# CHAPTER-II

Screening of
Antibacterial Activity
Results And Discussion

#### SCREENING OF ANTIBACTERIAL ACTIVITY

All the compounds in the present study were tested for their anti-bacterial activity using Kirby-Baur\* diffusion method against various gram positive and gram negative bacteria. The gram (+ve) bacteria studied were - Staphylococcus citreus; Staphylococcus aureus; Staphylococcus albus and gram (-ve) included Escherichia coli (E.coli); Pseudomonas aerugenosa Klebsiella pneumoniae.

All the above mentioned bacteria are pathogenic. Staph. citreus; Staph aureus and Staph albus cause spepsis in wounds and burns. They cause the majority of acute pyogenic lesions in man. Staph. aureus causes tonsillitis, pharyngitis, sinusitis, and pneumonia.

<u>E. coli</u> (-ve) causes diarrhoea or gastroenteritis particularly in infants, old children and adults. It also causes urinary tract infections, pyogenic infections and septicaemia; whereas pseudomonas (-ve) causes chronic diseases which are in the form of localised or generalised infections. Localised infections are common in wounds, bedsores, eye infections and urinary tract infections; <u>Klebsiella pneumoniae</u> causes urinary infections, abscesses, meningitis and septicaemia.

\* Text Book of Microbiology

by R. Anantnarayan & Jayram Panikar

Orient Longman, 2nd Edn.

# **Experimental**

The compounds reported in the present study were screened for their antimicrobial activity by Kirby-Bauer disc diffusion method.

The principle involved in this method is the diffusion of compound through a solid medium so that a gradient is established, the concentration being highest near the site of application and decreasing with distance.

#### Preparation of culture media:

All glasswares and other materials were sterilised. All media were adjusted to a correct  $H^{\dagger}$  ion concentration (pH). Since most of the bacteria grow in a slightly alkaline medium; the pH was adjusted between pH 7.2 - pH 7.6.

# Nutrient broth:

- i) Peptone 10 gm.
- ii) Meat extract (Lablemco): 10 g.
- iii) Water 1,000 ml.

These ingredients were mixed and allowed to dissolve. A precipitate of phosphate was removed by filteration. The medium was then sterilised at 15 1b for 20 minutes.

#### Nutrient agar :

To the nutrient broth 2 percent of agar was added at 15 1b for 20 mints. Autoclaved and filtered and sterlised.

A filter paper disc 6 mm in diameter available commercially was charged with the compound at 0.2 mg/ml concentration in acetone as solvent.

After overnight incubation, the degree of sensitivity was determined by measuring the zones of inhibition in mm. Acetone was

The sensitivity of the tested compounds was expressed as follows:

- i) Strong growth inhibitor (zone size 15-20 mm) + + 1
- ii) Moderate growth inhibitor (zone size 9-14 mm) + +
- iii) Less growth inhibitor (zone size 6-8 mm) -+
- iv) No growth inhibitor -

The rescults of screening of antibacterial activity are presented in table : 2

### ANTIBACTERIAL SCREENING RESULTS

### Type of Microorganism (Bacteria)

a)	SC -	Staphylococcus citreus	(gram +ve)
b)	SA -	Staphylococcus aureus	(gram +ve)
c)	SAL -	Staphylococcus albus	(gram +ve)
d)	EC –	Escherichia coli	(gram -ve)
e)	PA -	Pseudomonas aerugenosa	(gram -ve)
f)	KP –	Klebsiella pneumoniae	(gram -ve)

Table No. 2 Compound No. Name of the compound Antibacterial activity SC SA SAL EC PA KP <u>Series-I</u> IIa  $N_{10}$ - [Hydrazido]-Phenothiazine  $\hbox{$4-$Ary1-1-(N$_{10}-Phenothizinoy1)$ thiosemi-}\\$ IIIa -carbazide  $1-Pheny1-2-(N_{10}-Phenothiaziny1)-5-$ IVa mercapto-1,3,4 triazole  $5-Anilino-2-(N_{10}-Phenothiaziny1)-$ Va 1,3,4-oxadiazole  $5-Anilino-2-(N_{10}-Phenothiaziny1)-$ VIa 1,3,4-thiadiazcle Series-II IIb  $N_{10}$ -(Hydrazido methyl)-Phenothiazine IIIb 4-Pheny1-1-[2-(10-phenothiaziny1) acetyl]thiosemicarbazide  $1-Pheny1-2-[N_{1C}-Phenothiaziny1 methy1]$ IVb ++ -5-mercapto-1,3,4-triazole

Compound No. Name of the compound			Antibacterial activity						
		SC	SA	SAL	EC	PA	KP		
VЪ	5-Anilino-2-[N <sub>10</sub> -phenothiazinyl methyl] 1,3,4-Oxadiazole.	+	-	-	-		*****		
VIb	5-Anilino-2-[N <sub>10</sub> -phenothiazinyl methyl] -1,3,4-thiadiazole.	+	-	-		-			
Series-	<u>III</u>					,			
IIC	$N_{10}$ -[ -Hydrazido-ethy1]Phenothiazine	Manager	+	_		-	****		
IIIc	4-Pheny1-1-[1-oxc-2-(10-phenothiaziny1)-ethy1]-thiosemicarbazide		+	++	-	+++			
IVc	1-Pheny1-2-[<-10-phenothiaziny1-ethy1] -5-mercapto-1,3,4-triazole	+	+	-	-	-			
Vc	5-Anilino-2-[<-10-phenothiazinyl ethyl] -1,3,4-oxadiazole	•••	-	-	rest	-			
VIc	5-Anilino-2-[ <b>4</b> -10-phenothiaziny1 ethy1] -1,3,4-thiadiazole	++	_	***	+	<del>-</del>			
Series-	IV								
IId	N <sub>10</sub> -[Malonyl hydrazido] phenothiazine	+	_			++	-		
IIId	Bis-[4-pheny1-1-(1-oxo-2-N <sub>10</sub> -pheno-thiaziny1 methy1] thiosemicarbazide	+	+	+	+	+.			
IVd	N <sub>10</sub> -[Bis(1-Pheny1-5-mercapto-1,3,4-triazoly1)methy1]phenothiazine	++	-	+	++	++	*****		
Vd	10-[Bis(5-Anilino-1,3,4-oxadiazoly1) methyl]phenothiazine	-	-	++	++	-	- -		
VId	N <sub>10</sub> -[Bis(5-Anilino-1,3,4-thiadiazoly1)-phenothiazine	++	-	-	++	++	+		
Series	<u>- v</u> :								
I <sub>f</sub>	N <sub>10</sub> -[4-Acetamido-2-methy1 pheny1 sulphonamidoacetv1] phenothiazine	++	_		-	+			
Ig .	N <sub>10</sub> -[4-Acetamido-3-Methy1 pheny1 sulphonamido-acety1]phenothiazine	+	-	++	SHIVE	INIVER 3RARY	STY X		

Compound No. Name of the compound		Ar	Antibacterial activity						
		SC	SA	SAL	EC	PA	KP		
<sup>I</sup> h	N <sub>10</sub> -[4-Methyl phenyl sulphona acetyl] phenothiazine	mido	++	-	++	++	++		
I	N <sub>10</sub> -[Phenyl sulphonamido acet phenothiazine	y1] +	+++	_	++	++	+		