CHAPTER – III

ANTIMICROBIAL SECREENING

SCREENING OF ANTIBACTERIAL ACTIVITY

Most of the heterocyclic compounds exhibit antifungal and antibacterial activities. Some of them are synthesized by microorganism and are called antibiotics. Some heterocyclic compounds which are not synthesized by microbes, show antifungal and antibacterial activities to control microbes. Different dyes, sulphonamides, pyrazoles, thiadiazoles, indoles, quinolines, phthalazines found to show antibacterial activities and are of therapeutic use.

All the compounds included in the present study such as 3- arylidenehydrazido - 2 - phenylphthalhydrazides, 4 – arylidine-hydrazino 2-phenylphthalhydrazides, 2,3-diarylidenehydrazidophthal-hydrazides were tested for their antibacterial activity by Cup plate diffusion method[111] against gram positive and gram negative bacteria. The gram (+ve) bacteria selected for this purpose were S.aureus and S. Faecalis and gram (-ve) E. Coli and K. Pneumoniae.

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

E. Coli causes diarrhoea or gastroenteritis particularly in infants, old children and adults. It also causes urinary tract infections, pyogenic infections and septicaemia, where as pseudomonas causes chromic diseases which are in the form of localised or generalised infections. Localised infections are common in wounds, eye infections and urinary track infections, Klebsiella pneumoniae causes urinary infections, abscesses, meningitis and septicaemia.

Experimental

The compounds reported in the present study were screened for their antimicrobial activity by Kirby-Bauer disc diffusion method.[112] The principle involved in this method is the diffusion of a 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 sterillised. All media were adjusted to a correct H^+ ion concentration (pH). Since the most of the bacteria grow in a slightly alkaline medium; the pH was adjusted between pH 7.2 – 7.6.

Nutrient broth

i)	peptone	:	10 gm
ii)	Meat extract (Lablemco)	:	10 gm
iii)	Water	:	1,000 ml

These ingredients were mixed and allowed to dissolve. A precipitated phosphate was removed by filtration. The medium was then sterilized at 15 1b for 20 minutes.

Nutrient agar

To the nutrient broth, 2 percent of agar was added at 15 1b for 20 minutes, autoclaved, filtered and sterilized.

A filter paper disc of 6 mm in diameter available commercially was charged with the compound at 0.1mg/ml concentration in DMSO

as solvent. After overnight incubation, the degree of sensitivity was determined by measuring the zones of inhibition.

A solvent was also run to know the activity of blank (solvent). The standard drug was also screened under similar conditions for comparison.

RESULTS AND DISCUSSION

A) Antimicrobial screening of 3-Arylidenehydrazido-2-phenyl-phthalhydrazide (IV) and related derivatives :

The compounds (I, II, III and IV a-h) were tested in vitro for their antibacterial activity against S. aureus S. faecalis (gram positive bacteria) and E. coli and K. pneumoniae (gram negative bacteria) at 100 ppm.concentration using a tetracycline as a standard compound for comparison. The zones of inhibition were measured in mm and have been depicted in Table - 4.

The results of antimicrobial screening indicates that the compounds I, III, IVc, IVd, IVe, IVf exhibited moderate to good antibacterial activity against both types of bacterial species. Two generalizations can be made from the results. Firstly, in comparison with 2-phenylphthalhydrazide the 3-arylidenehydrazido-2-phenylphthal-hydrazides (IV) were found to be less active with the marginal difference. Secondly, the 3-arylidenehydrazido 2-phenyl-phthalhydrazides with the substitutent pattern R = chlorophenyl, hydroxyphenyl and nitrophenyl attached to the 2-phenyl-phthalhydrazide nucleus, were found to be more active than otherwise substituted compounds. Antimicrobial screening results also indicated

that there is no significant effect of chlorine atom in the compound (IV) on antibacterial activity.

B) Antimicrobial screening of 4-arylidenehydrazino-2-phenylphthal hydrazide (III) and related compounds :

The compounds (II and III a-i) were tested in vitro for their antibacterial activity against S. aureus, S.Faecalis (gram positive bacteria) and E. coli and K. pneumoniae (gram negative bacteria) using tetracycline as a standard compound. Most of the compounds in this series exhibited moderate to good antibacterial activity. Compound II, III, IIIc, IIId, IIIf, IIIh have shown spectacular activity against both types of bacterial species. Among these compound II is found to be the most active having considerable value as a drug.

The compounds having substitutents pattern R = chlorophenyl, nitrophenyl, hydroxyphenyl were found to exhibit moderate antibacterial activity. Rest of the compounds of this series exhibited lower activity against the bacterial species under study (Table 5).

C) Antimicrobial screening of 2,3-diarylidenehydrazidophthal-hydrazide (V) and related compounds :

The antibacterial activity was evaluated by testing the synthesized compound at 100 ppm concentration in DMSO against gram + Ve and gram – Ve bacteria. The compounds included in scheme III, Sr. No. I, II, IV, Va, Vc, Vd, Ve, Vf were found to be more active than rest of the compounds against bacteria under study. In this series also the compounds with the substituent pattern R = chlorophenyl, nitrophenyl and hydroxyphenyl were found to show significant antibacterial activity (Table – 6).

The results of antimicrobial screening indicated that the phthalhydrazide (I), its disodium salt (II), 2,3-dihydrazidophthal-hydrazide (IV) have also exhibited good antibacterial activity against both types of bacterial species than other compounds of series and have considerable medicinal value.

Table - 4 : Antimicrobial screening data of 2-phenylphthalhydrazide (I), 3-carbethoxy 2-phenylphthal-hydrazide (II), 3-hydrazido - 2-phenylphthaldrazide (III) and 3-arylidenehydrazido-2-phenyl-phthalhydrazides (IV).

(Diameter of zones of inhibition in mm)

Compound Sr. No.	R	Gram +ve Bacteria		Gram –ve Baacteria	
		S. aureus	S. faecalis	E. coli	K. pneumoniae
I	-	20	19	21	18
II	-	08	09	08	06
III	-	19	17	18	16
IV a	p-OCH_3· C_6H_4	09	08	06	07
IV b	o-OH, m-OCH_3· C_6H_3	12	13	12	14
IV c	o-Cl· C_6H_4	14	13	12	10
IV d	p-Cl· C_6H_4	13	15	14	14
IV e	o-NO_2· C_6H_4	12	16	16	14
IV f	p-$N(CH_3)_2$· C_6H_4	08	09	09	06
IV g	o-OH· C_6H_4	14	15	13	11
IV h	p-CH_3· C_6H_4	06	07	07	09
Standard Compound	Tetracycline	25	26	25	24

Zone of inhibition in mm :

15 – 20 mm strong growth inhibitor
09 – 14 mm moderate growth inhibitor
06 – 08 mm less growth inhibitor
00 – 00 mm no antibacterial activity.

Table – 5: Antimicrobial screening data of 4-hydrazino-2-phenylphthalhydrazide (II), 4-arylidenehydrazino-2-phenylphthalhydrazide (III) and related compounds :

(Diameter of zones of inhibition in mm)

Compound Sr. No.	R	Gram + ve Bacteria		Gram –ve Baacteria	
		S. aureus	S. faecalis	E. coli	K. pneumoniae
II	-	22	18	20	17
III a	p-OCH_3· C_6H_4	08	09	07	10
III b	o-Cl· C_6H_4	13	14	11	13
III c	p-Cl· C_6H_4	14	12	13	10
III d	o-OH· C_6H_4	14	15	12	15
III e	p-$N(CH_3)_2$· C_6H_4	08	09	06	07
III f	o-NO_2· C_6H_4	14	11	12	15
III g	o-OH, m-OCH_3· C_6H_3	10	08	07	06
III h	p-OH· C_6H_4	15	13	12	14
III I	3,4,5,-$(OCH_3)_3$· C_6H_2	06	10	08	09
Standard Compound	Tetracycline	25	26	25	24

Table – 6 : Antimicrobial screening data of phthalhydrazide (I), 2,3-disodiumphthalhydrazide(II), 2,3-dicarbethoxyphthalhydrazide (III), 2,3-diahydrazidophthalhydrazide (IV), 2,3-diarylidenehydrazidophthalhydrazide (V) and related compounds

(Diameter of zones in inhibition in mm)

Compound Sr. No.	R	Gram +ve Bacteria		Gram –ve Baacteria	
		S. aureus	S. faecalis	E. coli	K. pneumoniae
I	-	22	20	21	20
II	-	21	18	17	18
III	-	08	09	10	11
IV	-	20	18	19	18
V a	p-Cl · C_6H_4	16	15	14	15
V b	o-OCH_3 · C_6H_4	09	10	07	08
V c	o-NO_2 · C_6H_4	16	17	15	18
V d	p-$N(CH_3)_2$ · C_6H_4	14	12	16	15
V e	o-Cl · C_6H_4	17	16	14	15
V f	o-OH · C_6H_4	14	13	15	12
V g	p-OH· C_6H_4	15	14	13	13
V h	o-OH, m-OCH_3· C_6H_4	10	11	11	12
Standard Compound	Tetracycline	25	26	25	24