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Scientific Research

Antibacterial Activities of New Schiff Bases and Intermediate Silyl Compounds Synthesized from 5-Substituted-1,10-phenanthroline-2,9-dialdehyde

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Abstract

Schiff bases are known to possess anticancer, antibacterial, antifungal, antitubercular, anti-inflammatory, antimicrobial and antimalarial properties. In this paper antibacterial studies against variety of plants and human pathogenic bacteria with eight newly synthesized Schiff bases and several intermediate silyl compounds have been reported. The antibacterial activities of the synthesized compounds were primarily determined by paper disc diffusion method. The minimum inhibitory concentration (MIC) of each compound was also determined by tube dilution process. Seven different human pathogenic bacteria and eighteen different plant pathogenic bacteria were used for the antibacterial activity studies. While all synthesized compounds have shown significant antibacterial activity, one intermediate silyl compound has shown remarkably high antibacterial property. 5-substituted derivatives have shown relatively higher activity than non-substituted compounds. Polar substituent which increases hydrophilicity may have a positive impact on the antibacterial property.

Keywords

5-Nitro-1,10-phenanthroline Dialdehyde, 5-Bromo-1,10-phenanthroline Dialdehyde, Schiff Bases,

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1. Introduction

Schiff base, named after Hugo Schiff, is another name of iminie functional groups or azomethine groups (-C=N) that are classically formed by condensation of a primary amine with an aldehyde or ketone [1]. Since various Schiff bases and their complexes have been formed and exhibited promising activity against bacteria and other microorganism, biochemists are interested to study Schiff bases for their medical importance and use in design of medicinal compounds [2] [3]. Many Schiff bases have antimicrobial and antifungal activities [4]. Additionally, aryl groups or heterocyclic residues containing Schiff bases possess excellent biological activities [5] [6].

Phenanthroline and its derivatives have significantly antibacterial, antifungal and anticancer activity [7]. Phenanthroline metal complex interacts with DNA by cleavage of the DNA strand involving interaction of the metal center with a phosphate group and cleavage of the phosphate ester bond [8] [9]. Also, some phenanthroline Schiff bases and their complexes are found to be photocleavers of pUC19 DNA in visible light and cytotoxic in HeLa (human cervical cancer) and MCF-7 (human breast cancer) cells in visible light [10]. Phenanthroline complexes exhibited high cytotoxicity in cancer cell lines, even sometimes higher activity than cis-platin and substitution at 5- or 5,6-position increases cytotoxicity such as the S, S isomer of complex is 100-fold more cytotoxic than of cis-platin in L2110 murine leukemia cell lines [11].

Schiff bases of thiosemicarbazone are very important due to their antimicrobial action [12]. Also, Schiff bases and their metal complexes having amino thiol group (HN_2-NH-CS-R) show various biological activities. Thiosemicarbazone containing compounds can be used as drug against tuberculosis, leprosy [13] and tumor [14].

The biological study of Schiff bases containing 5-substituted-1,10-phenanthroline-2,9-dicarboxaldehyde with amines containing thione (C=S) groups has not been studied precisely. We, therefore, report here the antibacterial study of eight new Schiff bases containing 5-subntituted-1,10-phenanthroline moiety with different thiosemicarbazones. The intermediate silyl and aldehyde compounds are also studied against these bacteria.

2. Materials and Methods

Preparation of test microorganism

The sterilized (autoclaved for 30 min) medium was poured into sterilized petri dish (500 ml for 20 petri dishes) and solidified (1.5%, w/v agar) at room temperature for 30 min. 18 different types of plant pathogenic and 7 human pathogenic bacterial strains were used in this study of both gram positive and gram negative bacteria. A small amount of bacteria was collected from bacteria seed and streaked it onto an appropriate media plate for the bacteria. The plate was placed in a incubator for 24 h at 28˚C for plant pathogenic bacteria and at 37˚C for human pathogenic bacteria.

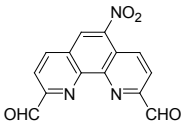
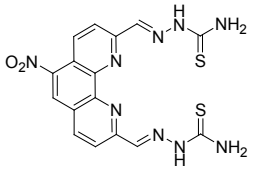
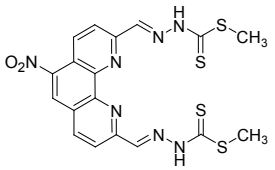
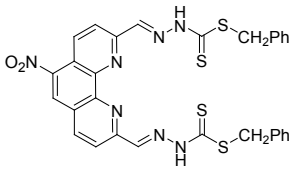
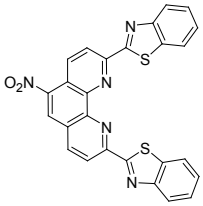
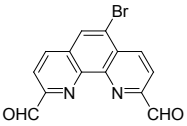
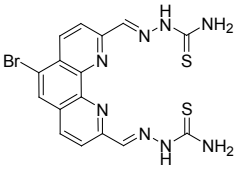
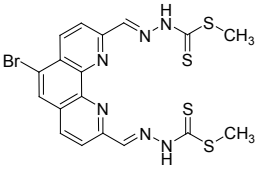
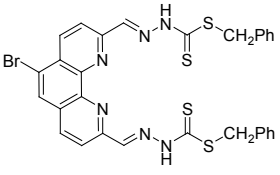
2.1. Preparation of Reagents

Fifteen compounds including eight new Schiff bases, four intermediates and one starting molecule were used for antibacterial screening test by using disk diffusion method (**Table 1**). The eight new Schiff bases were synthesized and characterized by using different spectroscopic methods including IR, ^{1}H NMR, ^{13}C NMR and Mass spectroscopy [15]. The concentration of the test compounds was 20 mg/ml. The concentration for standard, tetracycline (TC), was 10 mg/ml for screening test. Test compounds were dissolved in dimethylsulphoxide (DMSO). The name of the compounds and their structure are given in **Table 1**.

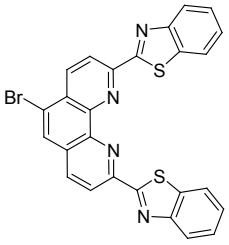
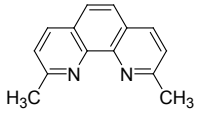
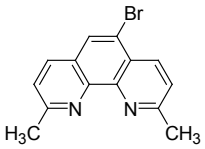
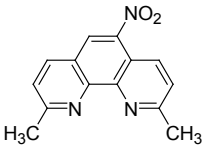
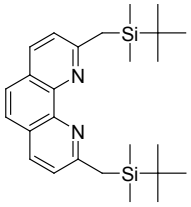
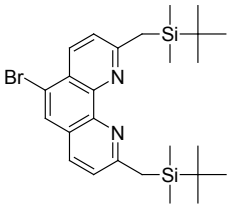
2.2. Antibacterial Activity Screening Test (Disc Diffusion Method) [16]-[18]

2-3 bacteria colonies were taken in appropriate liquid media and shook it in an incubator shaker for 5 h at 28˚C for plant pathogenic bacteria and at 37˚C for human pathogenic bacteria. The spinning speed maintained at 180 ppm. The bacteria solution was then diluted four times and spread 0.15 ml of this fresh pure culture on media plate with a help of spreader under aseptic condition. Sterile 6 mm whatman filter paper was used which was impregnated with 10 µl of sterile stock solution (20 mg/ml) of the compound. The filter paper with solution was

Table 1. Names of the compounds that are used in antibacterial study.

Compound Name	Compound Name	Structure
5-Nitro-[1,10]phenanthroline-2,9-dicarbaldehyde	A	
(2,2')-2'-(5-Nitro-1,10-Phenanthroline-2,9-diyl) bis(methan-1-yl-1-ylidene)bis-(hydrazinecarbodithioate)	B	
(2E,2'E)-Dimethyl-2,2'-(5-nitro-1,10-phenanthroline -2,9-diyl)bis(methan-1-yl-1-ylidene)bis (hydrazinecarbodithioate)	C	
(2,2')-Benzyl-2,2'-(5-Nitro-1,10-Phenanthroline-2,9-diyl)bis (methan-1-yl-1-ylidene)-bis (hydrazinecarbodithioate)	D	
2,9-Di-(benzo[d]thiazol-2-yl)-5-Nitro-1,10-Phenanthroline	E	
5-Bromo-1,10-phenanthroline-2,9-dicarbaldehyde	F	
(2,2')-2'-(5-Nitro-1,10-Phenanthroline-2,9-diyl) bis(methan-1-yl-1-ylidene)bis-(hydrazinecarbodithioate)	G	
(2E,2'E)-dimethyl-2,2'-(5-bromo-1,10-phenanthroline-2,9-diyl)bis (methan-1-yl-1-ylidene) bis(hydrazinecarbodithioate)	H	
(2,2')-Benzyl 2,2'-(5-bromo-1,10-Phenanthroline-2,9-diyl)bis(methan-1-yl-1-ylidene)-bis (hydrazinecarbodithioate)	I	

Continued

2,9-Di-(benzo[d]thiazol-2-yl)-5-bromo-1,10-phenanthroline	J	
2,9-dimethyl-1,10-phenanthroline	K	
5-Bromo-2,9-dimethyl-1,10-phenanthroline	L	
5-Nitro-2,9-dimethyl-1,10-phenanthroline	M	
2,9-Bis-[(tert-butyl-dimethyl-silanyl)-methyl]-[1,10]phenanthroline	N	
5-Bromo-2,9-bis-[(tert-butyl-dimethyl-silanyl)-methyl]-[1,10]phenanthroline	O	

placed in the middle of the bacteria spread media plate. The plates were incubated at appropriate temperature for 24 h. The diameter of the inhibition zone observed was measured in mm scale. Disc impregnated with sterile DMSO was used as a control and with sterile tetracycline (TC) was used as antibacterial reference standard. All tests were performed under sterile conditions in duplicate and repeated three times.

2.3. Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) indicates the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism after overnight incubation. To determine MIC, different variable concentrations of the compounds were used. Media plate and bacteria culture were prepared the same way that were done before in the disk diffusion method. After spreading about 0.15 ml diluted bacteria on the media plate, seven holes were punched on the plate for seven different solutions. 25 µL of each solution (T_c, T_0, T_1, T_2, T_3, T_4, T_5) was put in each hole according to the concentration and kept it in incubator at appropriate temperature for 1 day. The diameter of the inhibition zone was measured in mm scale and recorded the data. All the tests were repeated three times.

3. Results and Discussion

3.1. Antibacterial Activity Screening Test

The antibacterial study is based upon a comparison of inhibition of growth of bacteria by test compounds with that produced by standard antibiotic, Tetracycline (TC). Inhibition zone is the clear region around the paper disc saturated with an antimicrobial agent. The screening result showed that all the compounds exhibited noticeable antibacterial activity against different bacteria. The following Tables show the name of the compounds and antibacterial activity according to the inhibition zones in mm scale.

The antibacterial activity of compounds against 7 human pathogenic bacteria is presented in **Table 2**. The bar charts represent a comparison of the activities of 5-substituted Schiff bases with similar Schiff bases without the substituents. All compounds found to possess antibacterial activity against all human pathogenic bacteria. In fact, compounds showed higher activity for human pathogenic bacteria than plant pathogenic bacteria. Also, it is observed very high activity for silyl derivative, N (**Figure 1** and **Figure 4**). The silyl derivative showed higher activity than tetracycline (TC) in case of *Staphylococcus aures*, *Streptococcus pyogenes*, *Bacillus subtilus and Listeria monocytogenes*. **Figure 2** and **Figure 3** show graphical representations of antibacterial activity against human pathogenic bacteria. These bacterias are *E. Coli* MC4100, *E. Coli* DH5a, *Staphylococcus aures*, *Streptococcus pyogenes*, *Yersinia enterocoitica* 27729, *Listeria monocytogenes* and *Bacillus subtilus*.

The antibacterial activity of compounds against 18 plant pathogenic bacteria is presented in **Table 3** and **Figures 4-6**. While all compounds exhibited significant activity, the silyl derivative, N has higher activity in case of *Pectobacterium atosepticum* SCR1043, *Serratia odorifera* 33077, *Xanthomonous campestris* pv. *Pelargonii* Xcp42, *Xanthomonous campestris* pv. *Pelargonii* Xcp58 and *Pantoea sterwartii* DC28.

Nine different bacteria of Pseudomonas genre were tested. The result is presented in **Table 4**. In this case, nitro and bromo aldehyde (A and F) showed higher activity against *Pseudomonus Cichorii* 302959, *Pseudomonas syringae* 728*α* and Ps.Pv. *Morspruorum* 218-01 (**Figures 7-9**).

Table 2. Antibacterial activity of the compounds against human pathogenic bacteria.

Compound	Zone of Inhibition (mm)						
	E. Coli MC4100	*E. Coli* DH5*α*	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Yersinia enterocolitica* 27729	*Listeria monocytogenes*	*Bacillus subtilus*
Medium	LB	LB	TSB	TSB	LB	LB	TSB
Std. (TC)	24	24	29	30	40	33	37
A	15	14	15	16	15	18	27
B	7	9	8	8	11	9	11
C	9	10	01	9	11	9	12
D	8	10	8	10	11	12	11
E	7	9	7	8	11	9	11
F	10	10	14	15	12	16	29
G	7	9	7	10	10	--	8
H	8	9	8	11	10	8	8
I	7	10	8	11	11	11	11
J	8	9	9	11	10	16	10
K	10	9	15	15	13	30	29
L	10	10	9	10	9	22	14
M	10	10	12	12	11	14	11
N	9	10	21	32	10	29	58
O	8	8	10	12	10	21	14

Table 3. Antibacterial activity of nine plant pathogenic bacteria.

Compound	Zone of Inhibition (mm)								
	Pectobacterium carotovorum Eec7	*Dickeya dadantii* 3937	*Pectobacterium atosepticum* SCR 1043	*Serratia odorifera* 33077	*Pantoea sterwartii* DC283	*Xanthomonous campestris* pv. *Pelargonii* Xcp42	*Xanthomonous campestris* pv. *Pelargonii* Xcp58	*Pseudomonas corrugata* 301678	*Pseudomonas syringae tomatto* DC3000
Medium	LB	LB	LB	LB	LB	NY	NY	NY	KB
Std. (TC)	29	33	28	23	39	41	40	21	31
A	15	10	16	15	19	19	17	14	11
B	11	10	10	8	10	10	10	7	10
C	11	11	11	10	12	10	11	9	10
D	11	14	12	8	10	10	10	10	12
E	10	11	12	8	10	9	10	8	14
F	12	11	11	9	14	16	16	10	12
G	10	11	10	10	10	10	11	7	10
H	11	10	11	8	11	9	10	9	9
I	12	10	11	7	10	11	15	11	11
J	10	10	10	10	12	11	10	9	10
K	10	10	10	8	15	12	10	10	9
L	11	9	10	9	13	11	11	10	10
M	10	10	10	7	11	8	14	9	8
N	12	13	15	11	65	35	38	11	12
O	11	10	9	12	14	12	12	9	10

Table 4. Antibacterial screening test of nine bacteria of Pseudomonas genre.

Compound	Zone of Inhibition (mm)								
	P. syringae morspruorum 302756	*P. cichorii* 302959	*P. aeruginosa* PAO1	*P. Savastanoi*	*P. viridiflava*	*P. cichorii* 302699	*P. syringae* FF5	*P. syringae* 728*α*	Ps.Pv. *Morspruorum* 218-01
Medium	KB	KB	KB	KB	KB	KB	KB	KB	KB
Std. (TC)	32	27	10	40	25	29	27	26	25
A	13	50	12	15	13	12	11	13	15
B	7	7	7	10	7	7	7	8	7
C	7	7	7	10	9	8	10	8	8
D	7	7	7	10	7	9	11	7	8
E	7	--	7	10	--	7	7	--	11
F	13	37	10	12	11	10	12	24	19
G	7	7	7	9	8	8	8	7	8
H	7	7	8	19	--	7	7	9	7
I	7	7	7	10	7	8	8	8	11
J	8	7	8	10	7	7	8	7	10
K	--	--	7	9	7	8	7	7	--
L	9	8	7	9	8	8	7	8	8
M	8	8	7	9	8	8	8	8	8
N	9	9	--	9	9	7	11	11	12
O	7	8	7	9	7	8	9	7	9

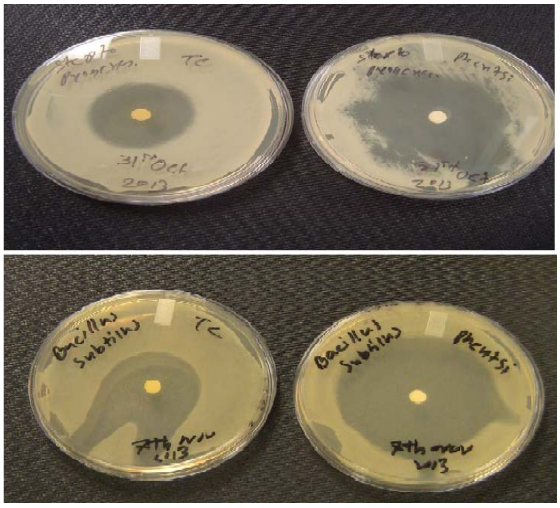


Figure 1. Photos of the antibacterial test of compound N and standard.

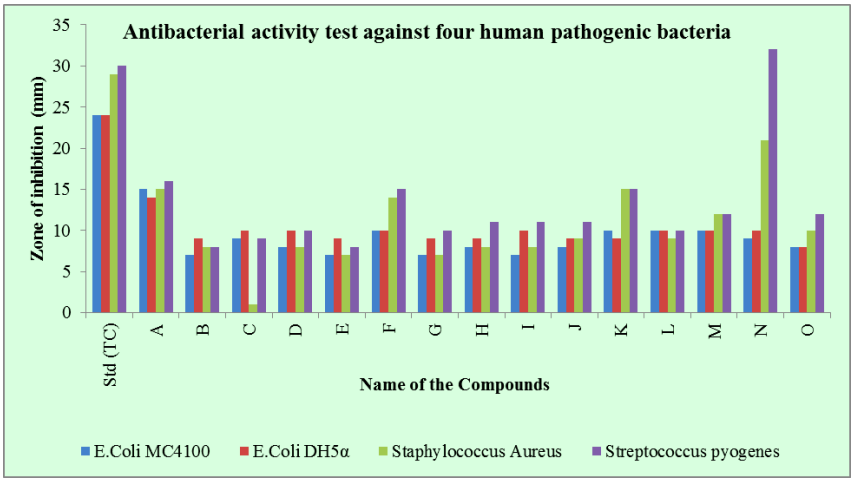


Figure 2. Antibacterial activity test against four human pathogenic bacteria.

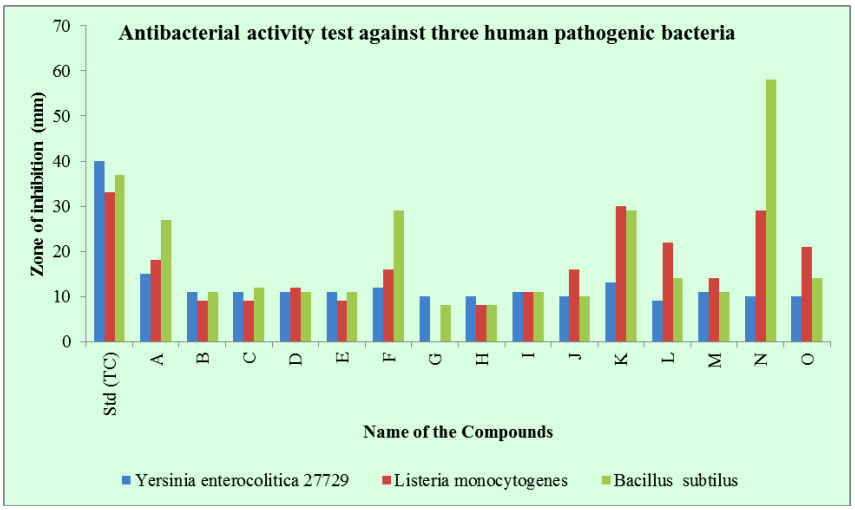


Figure 3. Antibacterial activity test against three human pathogenic bacteria.

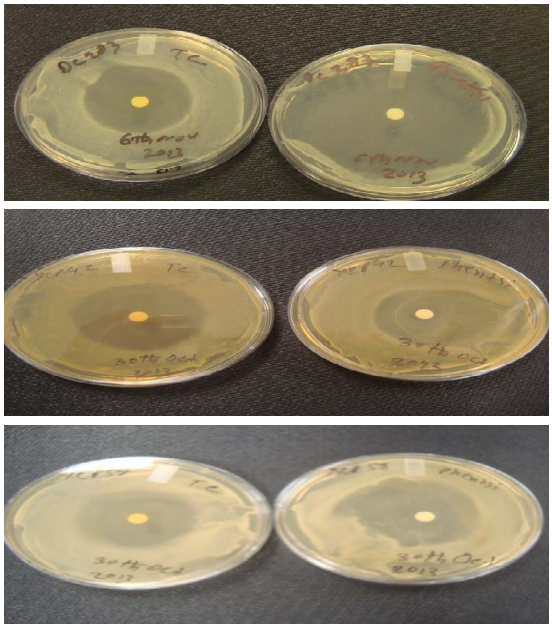


Figure 4. Some real pictures of the high activity of silyl methyl phenanthroline compound, N against *Pantoea sterwartii* DC283, *Xanthomonous campestris* pv. *Pelargonii* Xcp42, *Xanthomonous campestris* pv. *Pelargonii* Xcp58.

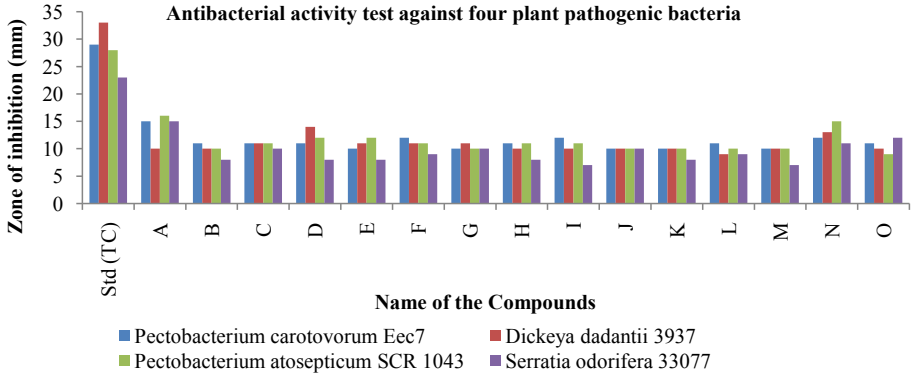


Figure 5. Antibacterial activity test against four plant pathogenic bacteria.

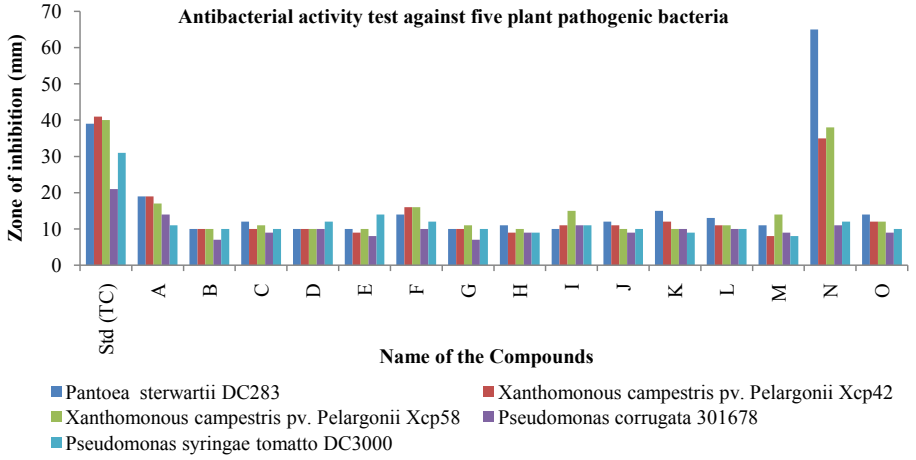


Figure 6. Antibacterial activity test against five plant pathogenic bacteria.

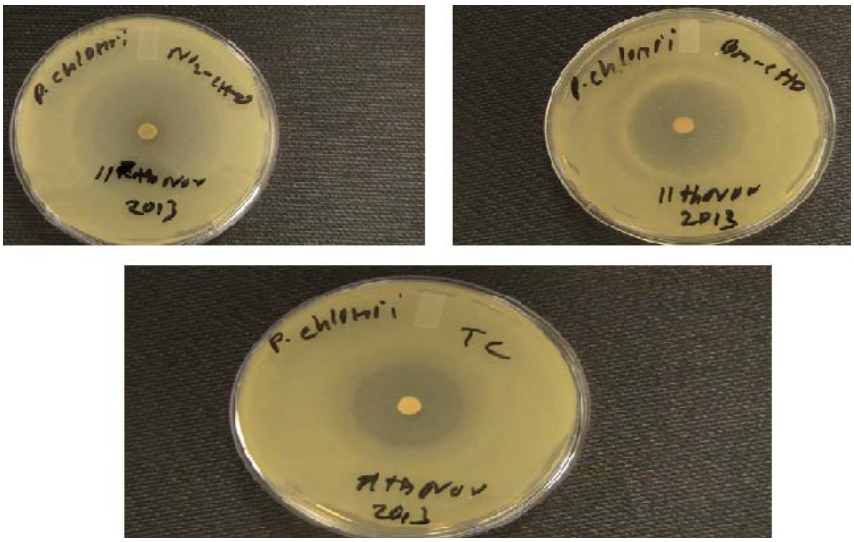


Figure 7. Photo of antibacterial test of bromo, F and nitro aldehyde, A against *Pseudomonas cichorii.*

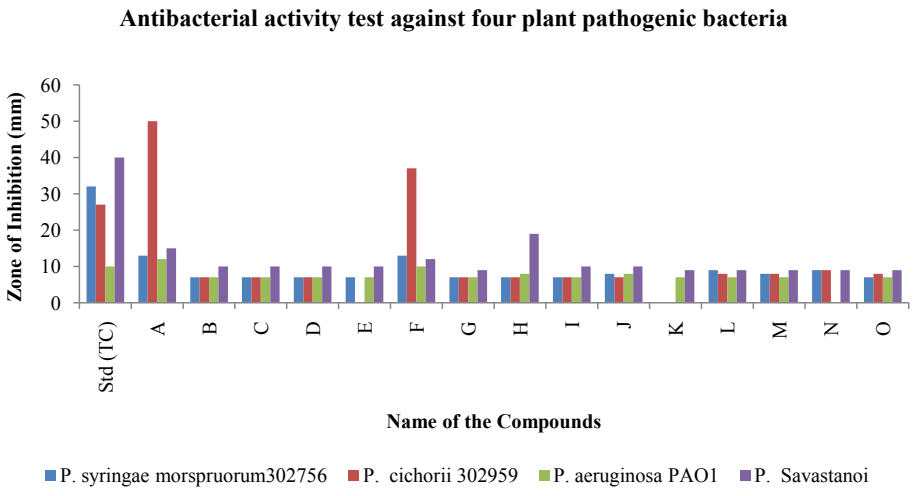


Figure 8. Antibacterial activity test against four plant pathogenic bacteria.

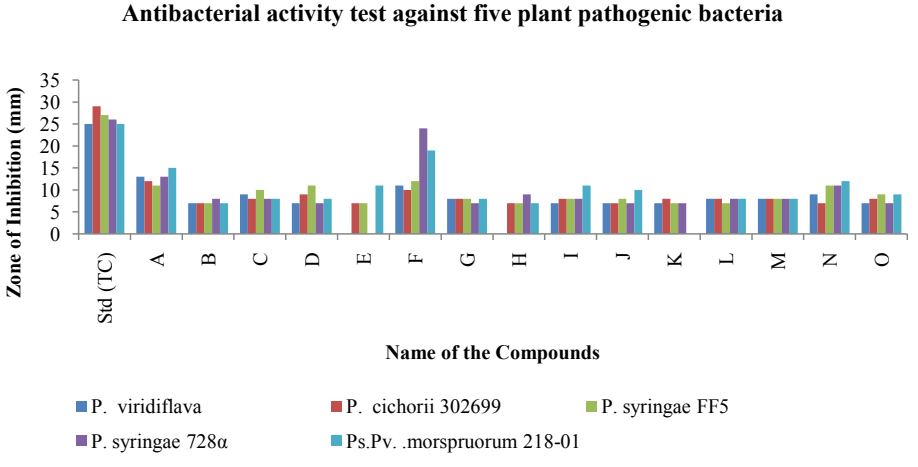


Figure 9. Antibacterial activity test against five plant pathogenic bacteria.

3.2. Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the minimum concentration of the test compound that inhibits the visual growth of the microorganism. MIC was determined for those compounds that showed higher activity on some bacteria. 5 Different concentrated solutions of the compounds were used to test MIC starting from 100 µg/ml to 5 mg/ml. In all cases, the MIC of silyl methyl phenanthroline, N (**Figure 10** and **Figure 11**) was found to be 100 µg/ml toward *Streptococcus pyogenes*, *Listeria monocytogenes*, *Bacillus subtilus*, *Xanthomonous campestris* pv. *Pelargonii* Xcp42, *Xanthomonous campestris* pv. *Pelargonii* Xcp58, *Pantoea sterwartii* DC283 (**Table 5**). Also, for nitro aldehyde, A, 100 µg/ml was the MIC for *Streptococcus pyogenes*, *Listeria monocytogenes*, *Xanthomonous campestris* pv. *Pelargonii* Xcp42, *Xanthomonous campestris* pv. *Pelargonii* Xcp58, *E. coli* MC4100, *Pseudomonas syringae Morspruorum* 302756 and 200 µg/ml was the MIC for *Bacillus subtilus* (**Figure 12** and **Figure 13**). All the results are shown in **Table 6** and **Table 7**. Moreover, 100 µg/ml was the MIC for bromo aldehyde, F against *Streptococcus pyogenes*, *Bacillus subtilus*, *Listeria monocytogenes*, *Xanthomonous campestris* pv. *Pelargonii* Xcp42, *Xanthomonous campestris* pv. *Pelargonii* Xcp58, *Pseudomonus Cichorii* 302959 (**Figure 14** and **Figure 15**). **Table 8** and **Figure 16** show summary of results.

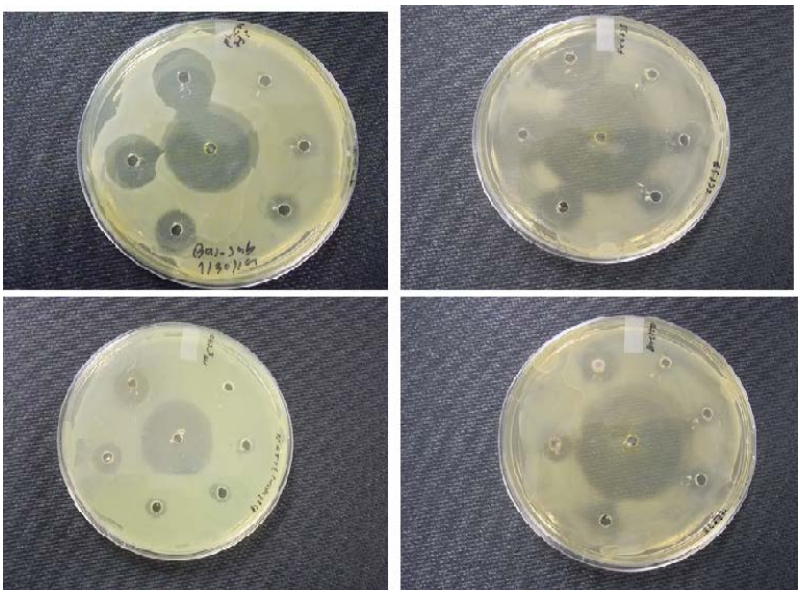


Figure 10. Pictures of minimum inhibitory concentration (MIC) test for the Compound N.

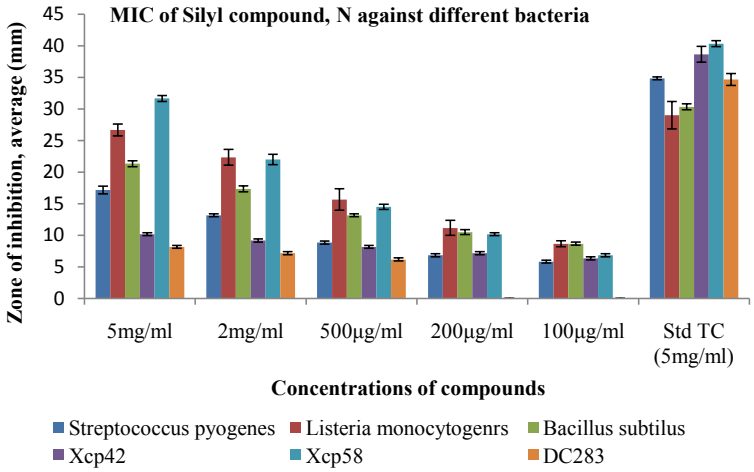


Figure 11. MIC of Silyl compound, N against different bacteria.

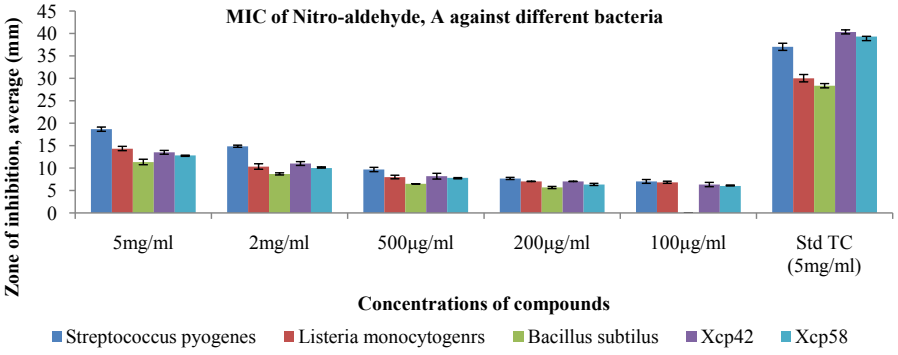


Figure 12. MIC of Nitro-aldehyde, A against five bacteria.

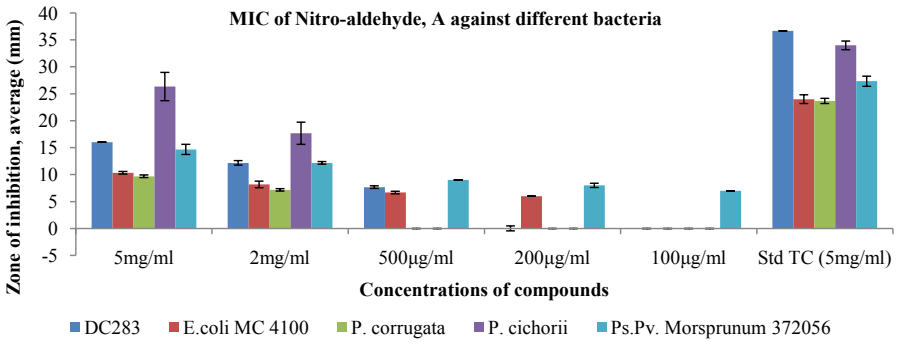


Figure 13. MIC of Nitro-aldehyde, A against six bacteria.

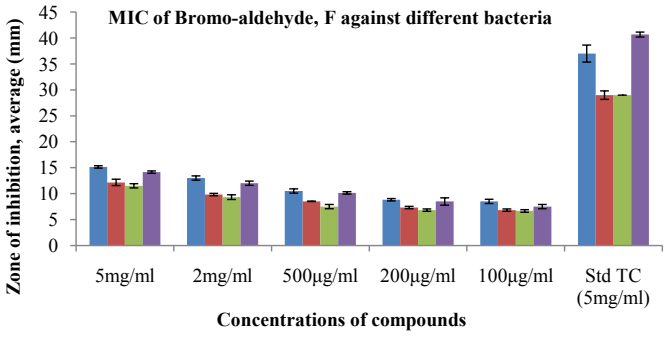


Figure 14. MIC of Bromo-aldehyde, F against four bacteria.

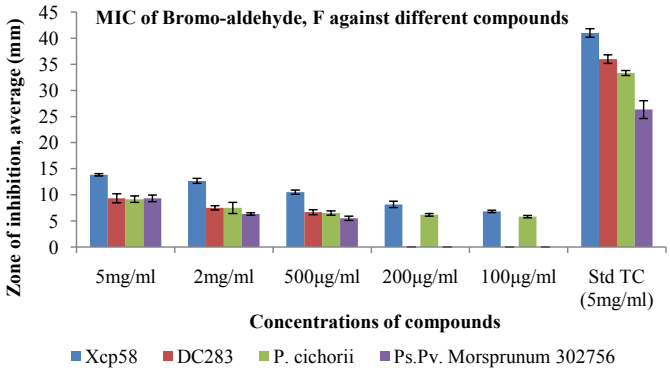


Figure 15. MIC of Bromo-aldehyde, F against four bacteria.

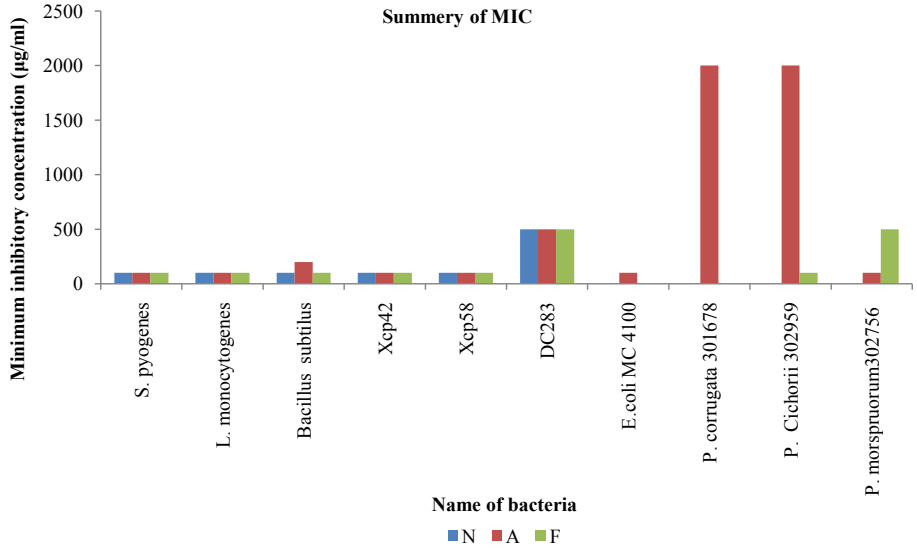


Figure 16. Overall summery of MIC test.

Table 5. Minimum inhibitory concentration (MIC) of Silyl methyl Phenanthroline compound, N.

Name of Bacteria	Zone of Inhibition (mm) of Silyl Methyl Phenanthroline, N					
	5 mg/ml	2 mg/ml	500 µg/ml	200 µg/ml	100 µg/ml	Std. TC (5 mg/ml)
Streptococcus pyogenes	17.17 ± 0.62	13.17 ± 0.24	8.83 ± 0.24	6.83 ± 0.23	5.83 ± 0.24	34.83 ± 0.23
Listeria monocytogenes	26.67 ± 0.94	22.33 ± 1.25	15.67 ± 1.7	11.17 ± 1.18	8.67 ± 0.47	29 ± 2.16
Bacillus subtilus	21.33 ± 0.47	17.33 ± 0.47	13.17 ± 0.24	10.5 ± 0.41	8.67 ± 0.24	30.33 ± 0.47
Xanthomonous campestris pv. *pelargonii* Xcp42	10.17 ± 0.24	9.17 ± 0.24	8.17 ± 0.24	7.17 ± 0.24	6.33 ± 0.24	38.67 ± 1.25
Xanthomonous campestris pv. *pelargonii* Xcp58	31.67 ± 0.47	22 ± 0.82	14.5 ± 0.41	10.17 ± 0.24	6.83 ± 0.24	40.33 ± 0.47
Pantoea sterwartii DC283	8.17 ± 0.24	7.17 ± 0.24	6.17 ± 0.24	0 ± 0	0 ± 0	34.67 ± 0.94

Table 6. Minimum inhibitory concentration (MIC) of nitro aldehyde, A compound.

Name of Bacteria	Zone of Inhibition (mm) of Nitro Aldehyde, A					
	5 mg/ml	2 mg/ml	500 µg/ml	200 µg/ml	100 µg/ml	Std. TC (5 mg/ml)
Streptococcus pyogenes	18.67 ± 0.47	14.83 ± 0.24	9.67 ± 0.47	7.67 ± 0.24	7 ± 0.41	37 ± 0.82
Listeria monocytogenes	14.33 ± 0.47	10.33 ± 0.62	8 ± 0.41	7 ± 0	6.83 ± 0.24	30 ± 0.82
Bacillus subtilus	11.33 ± 0.62	8.67 ± 0.24	6.5 ± 0	5.67 ± 0.24	0 ± 0	28.33 ± 0.47
Xanthomonous campestris pv. *Pelargonii* Xcp42	13.5 ± 0.41	11 ± 0.41	8.17 ± 0.62	7 ± 0	6.33 ± 0.47	40.33 ± 0.47
Xanthomonous campestris pv. *Pelargonii* Xcp58	12.83 ± 0.24	10 ± 0	7.83 ± 0.24	6.33 ± 0.24	6 ± 0	39.33 ± 0.94
Pantoea sterwartii DC283	16 ± 0.41	12.17 ± 0.24	7.67 ± 0.47	0 ± 0	0 ± 0	36.67 ± 1.25
E. coli MC4100	10.33 ± 0.24	8.17 ± 0.62	6.67 ± 0.24	6 ± 0	0 ± 0	24 ± 0.82
Pseudomonas corrugata 301678	9.67 ± 0.24	7.17 ± 0.24	0 ± 0	0 ± 0	0 ± 0	23.67 ± 0.47
Pseudomonus cichorii 302959	26.33 ± 2.62	17.67 ± 2.05	0 ± 0	0 ± 0	0 ± 0	34 ± 0.82
Pseudomonas syringae morspruorum 302756	14.67 ± 0.94	12.17 ± 0.24	9 ± 0	8 ± 0.41	7 ± 0	27.33 ± 0.94

Table 7. Minimum inhibitory concentration (MIC) of bromo aldehyde, F compound.

Name of Bacteria	Zone of Inhibition (mm) of Bromo Aldehyde, F					
	5 mg/ml	2 mg/ml	500 µg/ml	200 µg/ml	100 µg/ml	Std. TC (5 mg/ml)
Streptococcus pyogenes	15.17 ± 0.24	13 ± 0.41	10.5 ± 0.41	8.83 ± 0.24	8.5 ± 0.41	37 ± 1.63
Listeria monocytogenes	12.17 ± 0.62	9.83 ± 0.24	8.5 ± 0	7.33 ± 0.24	6.83 ± 0.24	29 ± 0.82
Bacillus subtilus	11.5 ± 0.41	9.33 ± 0.47	7.5 ± 0.41	6.83 ± 0.24	6.67 ± 0.24	29 ± 0
Xanthomonous campestris pv. *pelargonii* Xcp42	14.17 ± 0.24	12 ± 0.41	10.17 ± 0.24	8.5 ± 0.71	7.5 ± 0.41	40.67 ± 0.47
Xanthomonous campestris pv. *pelargonii* Xcp58	13.83 ± 0.24	12.67 ± 0.47	10.5 ± 0.41	8.17 ± 0.62	6.83 ± 0.24	41 ± 0.82
Pantoea sterwartii DC283	9.33 ± 0.85	7.5 ± 0.41	6.67 ± 0.47	0 ± 0	0 ± 0	36 ± 0.82
Pseudomonus cichorii 302959	9.17 ± 0.62	7.5 ± 1.08	6.5 ± 0.41	6.17 ± 0.24	5.83 ± 0.24	33.33 ± 0.47
Pseudomonas syringae morspruorum 302756	9.33 ± 0.62	6.33 ± 0.24	5.5 ± 0.41	0 ± 0	0 ± 0	26.33 ± 1.7

Table 8. Overall summary of MIC.

Name of the Bacteria	Minimum Inhibitory Concentration (µg/mL)		
	N	A	F
Streptococcus pyogenes	100	100	100
Listeria monocytogenes	100	100	100
Bacillus subtilus	100	200	100
Xanthomonous campestris pv. *pelargonii* Xcp42	100	100	100
Xanthomonous campestris pv. *pelargonii* Xcp58	100	100	100
Pantoea sterwartii DC283	500	500	500
E. coli MC4100	--	100	--
Pseudomonas corrugata 301678	--	2000	--
Pseudomonus cichorii 302959	--	2000	100
Pseudomonas syringae morspruorum 302756	--	100	500

4. Conclusion

Antibacterial studies of 15 different compounds including intermediate silyl and aldehyde compounds were carried out. These compounds were tested on 18 plants and 7 human pathogenic bacteria. The antibacterial activity screening test of the synthesized compounds was initially examined by paper disc diffusion technique. In screening test, the compounds showed higher antibacterial activity for human pathogenic bacteria. High activity was observed for silyl derivative as well. Compound N was even more active than the standard tetracycline (TC). Moreover, it has been found that the activity of the 5-substituted Schiff bases has shown higher activity than their non- substituted members. Finally, the minimum inhibitory concentrations (MIC) of the compounds were determined by tube dilution method. For most of the compounds MIC was 100 µg/ml.

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