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SYNTHESIS, AND CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF 2-(5-{[(1Z)-SUBSTITUTED METHYLENE] AMINO}-1, 3, 4-THIADIAZOL-2-YL) PHENOL DERIVATIVES

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ABSTRACT

Chemical modifications of drug molecules of a series having optimal activity is widely used and continue to be an important factor in new drug discovery studies. In order to obtain new, effective and safe drugs has led today's researchers to improve the existing drugs by increasing their potency, duration of action and by decreasing the toxic side effects. Structure activity studies show that variations in ring system or minor group extend distinct pharmacological effect upon the drug molecules. 1,3,4-Thiadiazoles are biologically important group of compounds having activities like antibacterial, antifungal, antiinflammatory, diuretic, antiulcer, antihelmintic other biological activities. Prompted by these reports, it was contemplated to synthesize new 1,3,4-thiadiazoles. Thus an attempt was made to synthesize 2-(5-{[(1Z)-substituted methylene]amino}-1,3,4-thiadiazol-2-yl)phenol derivatives in this present study . It is likely that the new derivatives with some modification in their chemical structure may result in some profound change in the pharmacological response. It may increase, decrease or alter the nature of the response.

Keywords: Aminophylline, Bulk drug, Formulation.

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INTRODUCTION

Organic chemists are frequently facing the problem of characterizing and elucidating the structure of organic compounds. In the field of natural products has the prospect of isolating such compounds from their sources in a pure state and then determining their structure [1]. On the other hand the synthetic organic chemists encounter new or unexpected compounds in the course of investigations into the applicability of new reagents or techniques or as by products of established Reactions [2].

All the reactions were carried out under prescribed laboratory conditions. All the reactions requiring anhydrous conditions were conducted in well dried apparatus. The solvents and reagents used in the synthetic work were of laboratory reagent grade and were purified by distillation and crystallization techniques wherever necessary and their melting points were checked with the available literature.

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MATERIALS AND METHODS

Melting points of newly synthesized compounds were determined by open capillary method and were uncorrected. Purity of the compounds was routinely checked by micro TLC.

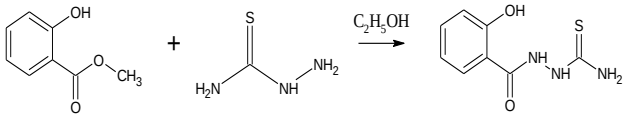
The IR spectra of the compounds were recorded on THERMONICOLET NEXUS-670spectrometer using KBr pellet. 1H NMR spectra were recorded in a ADVANCE-300MHz spectrometer using TMS as internal

standard. Mass spectra were recorded in NCMS-spectrometer.

The present work deals with the reaction between reduced Schiff's base with five different aldehydes such as benzaldehyde/ salicylaldehyde/ chlorobenzaldehyde/ nitrobenzaldehyde/ vanillin. The reaction was performed as follows. Methyl salicylate was treated with thiosemicarbazide. Later it is cyclized using conH2SO4 and ammonia to form Schiff's base, which is then reduced with five different aldehydes such as benzaldehyde/ salicylaldehyde/ chlorobenzaldehyde/ nitrobenzaldehyde/ vanillin.

Synthesis

Synthesis of 2-benzoyl hydrazine carbothioamide (III).

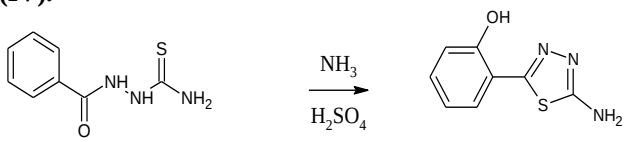


(I) (II) (III)

A mixture of methyl salicylate **(I)** (15ml, 0.01mol) and thiosemicarbazide **(II)** (15.2gms, 0.01mol) in ethanol was refluxed for 3hrs [3]. The resultant solution was cooled to room temperature and poured into crushed ice. A solid separates out and it is allowed to settle down during 0.5hrs. It is filtered and washed with water. The crude product thus obtained was recrystallized from chloroform.

PERCENTAGE YIELD = 85.3%
COLOUR = Pale brown
MELTING POINT = 160-161^{0}C

Synthesis of 2-(5-amino-1,3,4-thiadiazol-2-yl)phenol (IV).

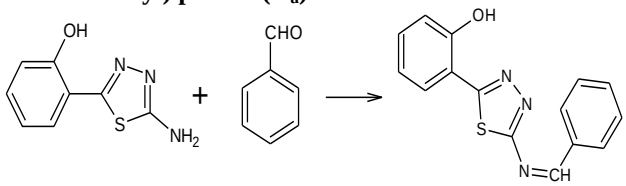


(III) (IV)

Compound (III) (0.01mol) was added slowly to concH_2SO_4 with stirring Maintaining the temperature below 0^{0}C. The temperature was maintained at 0^{0}C for another 1hr and the reaction mixture was allowed to stand at room temperature overnight [4]. The contents were warmed to 50, cooled and poured over crushed ice. The solid, thus obtained was washed with water and treated with a solution of ammonia. The solid thus obtained was collected washed with water, dried and crystallized from ethanol.

PERCENTAGE YIELD = 76,5%
COLOUR = Pale yellow
MELTING POINT = 179-180^{0}C

Synthesis of 2-(5-{(1Z)-methyleneamino}-1, 3, 4-thiadiazol-2-yl) phenol (V_a).

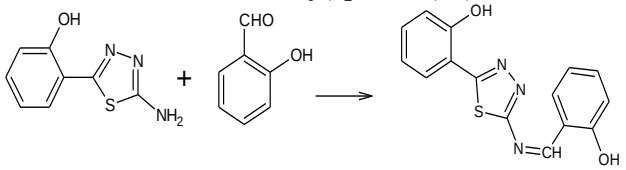


(IV) Benzaldehyde (V_a)

A mixture of compound **(IV)** (19.3gms, 0.01mol) and benzaldehyde in equimolar quantity using ethanol as solvent was refluxed for 3hrs. The resultant solution was cooled to room temperature and poured into crushed ice. A solid separates out and it is allowed to settle down during 0.5hrs. It is filtered and washed with water. The crude product thus obtained was recrystallized from water.

PERCENTAGE YIELD = 76. 5%
COLOUR = Creamy white
MELTING POINT = 179-180^{o}C

Synthesis of 2-(5-{[(1Z)-(2-hydroxyphenyl) methylene] amino}-1, 3, 4-thiadiazol-2-yl) phenol (V_b).

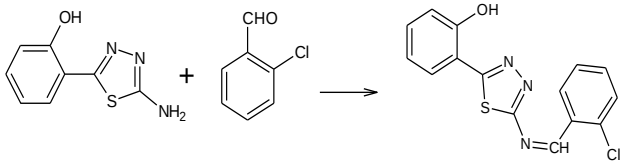


(IV) Salicylaldehyde (V_b)

A mixture of compound **(IV)** (19.3gms, 0.01mol) and salicylaldehyde in equimolar quantity using ethanol as solvent was refluxed for 3hrs. The resultant solution was cooled to room temperature and poured into crushed ice. A solid separates out and it is allowed to settle down during 0.5hrs. It is filtered and washed with water. The crude product thus obtained was recrystallized from water [5].

PERCENTAGE YIELD = 81.4%
COLOUR = Pale brown
MELTING POINT = 210^{0}C

Synthesis of 2-(5-{[(1Z)-(2-chlorophenyl)methylene]amino}-1,3,4-thiadiazol-2-yl)phenol (V_c).

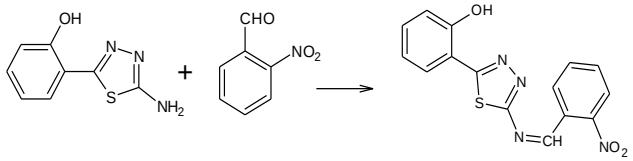


(IV) Chlorobenzaldehyde (V_c)

A mixture of compound **(IV)** (19.3gms, 0.01mol) and chlorobenzaldehyde in equimolar quantity using ethanol as solvent was refluxed for 3hrs. The resultant solution was cooled to room temperature and poured into crushed ice. A solid separates out and it is allowed to settle down during 0.5hrs. It is filtered and washed with water. The crude product thus obtained was recrystallized from water.

PERCENTAGE YIELD = 31%
COLOUR = Creamy white
MELTING POINT = 220^0C

Synthesis of 2-(5-{[(1Z)-(2-nitrophenyl)methylene]amino}-1,3,4-thiadiazol-2-yl)phenol (V_d).

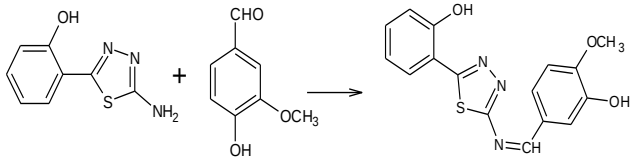


(IV) Nitrobenzaldehyde (V_d)

A mixture of compound **(IV)** (19.3gms, 0.01mol) and nitrobenzaldehyde in equimolar quantity using ethanol as solvent was refluxed for 3hrs. The resultant solution was cooled to room temperature and poured into crushed ice. A solid separates out and it is allowed to settle down during 0.5hrs. It is filtered and washed with water. The crude product thus obtained was recrystallized from water.

PERCENTAGE YIELD = 47%
COLOUR = Yellow
MELTING POINT = 265^0C

Synthesis of 2-[(Z)-{[5-(2-hydroxyphenyl)-1,3,4-thiadiazol-2-yl]amino}methyl]2-methoxyphenol (V_e).



(IV) Vanillin (V_e)

A mixture of compound (IV) (19.3gms, 0.01mol) and vanillin in equimolar quantity using ethanol as solvent was refluxed for 3hrs. The resultant solution was cooled to room temperature and poured into crushed ice. A solid separates out and it is allowed to settle down during 0.5hrs. It is filtered and washed with water. The crude product thus obtained was recrystallized from water.

PERCENTAGE YIELD = 63%
COLOUR = Creamy white
MELTING POINT = 278^0C

ANTIMICROBIAL ACTIVITY

Evaluation of antimicrobial activity

The antimicrobial activity can be evaluated by serial dilution test and disc diffusion test. Diffusion test used to determine the sensitivity of organism by measuring zone of inhibition. Serial dilution test is used to determine the minimum inhibitory concentration (MIC). MIC is the lowest concentration of a drug that inhibits the growth of particular organism under specified conditions. Initially the zone of inhibition carried out to evaluate the sensitivity of the organism towards the compounds. From the zone of inhibition data the organisms were selected for determination of MIC [6].

Disc diffusion test

Modified Kirby-Bauer method[51] was used for the evaluation of microbial sensitivity of the synthesized compounds. Circular paper disks were impregnated with the specific amount of test compounds and were placed on suitable agar medium (Muller Hinton agar), which was inoculated with the test organism. After incubation, the Petri dishes were observed for growth of inhibition zone around the disk. A "halo" or Zone of inhibition forms, where concentration of the diffused molecule is sufficient to inhibit microbial growth. The diameter of zone of inhibition is directly proportional to antimicrobial activity of the compound. The diameter of zone of inhibition was compared with that of standard antibiotics [7].

The size of zone of inhibition depends on rate of antibiotic diffusion, rate of bacterial growth and incubation condition, concentration of organism.

Cultivation of microorganism

The following bacterial cultures were used for the study.

1. *Bacillus subtilis* - Gram positive bacteria
2. *Staphylococcus aureous* - Gram positive bacteria
3. *Escherichia coli* - Gram negative bacteria
4. *Pseudomonas aeruginosa* - Gram negative bacteria

The following fungal cultures were used for the study.

- *Aspergillus niger*
- *Candida albicans*

Drugs control

Ampicillin (antibacterial)
Clotrimazole (antifungal)

Concentration All the test compounds were tested at 100 µg/ml.

Solvent: Dimethylformamide (DMF)

Preparation of paper discs

Paper disk of 6 mm diameter and 2 mm thickness was used for the test. These disks were found to absorb 0.02 ml of the solvent (DMF). These disks were

sterilized by autoclaving at 121°C (15lbs psig) for 15 minutes**.**

Preparation of culture medium:

It provides all essential nutrients for the growth of microorganism. Muller Hinton agar medium was used to incolate bacterial strains and Sabourands medium used for fungal strains.

The medium was prepared by dissolving the specified quantity of the dehydrated medium in purified water and was dispersed in 20ml volumes in to test tubes. The test tubes were closed with cotton plugs and were sterilized by autoclaving at 121°C (15 lb psig) for 15 minutes. The contents of tubes were poured aseptically in to sterile Petri plates (90mm diameter) and allowed to solidify.

Procedure

Fill Petri dishes to depth of 3-4 mm with a nutrient agar medium, which has previously been inoculated, with suitable inoculums of a susceptible test organism. The dishes should be selected with flat bottom and should be placed on a level surface to ensure that the layer of the medium will be of a uniform thickness each plate was divided into six equal positions along the diameter. Each portion was used to place one disk. Four disk of each sample was placed on four portions, two disks were placed one each with ciprofloxacin disk and a disk impregnated with the solvent.

All plates were kept in the refrigerator for 30 min to allow the diffusion of sample to the surrounding agar medium. The Petri dishes were incubated at 30°C for 18 h. Diameter of the zone of inhibition was measured and the average diameter for each sample was calculated. The diameter obtained for the test samples were compared with that produced by standard ciprofloxacin.

Similar procedure was carried out for the evaluation of antifungal activity of using Sabourauds dextrose agar medium and, clotrimazole, 10 µg discs as standard drug. Antifungal activity was tested against *Candida albicans and Aspergillus niger*

Determination of MIC by serial dilution method

MIC of the synthesized compounds were determined by tube dilution techniques[52]Serial dilution of the substance under examination was placed into culture tubes containing suitable medium and inoculated with the test organism. After incubation, the minimum concentration of test compound that inhibited the growth of the organism was observed.

Cultivation of microorganism

The following bacterial cultures were used for the study

1. *Bacillus subtilis* - Gram positive bacteria
2. *Staphylococcus aureous* - Gram positive bacteria
3. *Escherichia coli* - Gram negative bacteria
4. *Pseudomonas aeruginosa* - Gram negative bacteria

The following fungal cultures were used for the study.

- *Aspergillus niger*
- *Candida albicans*

Drugs control

Ampicillin (antibacterial)
Griseofulvin (antifungal)

Concentrations

Solvent: Dimethylformamide (DMF)

The media were prepared by dissolving the specified quantity of dehydrated medium (Hi-medium) in purified water. The medium was distributed 4 ml quantities into test tubes. The tubes were closed with cotton plug and sterilized by autoclaving at 121 ^{o} C (15lbs psig) 15 min.

Procedure

All the synthesized compounds were dissolved separately to prepare a stock solution containing 1000 µg/ml of DMF. 32 mg of different synthesized compounds were dissolved in 2 ml of the DMF and 1 ml of this solution was aseptically transferred to the sterile nutrient broth medium and made up to 16 ml with sterile nutrient media, thus 1 ml of the resulted solution gives 1000 µg/ml. 1 ml of the above solution was transferred to 1 ml of DMF to give half the concentration of first. Thus successive concentrations like 250, 125, 62.5 and so were prepared in a similar manner up to 6 dilutions from sixth one ml of the solution is discarded. The tubes were mixed well after each addition.

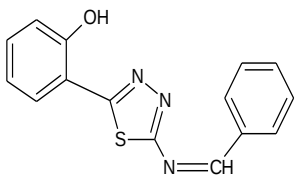
All the tubes were inoculated with one loopful of one of the test organism. The process was repeated with different test organisms. A positive control and a negative control were also prepared to confirm the nutritive property and sterility, respectively of the prepared medium. The tubes were incubated 37°C for 24 hours. The presence or absence of growth of organism was observed after incubation compared with that of standard drug (ampicillin) .

Similar procedure was carried out for the evaluation of antifungal activity using Sabourauds dextrose agar medium by standard drug (Griseofulvin).

RESULTS AND DISCUSSION

Spectral data of synthesized compounds

Spectral data of 2-(5-{(1Z)-methyleneamino}-1, 3, 4-thiadiazol-2-yl) phenol (Va).



The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 1.

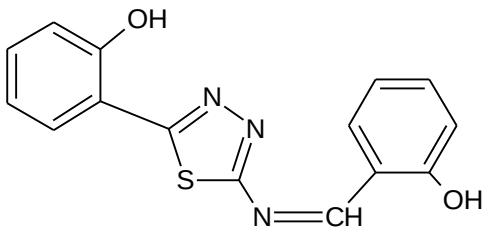
Proton Magnetic Resonance Spectrum:

The H^1NMR spectrum was recorded on ADVANCE-300MHz spectrometer using TMS as internal standard and $CDCl_3$ as solvent is represented in figure 2.

Mass Spectrum:

The mass spectrum of the compound was recorded on NCMS spectrometer is given in figure 3. The mass spectrum Showed base peak at 210 corresponding to M peak indicating molecular weight of the compound 281.

Spectral data of 2-(5-{[(1Z)-(2-hydroxyphenyl)methylene]amino}-1, 3, 4-thiadiazol-2-yl) phenol (Vb).



The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 4.

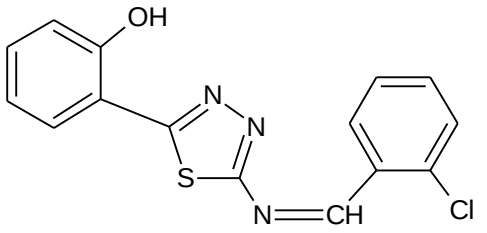
Proton Magnetic Resonance Spectrum:

The H^1NMR spectrum was recorded on ADVANCE-300MHz spectrometer using TMS as internal standard and DMSO as solvent is represented in figure 5.

Mass Spectrum:

The mass spectrum of the compound was recorded on NCMS spectrometer is given in figure 6. The mass spectrum Showed base peak at 210 corresponding to M peak indicating molecular weight of the compound 297.**M+1 peak.**

Spectral data of 2-(5-{[(1Z)-(2-chlorophenyl)methylene]amino}-1, 3, 4-thiadiazol-2-yl) phenol (Vc).



The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 7.

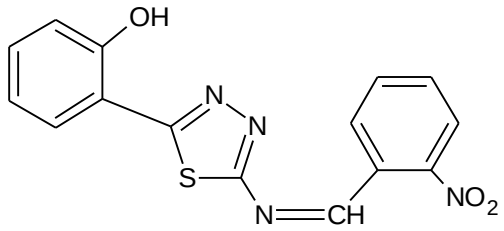
Proton Magnetic Resonance Spectrum:

The H^1NMR spectrum was recorded on ADVANCE-300MHz spectrometer using TMS as internal standard and Acetone as solvent is represented in figure 8.

Mass Spectrum:

The mass spectrum of the compound was recorded on NCMS spectrometer is given in figure 9. The mass spectrum Showed base peak at 210 corresponding to M peak indicating molecular weight of the compound 315.7.

Spectral data of 2-(5-{[(1Z)-(2-nitrophenyl)methylene]amino}-1, 3, 4-thiadiazol-2-yl) phenol (Vd).

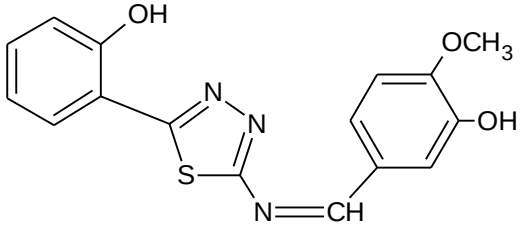


The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 10.

Proton Magnetic Resonance Spectrum:

The H^1NMR spectrum was recorded on ADVANCE-300MHz spectrometer using TMS as internal standard and $CDCl_3$+DMSO as solvent is recorded in figure 11.

Spectral data of 2-[(Z)-(2-hydroxyphenyl)-1, 3, 4-thiadiazol-2-yl) phenol (Ve)



The IR spectrum of the compound was recorded on THERMONICOLET NEXUS-670spectrometer by KBr method is given in figure 12.

Antimicrobial Screening

A drug which kills or inhibits the growth of microbes is known as antimicrobial agent. Invitro tests are used as screening procedure for new agents to test the susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. Due to development of sulphonamides

and penicillin's invitro measurement of susceptibility of microbes to chemotherapeutic agents have been used.

A drug is considered to be bacteriostatic or fungistatic when they inhibit the growth of bacteria or fungi respectively, and bactericidal or fungicidal due to its ability to kill bacteria or fungi. Important factors for antimicrobial activity are size of the inoculum, metabolic state of microbes, pH, temperature, and duration of interaction, concentration of inhibitor and presence of interference substances. The development of resistance among various pathogenic microbes towards antibiotics has increased the impetus for investigating new antimicrobial agents. When a compound was found to have positive therapeutic index, anew series of related compounds are synthesized in the hope that one of them would be more effective than the existing one.

Antibacterial activity was carried out on four bacterial strains of which two are gram positive and other two are gram negative bacteria: *Bacillus Subtilis , Staphylococcus Aureus, Escheria Coli, Pseudomonas Aeruginosa*. Antifungal activity was carried out on two fungal strains:*Candida Albicans* and *Aspergillus Niger.*

DISC DIFFUSION METHOD

(a). Antibacterial activity

All the synthesized compounds have shown potent to weak antibacterial activity. Compounds V_b, V_e showed potent antibacterial activity against B.substilis and P.aureginosa. V_c, V_d showed moderate antibacterial activity when compared to the standard.

Antifungal activity

From the above results it is evident that all the compounds showed potent to weak antifungal activity. Vc and Vd are having more potent antifungal activity against C.albicans and A.niger. Va and Ve showed moderate antifungal activity compared to the standard. Vb showed weak antifungal activity when compared to the standard drug.

Serial dilution method:

(a). Antibacterial activity studies:

The results showed that Vb have a MIC of 62.5µg/ml against Staphylococcus aureus, which is considered as a good activity when compared to standard compound. Compounds Vc, Vd showed moderate activity at 125µg/ml for Bacillus subtilis and Escherichia coli.

Compounds Ve, Vb have good activity with a MIC of 125µg/ml

Staphylococcus aureus and Escherichia coli. All the compounds showed MIC of 500µg/ml for Escherichia coli.

Compound Vd have good activity against with a MIC of 125µg/ml against Pseudomonas aureginosa and Staphylococcus aureus.

All the other compounds showed MIC of 500µg/ml for Pseudomonas aureginosa.

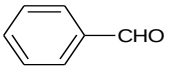
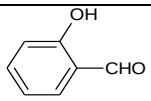
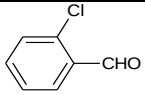
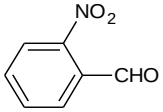
Compounds Vd and Ve have good activity with a MIC of 62.5µg/ml

Against Staphylococcus aureus. All the other compounds showed an MIC of 250µg/ml against Staphylococcus aureus.

Antifungal activity studies

Compound Vc showed excellent antifungal activity (125 µg/ml) against Candida *albicans* and *Aspergillus niger*. Compound Vd and Ve showed good antifungal activity against above organism. Compounds Va and Vb showed poor antifungal activity (1000µg/ml) compared to the standard.

Table 1. Physical Data Of Synthesised Compounds

Compound no	Molecular Formula	R	Molecular wt	% Yield	Melting point ^{0}c
Va	$C_{15}H_{11}N_3OS$		281	76.5%	179-180
V_b	$C_{15}H_{11}N_3O_2S$		297	81.4%	209-210
V_c	$C_{15}H_{10}N_3OSCl$		315.7	31%	219-220
V_d	$C_{15}H_{10}N_4O_3S$		326.3	47%	264-265

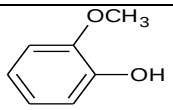
V_e	$C_{16}H_{13}N_3O_3S$		327.3	63%	277-278

Table 1 (a): IR frequencies:

Compound	Types of vibration	Wave number [Cm^{-1}]
Va	Phenolic (OH) Aromatic (CH) Aliphatic (OH) C=N N=C=S	3421.79 3154.07 2979.29 1590.49 1465.94

Table 1 (b) Proton Magnetic Resonance Spectrum:

Compound	Types of proton	Nature of signal	Δ value (ppm)	Number of protons
Va	Aromatic CH along with phenolic OH Imine CH	Multiplet Singlet	6.3-8.0 10.1	10 1

Table 2 (a): IR frequencies:

Compound	Types of vibration	Wave number [Cm^{-1}]
Vb	Phenolic (OH) Aromatic (CH) Aliphatic (OH) C=N N=C=S	3426.42 3166.74 2925.34 1609.66 1462.96

Table 2 (b) Proton Magnetic Resonance Spectrum:

Compound	Types of proton	Nature of signal	Δ value (ppm)	Number of protons
Vb	Aromatic CH along with phenolic OH Imine CH	Multiplet Singlet	6.7-8.4 9.8-10	10 1

Table 3 (a): IR frequencies:

Compound	Types of vibration	Wave number [Cm^{-1}]
Vc	Phenolic (OH) Aromatic (CH) Aliphatic (OH) C=N N=C=S C-Cl	3412.81 3151.88 3020.44 1608.23 1464.14 749.3

Table 3 (b) Proton Magnetic Resonance Spectrum:

Compound	Types of proton	Nature of signal	Δ value (ppm)	Number of protons

Vc	Aromatic CH along with phenolic OH	Multiplet	7.3-8.7	9
	Imine CH	Singlet	10.6-10.9	1

Table 4 (a): IR frequencies:

Compound	Types of vibration	Wave number [Cm^{-1}]
Vd	Phenolic (OH)	3421.10
	Aromatic (CH)	3024.38
	Aliphatic (OH)	2970.61
	C=N	1538.85
	NO_2	1514.37
	N=C=S	1471.37

Table 4 (b) Proton Magnetic Resonance Spectrum:

Compound	Type of proton	Nature of signal	Δ value (ppm)	Number of protons
Vd	Aromatic CH along with phenolic OH	Multiplet	7.2-8.6	9
	Imine CH	Singlet	11.5	1

Table 5 (a): IR frequencies:

Compound	Types of vibration	Wave number [Cm^{-1}]
Ve	Phenolic (OH)	3435.17
	Aromatic (CH)	3034.39
	Aliphatic (OH)	2923.55
	C=N	1591.93
	N=C=S	1462.15

Table 5 (b) Proton Magnetic Resonance Spectrum:

Compound	Types of proton	Nature of signal	Δ value (ppm)	Number of protons
Ve	Aromatic CH along with phenolic OH	Multiplet	6.9-8.1	9
	Imine CH	Singlet	10.3-10.5	1
	OCH_3	Singlet	3.1-3.2	3

Table 6. Antibacterial activity

S.No	Compounds	Diameter of zone of inhibition (mm)			
		B.substilis	S.aureus	P.aeruginosa	E.coli
1	Va	11	13	12	8
2	Vb	17	15	16	12
3	Vc	11	10	13	10
4	Vd	12	13	14	9
5	Ve	14	16	18	12
6	Standard Ampicillin (1mg/ml)	16	14	17	13
7	DMF	-	-	-	-

Table 7. Antifungal activity

S.No	Compounds	Diameter of zone of inhibition (mm)	
		C.Albicans	A.Niger
1	Va	10	12
2	Vb	8	7
3	Vc	13	17
4	Vd	16	18
5	Ve	11	14
6	Standard Ketoconazole (mg/ml)	14	17

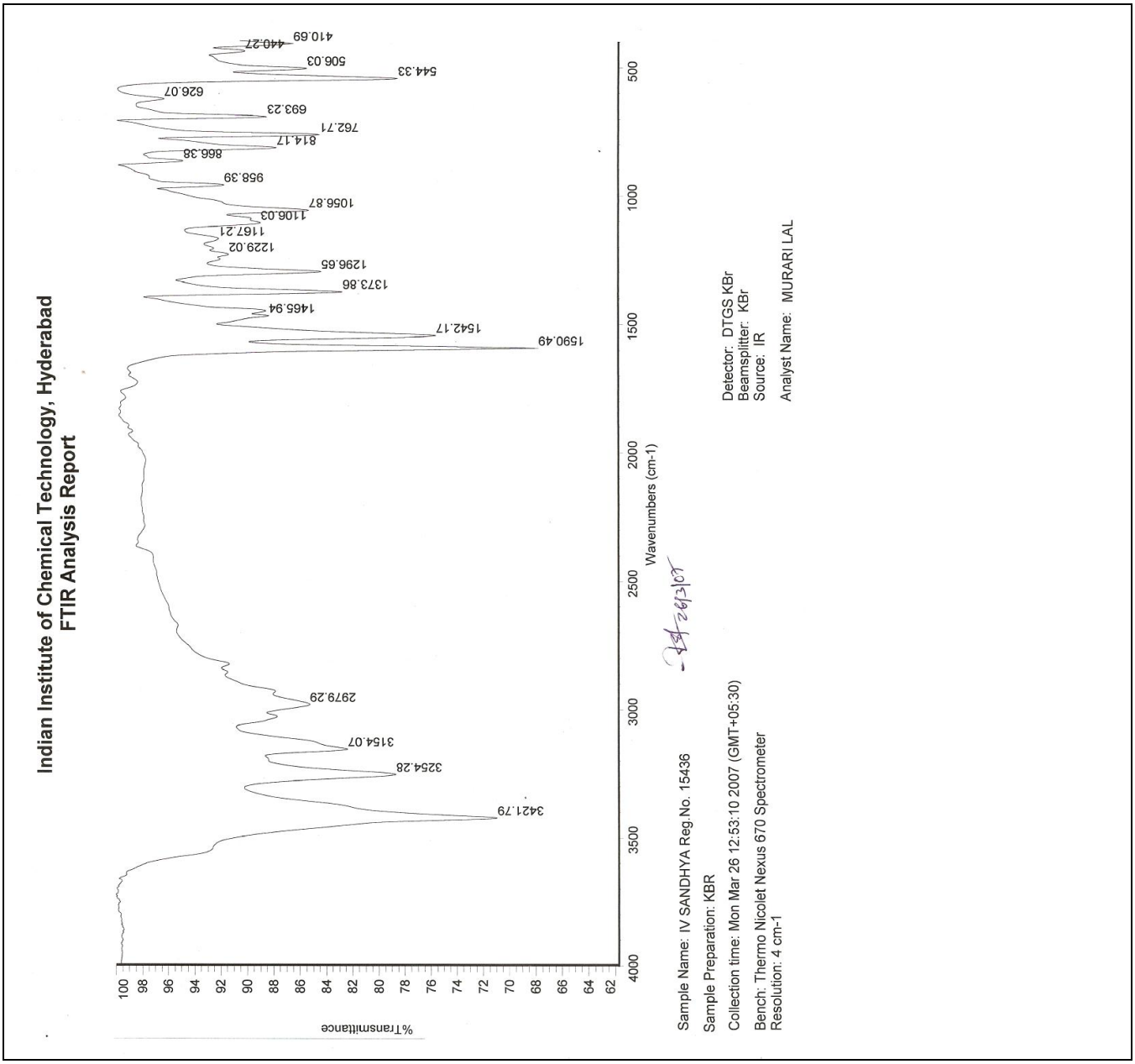


Table 8. Data for Minimum Inhibitary Concentration for Antibacterial activity

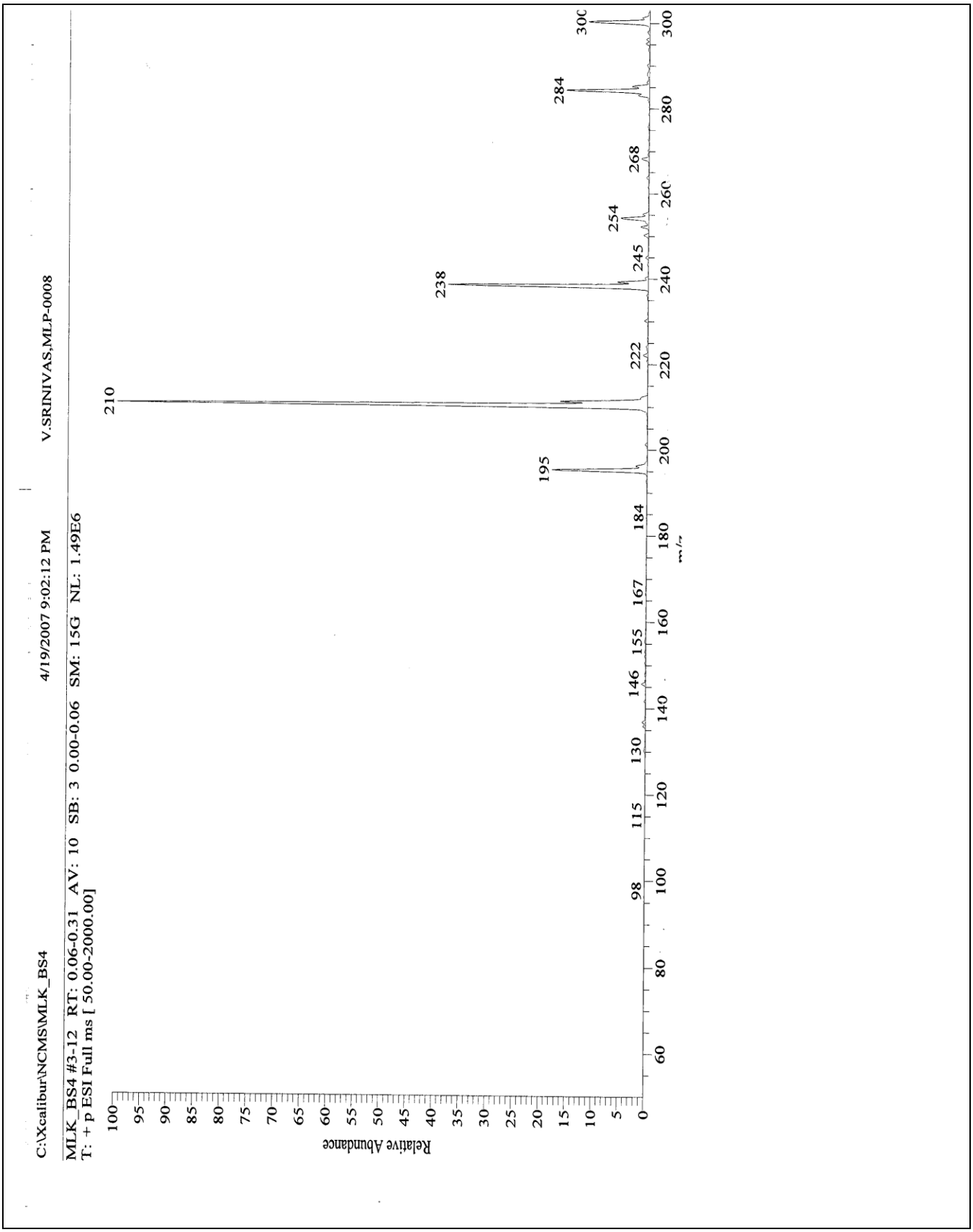
No.	Compound	**Bacillus subtilis**						*Escherichia coli*						*Pseudomonas aureginosa*						**Staphylococcus aureus**					
		Concentration (µg/ml)						Concentration (µg/ml)						Concentration (µg/ml)						Concentration (µg/ml)					
		1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	3125	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25
1	Va	-	-	-	+	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+
2	Vb	-	-	-	-	+	+	-	-	-	-	+	+	-	-	+	+	+	+	-	-	-	+	+	+
3	Vc	-	-	-	+	+	+	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	+	+	+
4	Vd	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	+	+	+
5	Ve	-	-	-	-	-	+	-	-	-	+	+	+	-	-	-	-	+	+	-	-	-	-	-	+
6	+ve control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	-ve control	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Ampicillin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

'+' Indicates presence of growth **'-' Indicates absence of growth**

Table 9. Minimum Inhibitary Concentration for Antifungal activity

No.	Compound No.	**Candida albicans**						**Aspergillus niger**					
		Concentration (µg/ml)						***Concentration (µg/ml)***					
		1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25
1	Va	-	-	+	+	+	+	-	-	-	+	+	+
2	Vb	-	-	+	+	+	+	-	-	-	+	+	+
3	Vc	-	-	-	+	+	+	-	-	-	-	+	+
4	Vd	-	-	-	+	+	+	-	-	-	-	+	+
5	Ve	-	-	-	-	+	+	-	-	-	-	+	+
6	+ve control	+	+	+	+	+	+	+	+	+	+	+	+
7	-ve control	-	-	-	-	-	-	-	-	-	-	-	-
8	Griseofulvin	-	-	-	-	-	-	-	-	-	-	-	-

'+' Indicates presence of growth **'-' Indicates absence of growth**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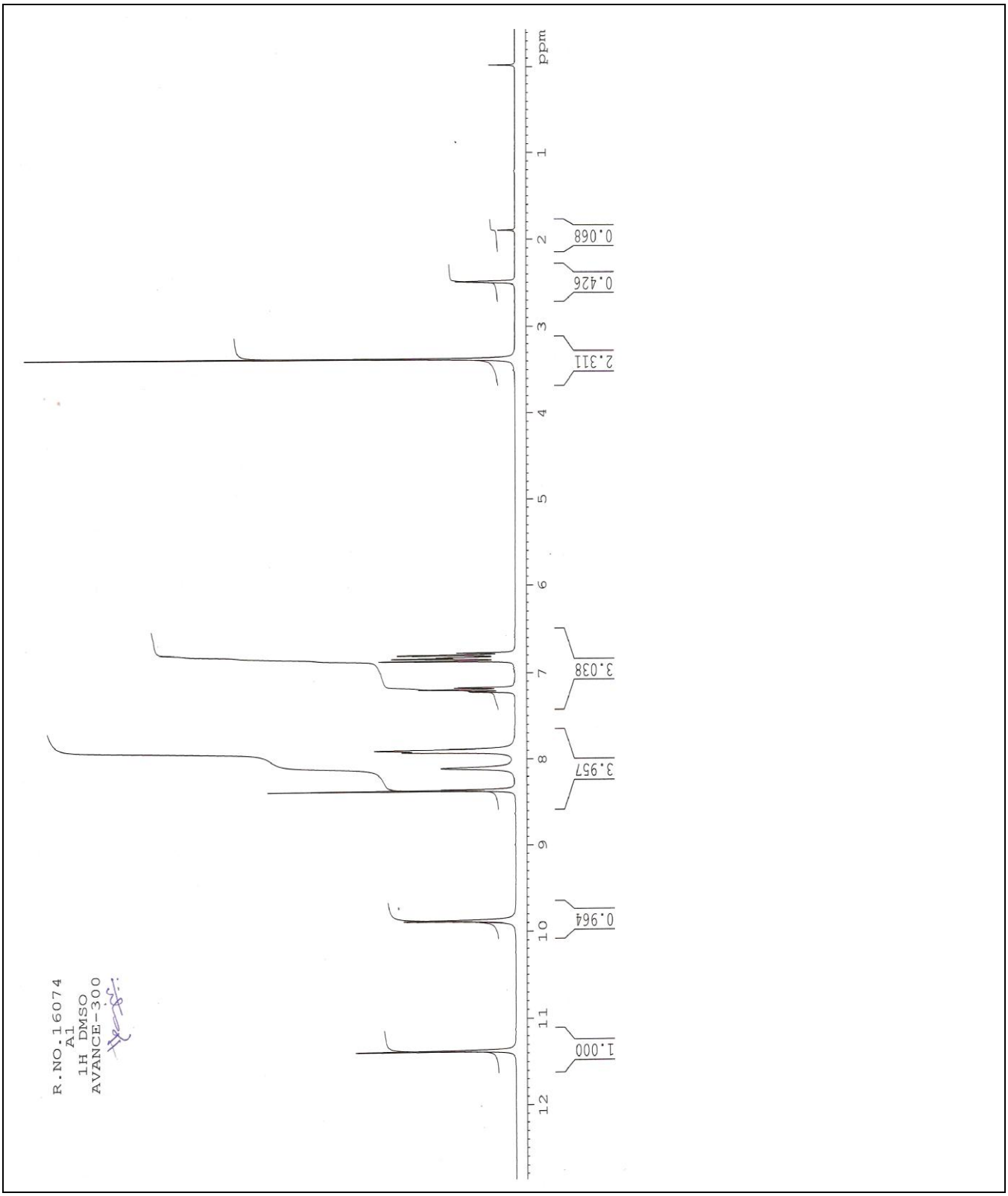
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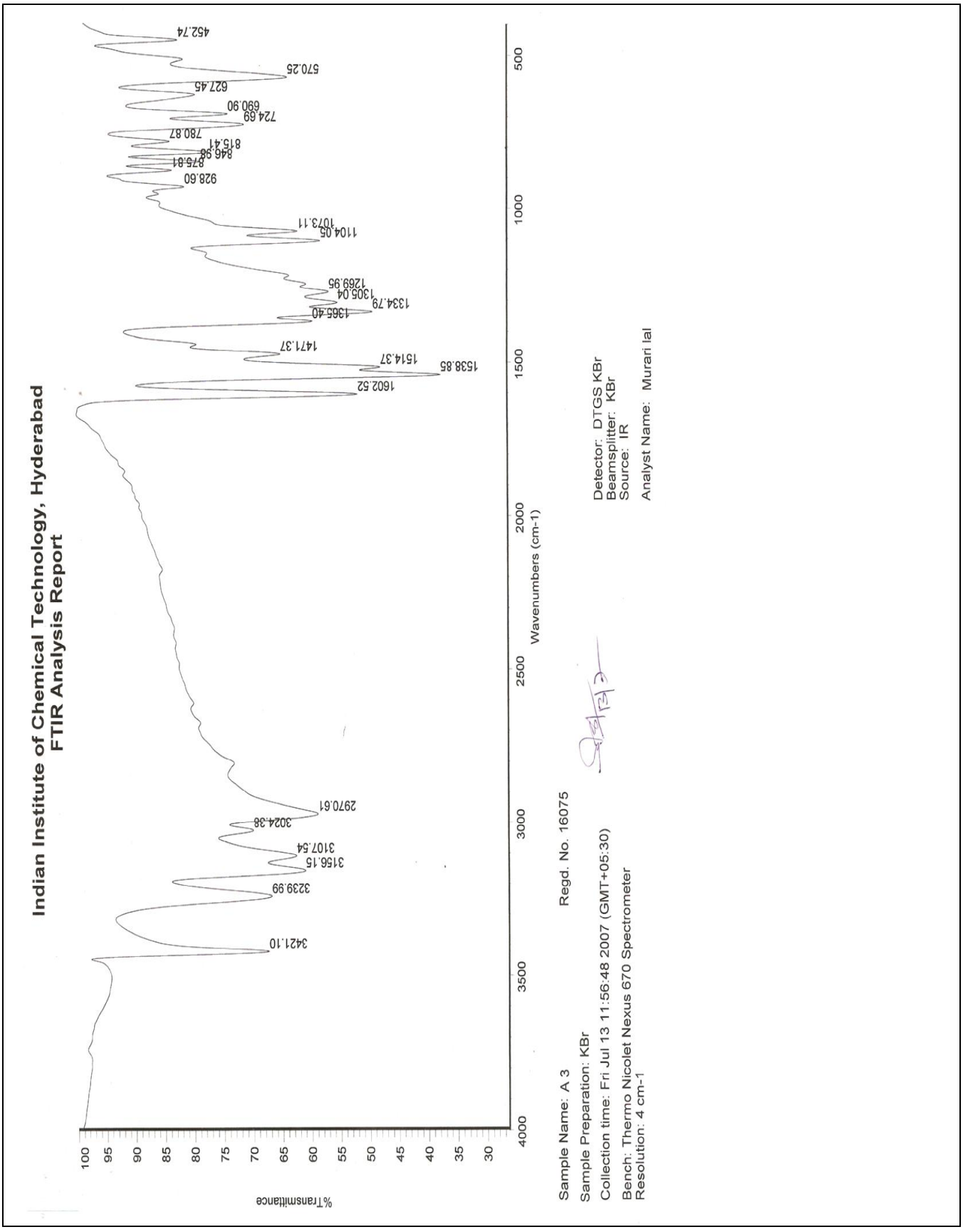
Indian Institute of Chemical Technology, Hyderabad
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35
30
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Collection time: Fri Jul 13 11:56:48 2007 (GMT+05:30)
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Beamsplitter: KBr
Source: IR
Analyst Name: Murari lal

CONCLUSION

This thesis deals with the synthesis, characterization and anti microbial screening of 2-(5-{[(1Z)-substituted methylene] amino}-1, 3, 4-thiadiazol-2-yl) phenol derivatives.

The first chapter of the thesis deals with a brief introduction to therapeutic agents such as Thiadiazoles.

The second chapter of the thesis deals with literature survey on the investigation carried out by earlier workers on the synthesis and evaluating heterocyclic compound 1,3,4-Thiadiazoles.

The third chapter explains the scope and object of the present investigation in detail. In particular, it explains how Thiadiazoles are an important structural feature for biologically active compounds and the structure of five novel compounds proposed to be synthesized and investigated in the present work for their antimicrobial activity.

The fourth chapter of the thesis explains in detail the experimental procedures that are adopted in the present investigation.

The fifth chapter the thesis deals with the results obtained in the present study along with detailed discussion on the result supported by reaction schemes, tables, figures etc.

The following are some of the important findings in the present study:

1. Thiadiazole derivatives prepared in good yields
2. All the synthesized compounds exhibited antibacterial and antifungal activities but at various MIC levels.
3. Compounds Vb, Ve showed potent antibacterial activity against Bacillus subtilis and Pseudomonas aureginosa but have moderate activity on Escherichia coli and Staphylococcus aureus.
4. Compound Vc exhibited moderate activity on all the bacterial strains under study.
5. Compound Vc exhibited less activity on all the bacterial and fungal strains under study.
6. Compounds Vd and Ve showed good antifungal activity.

The synthesised compounds along with the antimicrobial activity are believed to exhibit various other activities such as antibacterial, antifungal, antiinflammatory, diuretic, antiulcer, antihelmintic other biological activities. Apart from all of these several investigations are going on with Thiadiazole moiety in the field of drug discovery against diuretic activity. So, the newly synthesized compounds if evaluated for their diuretic activity will be a meaningful effort.

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