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ANTIBACTERIALACTIVITYOFCHEMICALLYSYNTHESIZEDNOVELSULFONAMIDECOMPOUNDS

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ABSTRACT: When first exposed to a new antibiotic, the susceptibility of microbes tends to behigh and their mortality rate is also high. The surviving microbes usually have some geneticcharacteristics that accounts for their survival. Their progeny are similarly resistant. A largerange of gram negative and gram positive bacteria show resistant to various antibiotics. Here,antibacterial activity of some chemically synthesized compounds was checked on common op-portunistic pathogens like Staphylococcous aureus, Bacillus subtilis, Escherichia coli, Pseudomo-nas aeruginosa. Two different techniques, agar well method and broth dilution method were usedto check antibacterial activity of chemically synthesized compounds and minimum inhibitoryconcentration(MIC).

Key words: - Staphylococcous aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aerugi-nosa,MinimumInhibitory Concentration (MIC),Ciprofloxacin.

INTRODUCTION:

Ever since antibiotics were introduced into clinical practice, bacterial pathogens have been developing resistance which reduce or eliminates their effectiveness. In addition, opportunistic pathogens withinnate resistance to antibiotics have become emerging problems particularly in hospital setting. So we require drugs which are capable to eliminate broad spectrum of bacteria. To check the antibacterial activity of chemical compounds different types of methods are used. Agar dilution and broth dilution are the most commonly used techniques to determine the minimal inhibitory concentration (MIC) of antibacterial agents. Other than that agar well diffusion and paper disc methods are also used to check MIC. MIC (Minimum Inhibitory Concentration) is used to check the minimum concentration of antibacterial agents that kills the present test organism.

Bacteriahavedevelopeddifferentmechanismstoescapetheeffectofantibacterial drugs. Asan example, mithicillin resistant Staphylococcus aureus (MRSA) was once thought to be problematic only in health care setting, but now community associated MRSA infections are becoming more common.

Some chemical compounds were synthesized that are capable to inhibit the growth of bacteria. It is necessary to check antibacterial activity of these compounds on some common opportunistic bacteria like gram positive Staphylococcus aureus and Bacillus subtilis and gramnegative Escherichiacoli & Pseudomonasaeruginosa. These bacteria mainly associated with human body and helpful in someways.

MATERIALS:

BACTERIALSTRAINS:-The bacteria used in study were:

Grampositive bacteria–[1]Staphylococcusaureus [2]Bacillussubtilis

Gramnegative bacteria–[1]Escherichiacoli [2]Pseudomonas aeruginosa

All these bacterial strains were obtained from the Department of Micro-biology, M. G. Science Institute, Ahmedabad. These all the bacterial strains weremaintained on nutrient agar slant at

4^0C and suspension prepared by picking upwellisolated colony from nutrient agar slant.

MEDIA:-

Simplenutrientagarwasusedinthisstudybecauseit supportedthe growth of themajority bacteria.

Composition of nutrientagar medium:-

Ingredients	Concentration (gm/100ml)
Bacteriologicalpeptone	1
Meatextract	0.3to0.5
NaCl	1.5
Agaragarpowder	2to 2.5
Distilledwater	100
pH	7.2to7.6

Chemical compounds:-Chemical compounds which are used to check anti-bacterial activity were chemically ynthesized at L.M.College of Pharmacy.

Other equipments:-5ml and 1ml sterilized pipettes, sterilized conical flask, test tubes and15×100mm Petriplates, cupborer, cotton plugs for test tubes, alcohol, etc.

Instruments	Company'sname
Incubator(37℃)	Ambassador laboratory equipments
Hot air Oven	Ambassador laboratory equipments
Autoclave	Sharda scientific instruments
Zone Reader	DBK industrial laboratory
Precision Balance	Swisser instruments

III. Minimum Inhibitory Concentration[mic]

Minimum Inhibitory Concentration (MIC) is the lowest con-centration of a chemical, usually a drug, which prevents visible growth ofthe test bacterium. The aim of the broth dilution method is to determinethe lowest concentration of the assayed antibacterial agent that, underdefined test conditions, inhibits the visible growth of the bacterium beinginvestigated.InMIC,resultshavebeengradedintosusceptible,intermediate or resistant to a particular antibacterial agent by using abreak point. Break point is a chosen concentration of an antibiotic whichdefines whether a bacterial species is susceptible or resistant to the anti-biotic. If the MIC is less than or equal to the susceptibility break point thebacteriumisconsideredsusceptibletotheantibiotic.Inbrothdilution,the test organism is inoculated into liquid (broth) medium in the presenceof different concentrations of antibacterial agent. Growth is assessed afterincubation for a defined period of time (24 hours) and MIC value is ob-served.

Results of MIC:-By studying the table no.3.1, it isobserved that Ps.aeruginosa does not give the inhibition towards some chemical compounds like LMAC 02, LMAC 03, LMAC 05 and LMAC 10 whereas B.subtilisgivestheMICinconcentrationasloweras5 µginchemicalcompoundsLMAC 01, LMAC 04, LMAC 06 and LMAC 07. So it is concluded that in caseof Ps.aeruginosa MIC is higher than 100µg in some cases. E.coli also does notgive the results in chemical compounds LMAC 03 and LMAC 04. In the caseof S.aureus MIC is between 15µg to 20µg but in some chemical compounds results are observed lower as well.

IV. METHOD

Stocksolution&dilutionpreparation:-

Here the chemical compound is water insoluble, that's why thestock solution and different dilutions were prepared in Dimethyl sulfoxide(DMSO).

The stock solution was prepared by adding 0.01 gram compoundin 5 ml DMSO. Then the three different dilutions of the stock solution werepreparedto get different concentration of chemical compound. These dilu-tions were used

to check bacterial susceptibility towards the chemicalcompounds.

Agarwellmethod:-

- Firstly, prepare bacterial suspension of given samples which are used to check antibacterial activity
- Here, pour plate method is used. 20-25 ml melted agar is sufficient to pour the15×100mmPetriplates. So 20ml meltedagar was inoculated

with 2 ml of previously prepared bacterial suspension. Then it pouredinsterilized Petri plates. Allow itto solidify.

- Draw four sectors on the bottom of the plate and label each sector. Keepone as control. Likewise prepares all the Petri plates. Here, four Petriplates were used to check the antibacterial activity of each bacteriumby given each chemical compound. One Petri plate is used as control ineach set to identify the perfect growth of each bacterium. It givesbetterstatisticalresults.
- Sterilize the cupborer by dippingit in alcoholfollowed by flaming(toburn off alcohol). By using the cup borer makes perfect wells in the previ-ouslyseeded plates in each quadrant.
- Fill each well with previously prepared different dilutions of the chemicalcompounds by using sterilized pipettes. Take great care so as to avoidoverflowingor spilling the chemicals.
- Then incubate all the plates at 37℃for 24 hours. Next day observe theresultsin terms of zone of inhibition.

RESULTDISCUSSIONANDCONCLUSION

Here, ciprofloxacin is used as control to check antibacterial activityof chemical compounds. All the four organisms which were usedin thisstudy, give the zone of inhibition. Gram negative organisms like E.coli andPs.aeruginosa give the zone of inhibition between the range of 22mm to34mm and 20mm to 35mm, respectively. Both Gram positive organisms,S.aureus and B.subtilis give the zone of inhibition in the range of 32mm to38mmand 35mm to 40mm, respectively.

Byobservingthetable5.1,theinterpretationobtaine disthatB.subtilis gives the highest zone of inhibition against the ciprofloxacin andE.coli gives the lowest zone of inhibition compared to other three organ-isms. By studying the figure 5.2 it is concluded that B.subtilis gives thehighestzone of inhibition.

In LMAC 01 chemical compound, the zone of inhibition is observedin the range of 16mm to 22mm. Gram negative organisms like E.coli andPs.aeruginosa give lower zone of inhibition as compared to gram positivebacteria. B.subtilis gives the highest zone of inhibition against LMAC 01chemicalcompound compared toother three organisms.

As observed in table 5.2 and figure 5.3 and 5.4, the compoundLMAC 01 is showing good antibacterial activity against all the four organ-isms. Hence, chemical compound LMAC 01 is carried out broad spectrumantibacterial activity. It is also concluded that the compound shows dosedependent activity. The zone of inhibition is increased when higher dose isappliedas seen in table 5.2. andfigure 5.4.

By observing the table 5.3 and figure 5.5, the interpretation ob-tained is that Ps. aeruginosa is not able to show susceptibility towardsLMAC 02 chemical compound but the same compound is exhibiting goodzone of inhibition in the case of gram positivebacteria.. Hence, it can beinferred that compound LMAC 02 is not effective for all gram negative bac-teria. As observed in table 4.3, the compound LMAC 02 does not show thedose dependent activity in S.aureus. Other two organisms show dose de-pendent activity. So it is concluded that LMAC 02 compound is not as ef-fective as LMAC 01 compound. Figure 5.6 shows the comparison betweenLMAC01 and LMAC02 compounds with ciprofloxacin.

By observing the table 5.4 and figure 5.7, the interpretation obtained is thatthe chemical compound LMAC 03 is showing good antibacterial activity againstB.subtilis and S.aureus as the zone of inhibition is observed which is quite large.But the same compound is not exhibiting any zone of inhibition against E.coliand Ps.aeruginosa. Hence, it can be concluded that LMAC 03 chemical com-poundisnoteffectiveagainstgramnegativebacteria asobservedinfigure5.8.Sothis compoundshows narrowspectrum antibacterial activity.

E.coli does not give the zone of inhibition against chemical compound LMAC04asobservedintable5.5.,whereasotherthree bacteria,Ps.aeruginosa,S.aureusandB.subtilisar esusceptibleagainstLMAC04chemicalcompounda nd give the zone of inhibition between the range of 16mm to 28mm as shownin figure 5.10 and table 5.5. The compound shows dose dependent activity. Thezoneofinhibitionisincreasedinsize astheconcentrationofdoseincreases.

By observing the table5.6and figures 5.11 and 5.12, the interpretation ob-tained is that the B.subtilis, S.aureus and E.coli show the susceptibility againstchemical compound LMAC 05 but it does not effect the Ps.aeruginosa. All theotherthreeorganismsgivethezoneofinhibition betweentherangeof18mm to 24mm. Here, S.aureus show the highest zone of inhibition. B.subtilisdoesnot showdose dependentactivity againstLMAC 05compound.

Chemical compound LMAC 06 is effective against both gram positive as well asgram negative organisms as shown in table 5.7 and figures 5.13 and 5.14. Sur-prisingly,here Ps.aeruginosa gives the highest diameter of inhibition. All organ-isms show dose dependentactivity. The zone of inhibition is increased as theconcentration of dose increases. So it isconcluded that chemical compoundLMAC 06 is broad spectrum and used against both grampositive as well asgramnegative.

By checking the table 5.8 and figures 5.15 and 5.16 thoroughly,it is ob-served that the LMAC 07 chemical gives the nearly same results which are givenby chemical compound LMAC 06. Here, E.coli gives the highest zone of inhibi-tion. So it is also broad spectrum chemical compound. So after observing allthe chemical compounds, it is concluded thatLMAC 06 and LMAC 07 are broadspectrum chemical compounds LMAC 03, LMAC 04 and LMAC 05 arenarrowspectrumchemical compounds.

ChemicalcompoundLMAC08iseffectiveagainstE.c oli,S.aureusand B.subtilis as observed in table 5.9 and figure 5.17. Ps.aeruginosa does not givethe zone of inhibition against this chemical compound. Here, diameter of zone ofinhibition is smalleras compared toprevious compounds. Byobserving thetable

5.9 and figures 5.19 and 5.20, the interpretation obtained is that the all four or-ganisms show the susceptibility against LMAC 09 compound. In the chemicalcompound LMAC 09, the zone of inhibition observed between the range of 15mmto 20mm which is very small compared to other chemical compounds. So it isconcluded that LMAC 09 compound is effective against both gram positive andgramnegative bacteria.

The chemical compound LMAC 10 is not able to resist Ps.aeruginosa.Other than Ps.aeruginosa, all the organisms give the zone of inhibition againstLMAC 10 compound between the range of 12mm to 18mm which is very smallasshown in the table 5.11and figure 5.21 and 5.22.

So after observing all the tables and figures, it is concluded that in themost of the observations

gram positive bacteria like Bacillus subtilis and Staphy-lococcusaureusareabletogivethezoneofinhibitionandsusceptibleagainstthe chemical compounds. But gram negative bacteria like Escherichia coli andPseudomonas aeruginosa are do not able to give the zone ofinhibitions againstmany antibacterial agents. So some are broad spectrum and some are narrowspectrumantibacterial agents.

VI. Summary:Here, agar well method was used to check antibacterial activityof different chemically synthesized compounds and broth dilution method wasused to check Minimum Inhibitory Concentration. For that two gram positivebacterial strains like B.subtilis and S.aureus and two gram negative bacterialstrains like Escherichia coli and Pseudomonas aeruginosa were used to checktheir susceptibility against chemical compounds. As control, ciprofloxacin drugwas used. Stock solution was prepared from chemical compounds and then tolower down the concentration and for getting different concentration, dilu-tions of stock solution was prepared. Then there was preparation of result tablesand graphs to compare the chemical compounds with each other and with cipro-floxacinwhich was use as control.

VII. ACKNOWLEDGEMENT

AuthorsacknowledgesupportfromL.M.CollegeofPharmacyandM.G.Scienceinstitute.

VIII. CONFLICTOFINTEREST

Authorsnothaveconflictofinterest.

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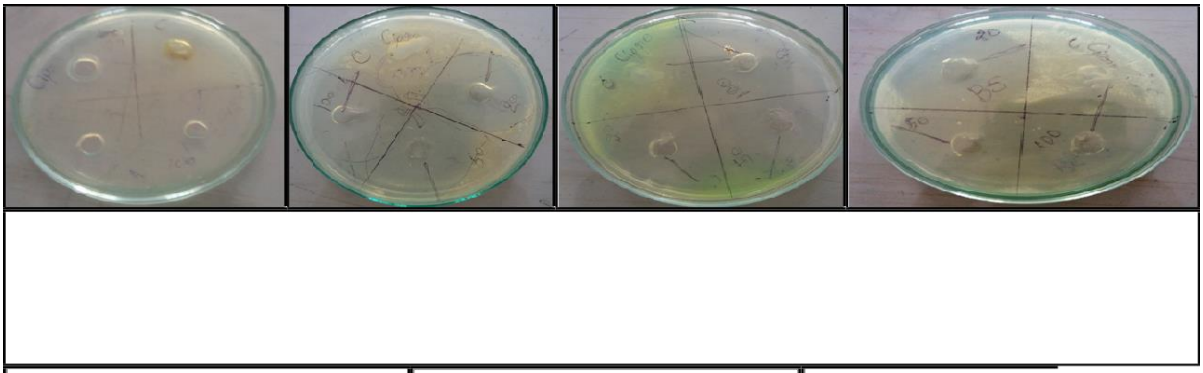
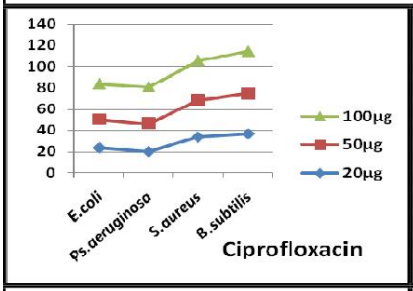
TableNo.3.1:-Results of MIC

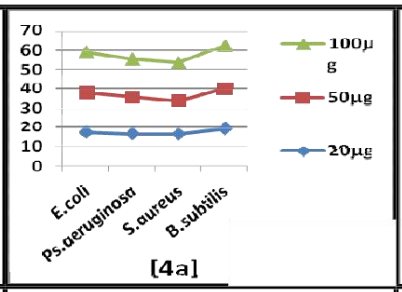
DATE	CHEMICAL COMPOUNDS	*E.coli*	*Ps.aeruginosa*	*S.aureus*	*B.subtilis*
30/5/19 to	**LMAC01**	10µg	22µg	5 µg	5 µg
1/6/19to	**LMAC02**	10µg	-	15µg	10µg
4/6/19	**LMAC03**	-	-	20µg	10µg
5/6/19	**LMAC04**	-	20µg	10µg	5 µg
6/6/19	**LMAC05**	15µg	-	10µg	10µg
7/6/19	**LMAC06**	15µg	10µg	13µg	5 µg
8/6/19	**LMAC07**	15µg	5 µg	10µg	5 µg
9/6/19	**LMAC09**	10µg	20µg	20µg	10µg
10/6/19	**LMAC10**	10µg	-	20µg	10µg

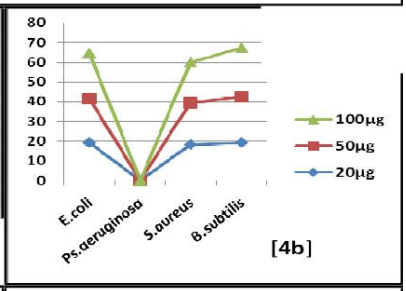
TableNo.5.1:-Results of antibacterial activity of ciprofloxacin which isused as control.

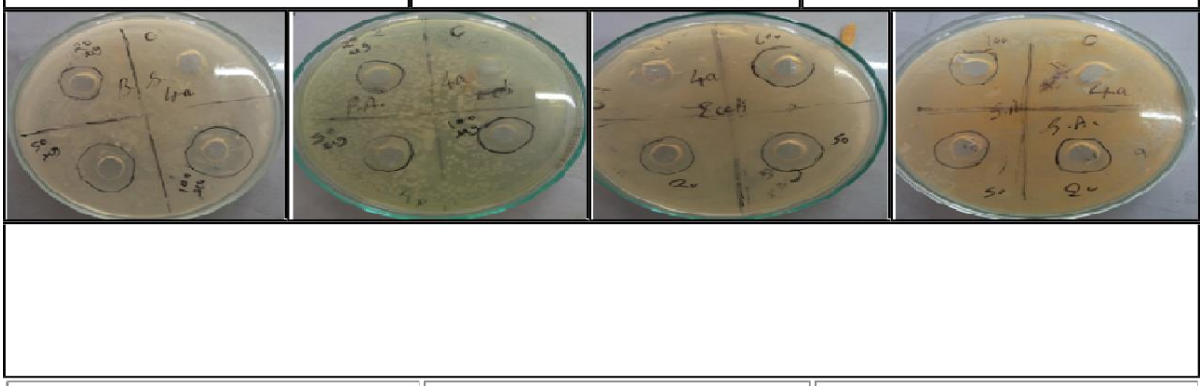
DATE	ORGANISM	CHEMICAL COMPOUND	CONCENTRATIONS OF CHEMICAL COM-POUNDS	NEXT DAY OBSERVATION (DIAMETER OF ZONE OF INHIBITION)
28/5/19	*E.coli*	Ciproflox-	20µg	20µg=23.6mm
		acin	50µg100µg	50µg = 26.6mm100µg=33.4mm
28/5/19	*Ps. aeruginosa*	Ciproflox-	20µg	20µg=20mm
		acin	50µg100µg	50µg = 26mm100µg=34.8mm
28/5/19	*S.aureus*	Ciproflox-	20µg	20µg=33.6mm
		acin	50µg100µg	50µg = 35mm100µg=37mm
28/5/19	*B.subtilis*	Ciproflox-	20µg	20µg=36.6mm
		acin	50µg100µg	50µg = 38.2mm100µg=39.8mm

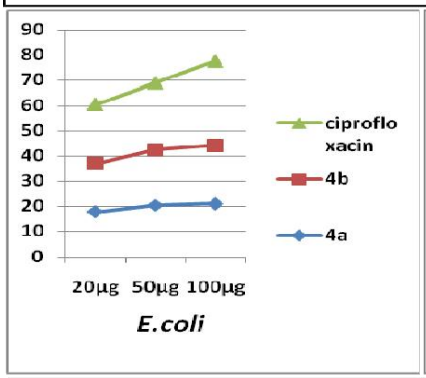



140
120
100
80
60
40
20
0
E.coli
Ps.aeruginosa
S.aureus
B.subtilis
100µg
50µg
20µg
Ciprofloxacin


70
60
50
40
30
20
10
0
E.coli
Ps.aeruginosa
S.aureus
B.subtilis
100µg
50µg
20µg
[4a]


80
70
60
50
40
30
20
10
0
E.coli
Ps.aeruginosa
S.aureus
B.subtilis
100µg
50µg
20µg
[4b]

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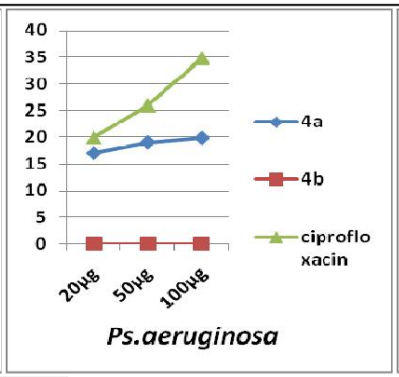
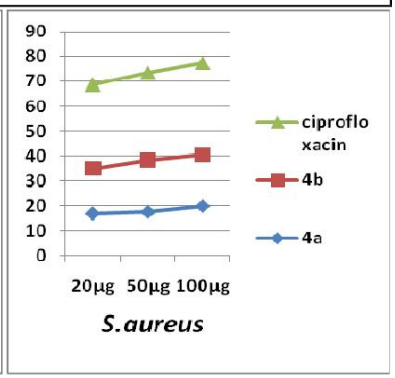
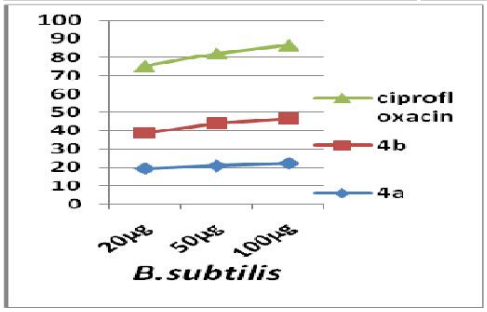
80
70
60
50
40
30
20
10
0
20µg 50µg 100µg
ciproflo xacin
4b
4a
E.coli


40
35
30
25
20
15
10
5
0
20µg
50µg
100µg
4a
4b
ciproflo xacin
Ps.aeruginosa


90
80
70
60
50
40
30
20
10
0
20µg 50µg 100µg
ciproflo xacin
4b
4a
S.aureus


100
90
80
70
60
50
40
30
20
10
0
20µg
50µg
100µg
ciprofl oxacin
4b
4a
B.subtilis