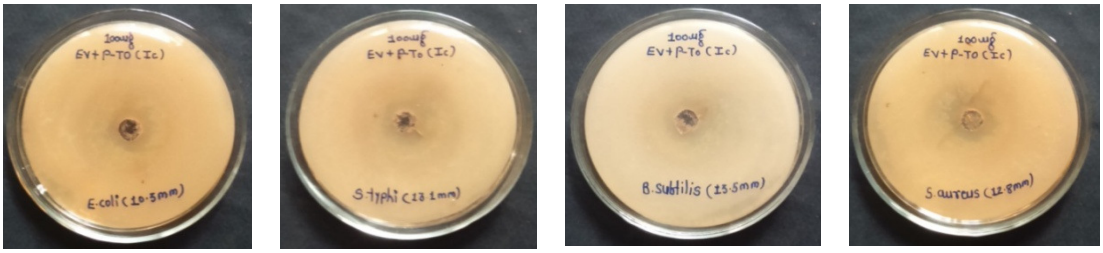
E. coli ***S. aureus***

Figure-2
Zone of inhibition of azo compound 1a



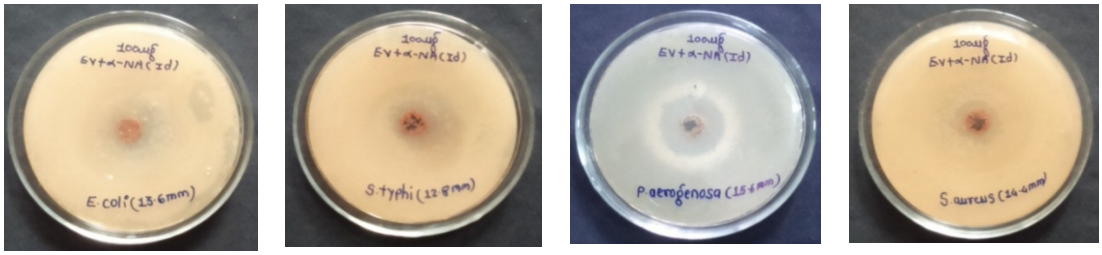
E. coli ***S. typhi*** ***B. subtilis*** ***S. aureus***

Figure-3
Zone of inhibition of azo compound 1b



E. coli ***S. typhi*** ***B. subtilis*** ***S.aureus***

Figure-4
Zone of inhibition of azo compound 1c



E. coli ***S. typhi*** ***P. aeroginosa*** ***S.aureus***

Figure-5
Zone of inhibition of azo compound 1d

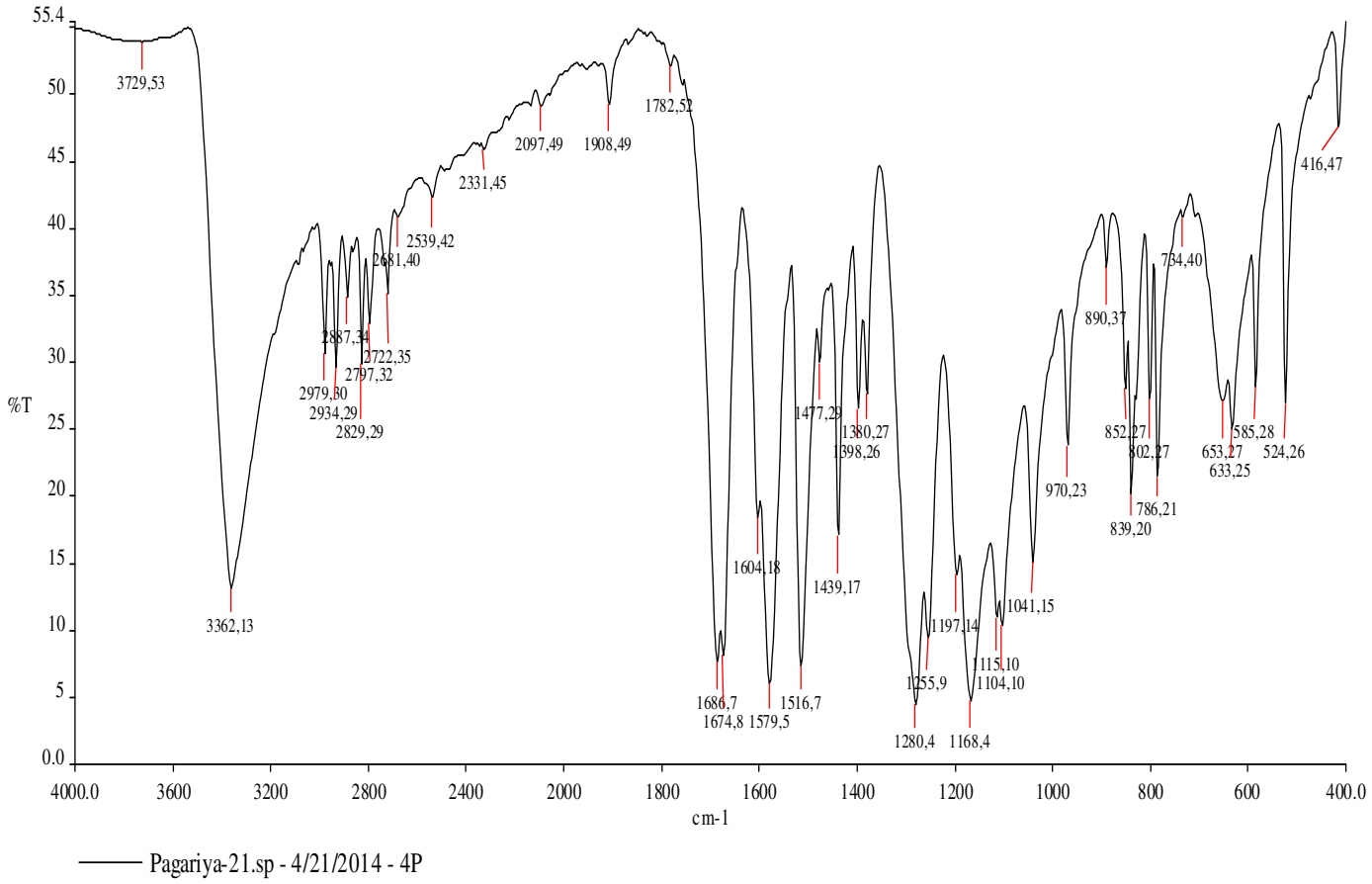


Figure-6
FTIR spectrum of azo compound 1a

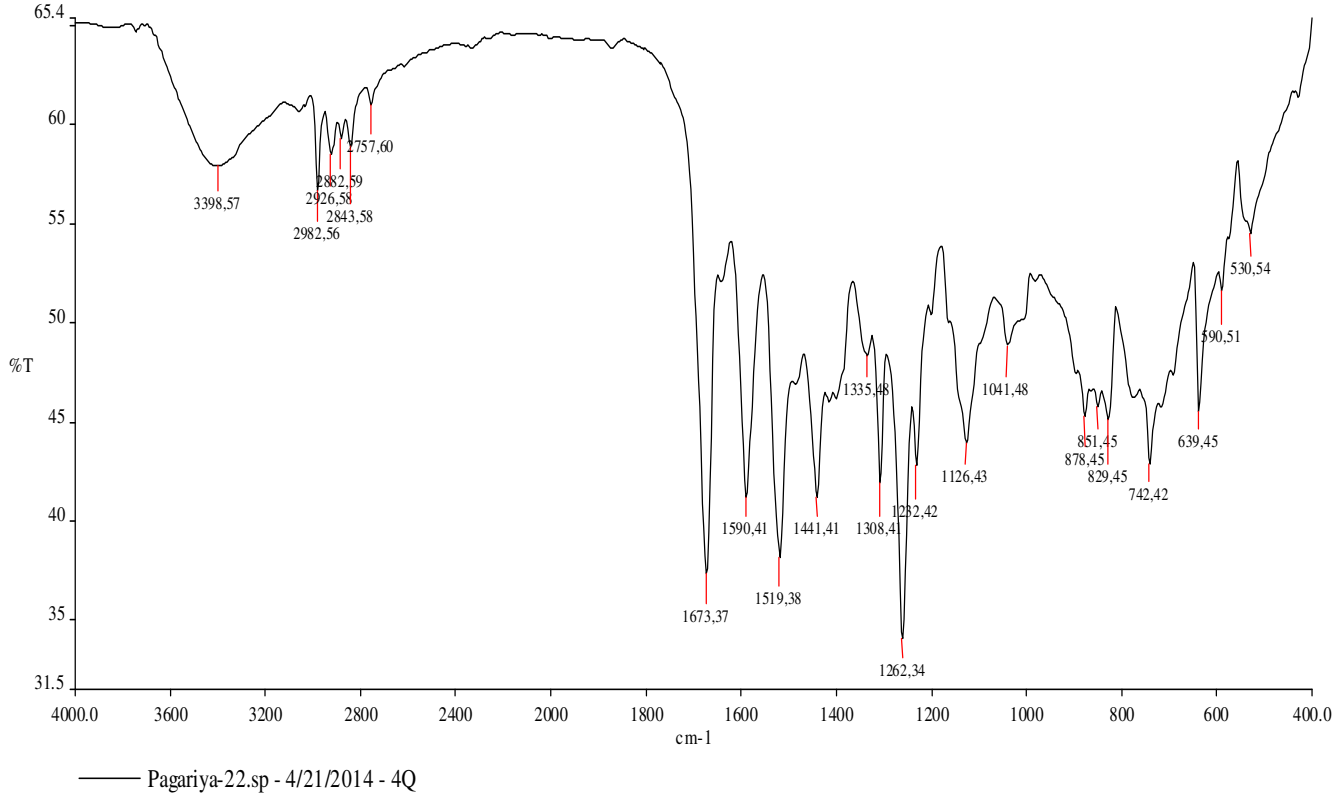


Figure-7
FTIR spectrum of azo compound 1b

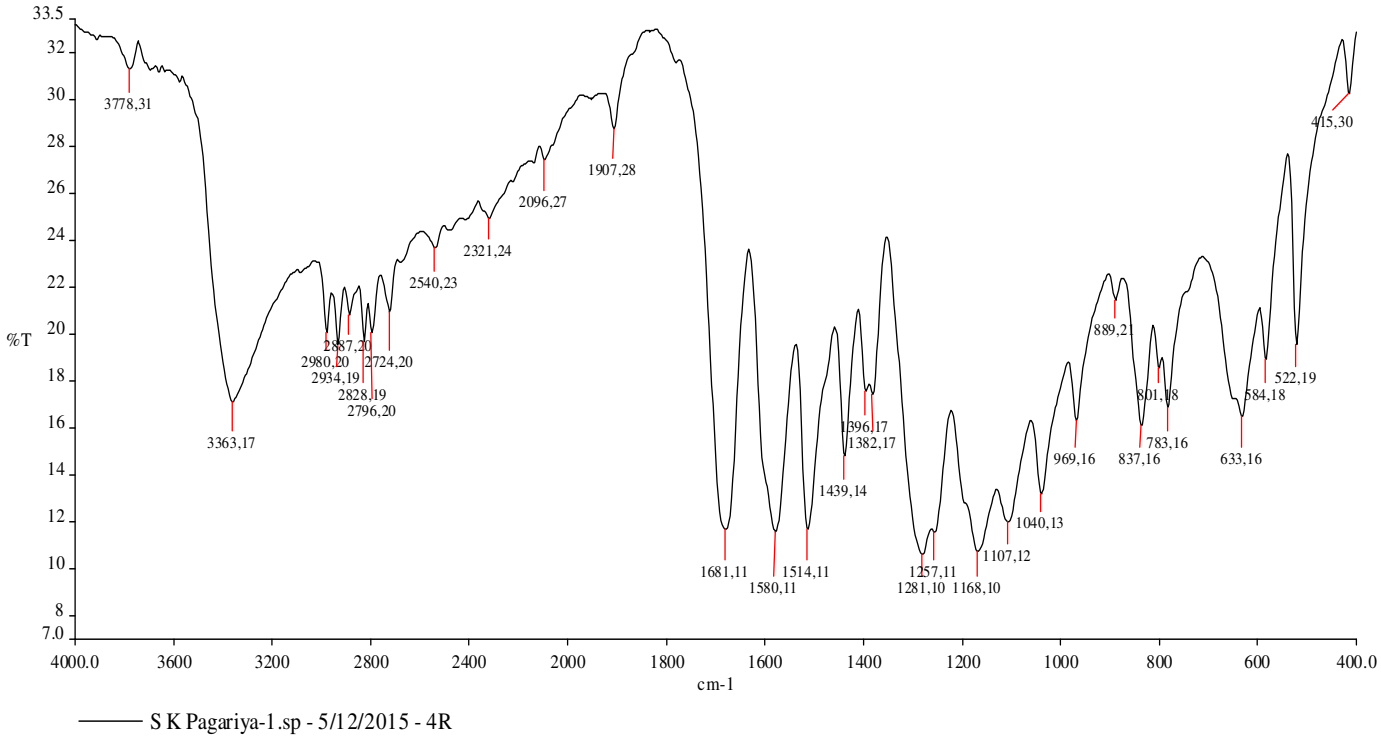


Figure-8
FTIR spectrum of azo compound 1c

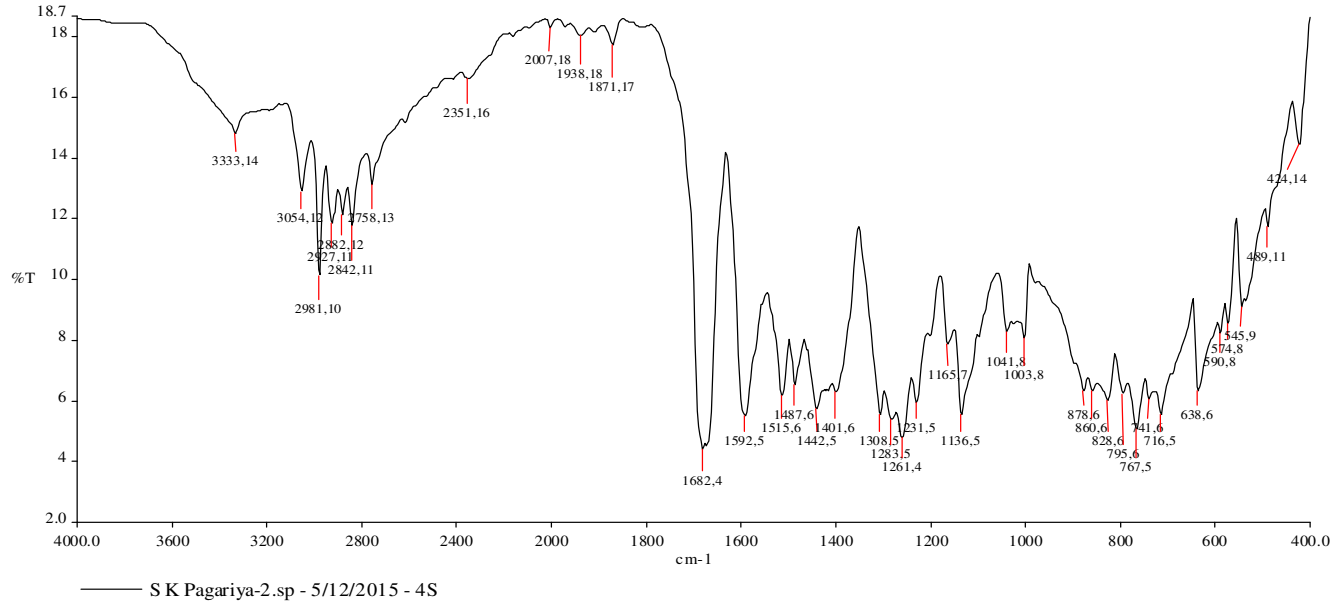


Figure-9
FTIR spectrum of azo compound 1d

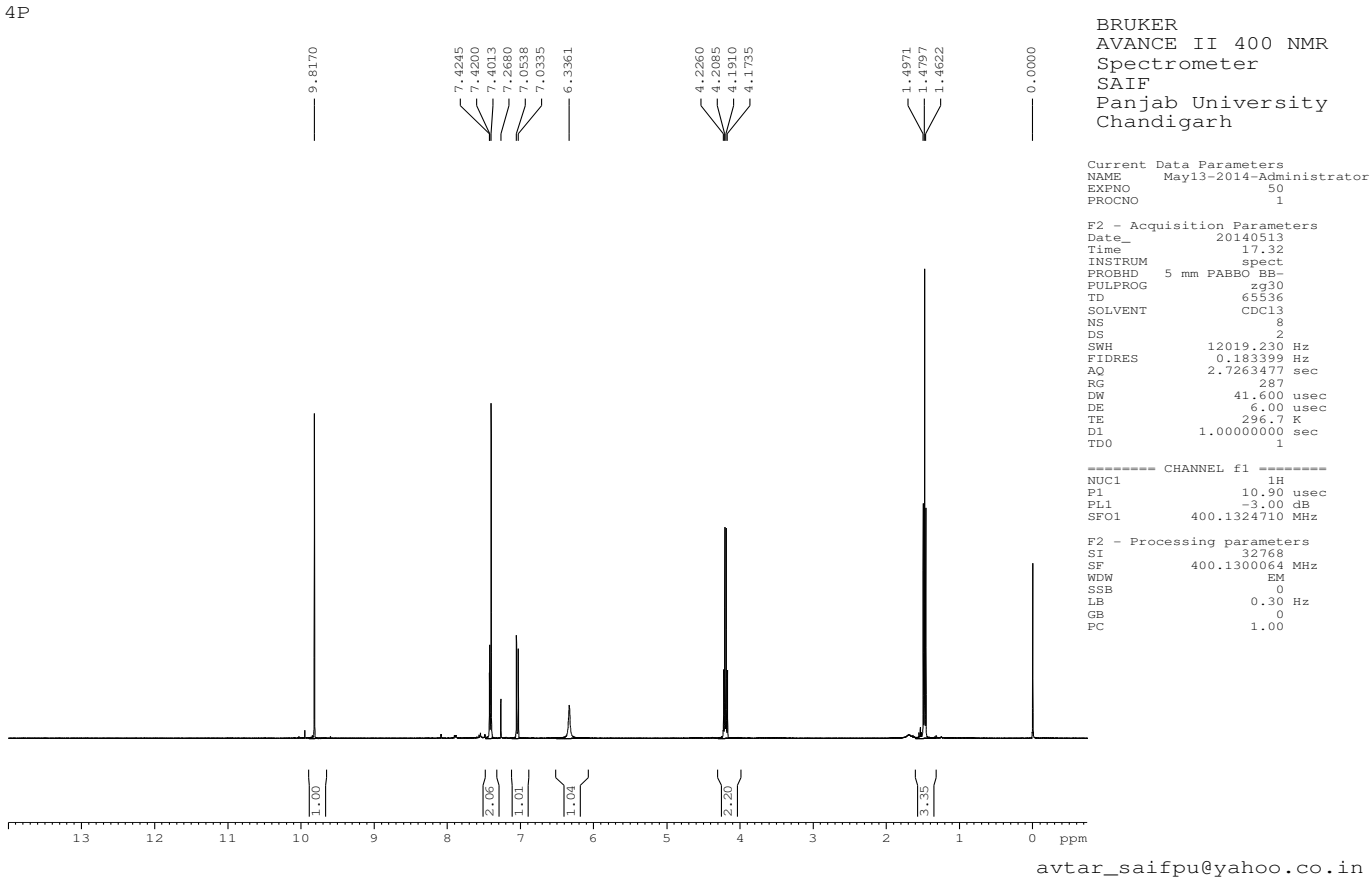


Figure-10
H^1 NMR spectrum of azo compound 1a

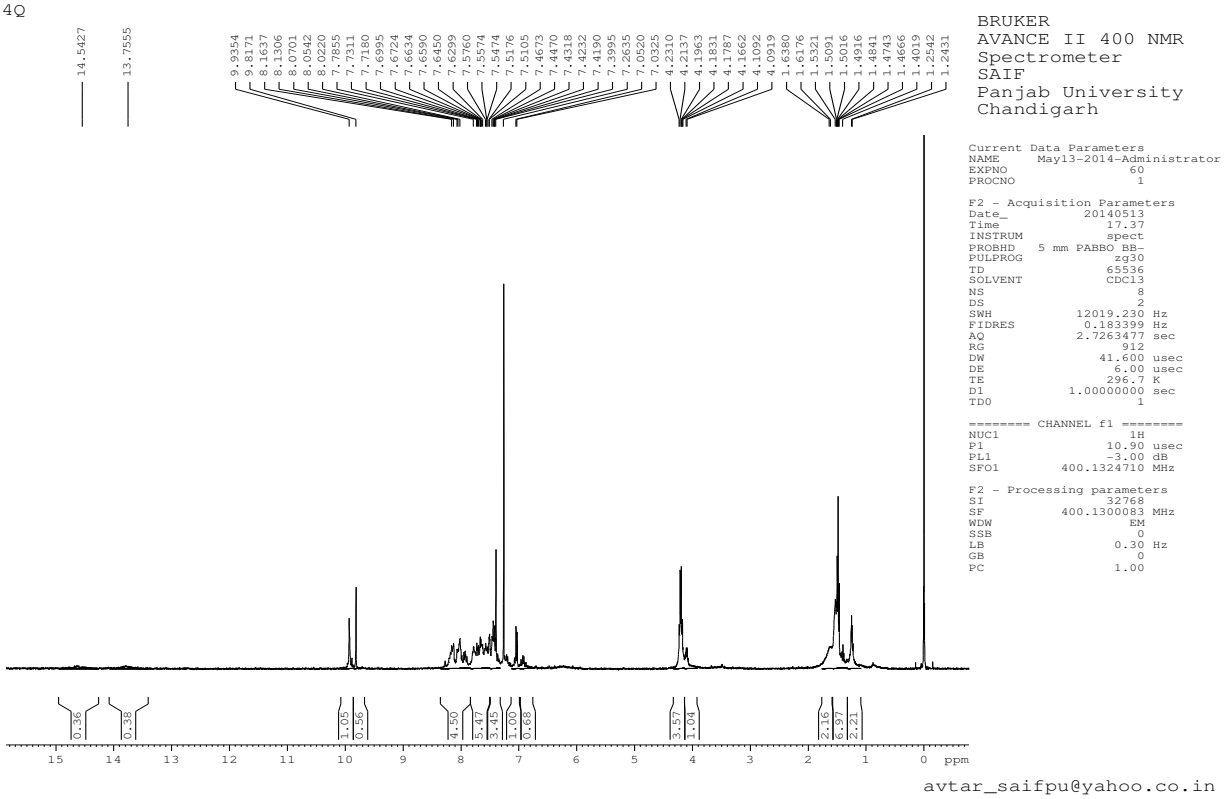


Figure-11
H[1] NMR spectrum of azo compound 1b

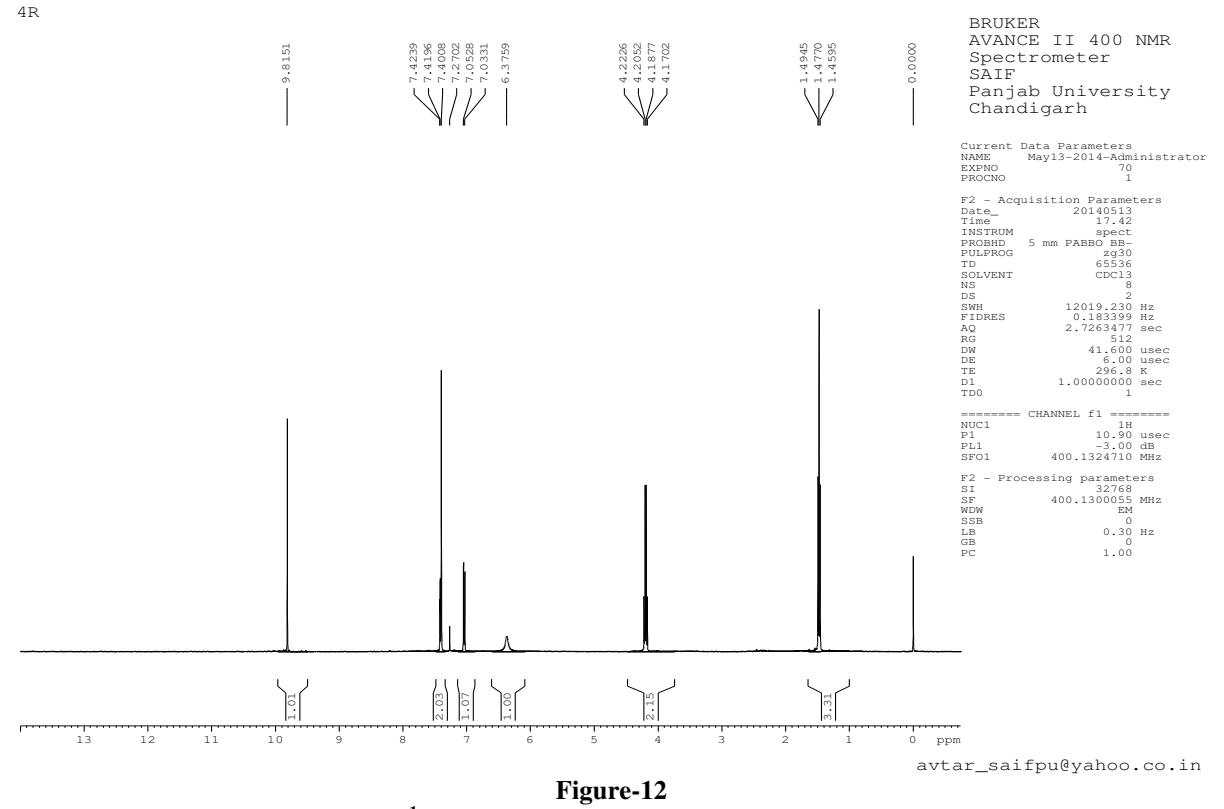


Figure-12
H[1] NMR spectrum of azo compound 1c

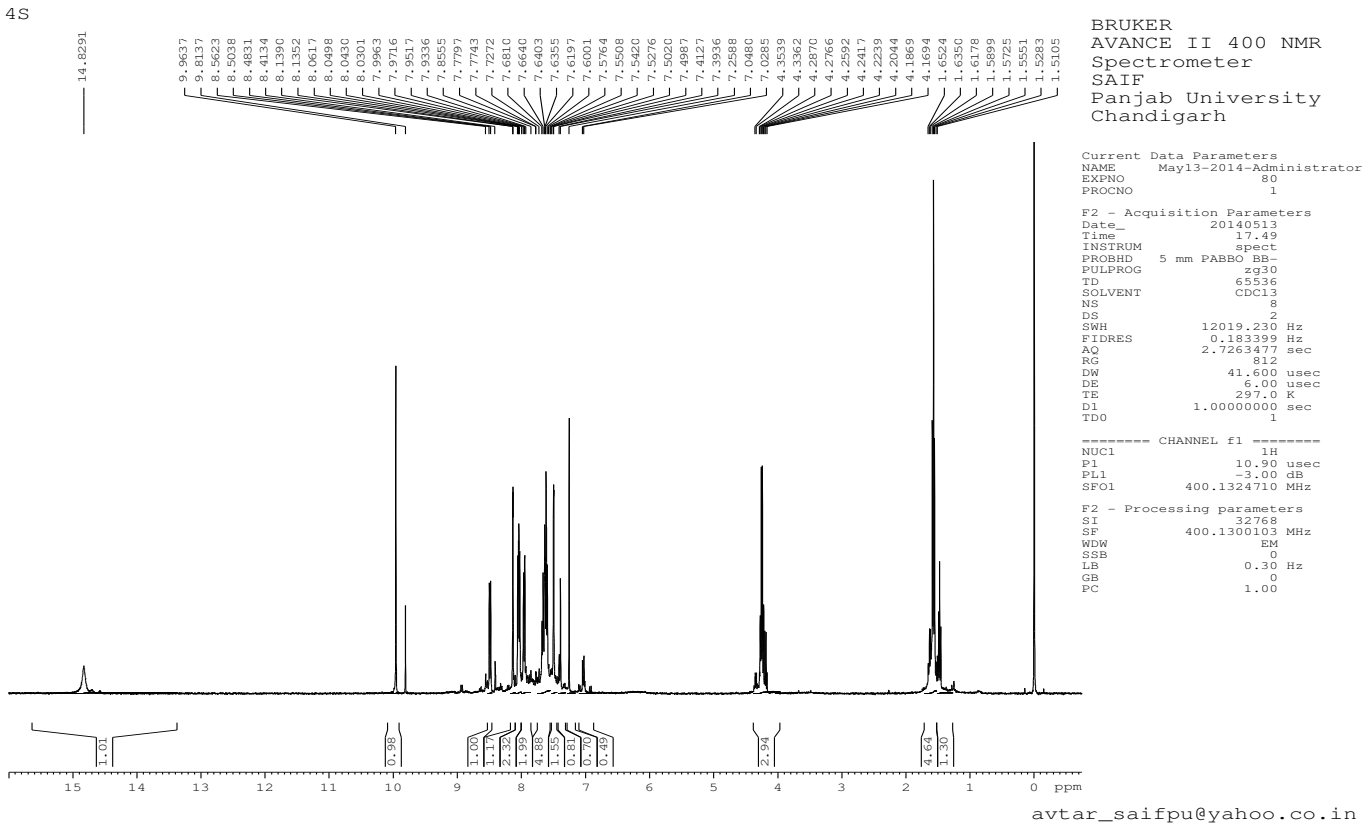


Figure-13
H[1] NMR spectrum of azo compound 1d

Conclusion

The current investigation reveals that, there was a miraculous inhibition of compound 1d shows antibacterial activity against all the tested organisms while the compound 1a was found to be most resistant by the pathogens. The highest zone of inhibition was obtained for 1d against *Pseudomonas aeroginosa* (15.6 mm) while lowest zone was recorded for 1a against *Staphylococcus aureus* (9.6 mm). The compounds 1b and 1c shows moderate inhibition of growth against tested pathogens.

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