CHAPTER - III

ANTIMICROBIAL AND ANTIFUNGAL SCREENING

CHAPTER-III

ANTIMICROBIAL AND ANTIFUNGAL SCREENING

1 Experimental, antimicrobial and antifungal screening data of the compounds

2 Results and discussions

3 References

4 List of publications

ANTIMICROBIAL AND ANTIFUNGAL SCREENIG OF THE COMPOUNDS :

INTRODUCTION

A large number of heterocyclic compounds show antibacterial and antifungal activities. Some of them are synthesised by microorganism and are called as antibiotics. Some other compounds which are not synthesised by microbes but show good antibacterial and antifungal activities used for controlling microbes and in the treatment of infections and diseases. Many dyes, sulphonamides, quinolines, pyrazoles, thiadiazoles, indoles show antibacterial and antifungal activities and are of therapeutic use.

The antibacterial and antifungal activity of newly synthesised compounds are observed by incorporating these compounds in the nutritional media used for the cultivation of various test microbes. The microbes usually pathogens. The microbial activity is examined by studying the growth inhibition pattern of the microbes on media containing these compounds. The methodology employed for testing consists of small paper discs previously impregnated with specific compound with known concentration. The sentivity of pathogens to different synthetic compounds is determined by measuring the diameter of the growth inhibition zones.

Thus, the compounds in the present study were tested for their antibacterial activity using paper disc method[162] and for

antifungal activity by turbidometric method.*

By using paper disc method the antibacterial activity was observed against Staphylococcus aureus (gram +ve) and Escherichia coli (gram-ve) bacteria. These bacterial species are pathogenic. S.aureus causes septic in wounds, burns etc. and are acute pathogenic lesions in man. It also causes tonsillistis, pharyngitis, sinusitis and pneumonia. E.coli causes diarrhoea or gastroenteritis perticularly in infants, children and adults. It also causes urinary track infections, pyrogenic infections and septicaemia etc.

EXPERIMENTAL PROCEDURE :

The compounds reported in the present study were screened for their antibacterial activity by paper disc method. The compound is allowed to diffuse through a solid medium, so that a gradient is estabblished, the concentration being decreasing with distance. The test bacterium is seeded in the medium and its sensitivity to the synthesised compound was determined by measuring the zones of growth inhibitions.

PREPARATION OF MEDIUM AND MATERIAL REQUIRED :

All the glasswares and other materials were sterilised. All media were adjusted to a correct hydrogen ion concentration (pH) between 7.1 to 7.5.

NUTRIENT MEDIUM COMPOSITION :

1	Peptone	:	5 gm
2	Agar Agar powder	:	10 gm
3	Meat extract	:	5 gm

4	Sodium chloride	:	2.5 gm
5	Distilled water	:	500 ml.

MATERIAL :

i) Nutrient medium (20 ml for each petri dish)

ii) Sterile petri dishes.

iii) Sterile pipettes.

iv) Old grown culture (24 hrs.) in test tube.

v) Solution of the compound of known concentration.

Nutrient medium is sterilised by autoclaving at 121^{o} C and at 15 lb/sq.inch pressure for 20-25 min. It was then poured into sterilised glass plate (20ml per plate) and cooled at room temperature. A suitable dilution of growth culture of the test bacteria was spread over media and plate dried at 37^{o} C for 0.5 hr. A filter paper discs (6 mm diameter, commercially used) charged with the compound at 10 mg/ml concentration in acetone and applied with sterile forceps after 24 hrs. of incubation. The degree of sensitivity was determined by measuring growth inhibition zones around the disc.

Similarly, one plate with Oxytetracycline (std.compd.) and other with acetone were charged and incubated for 24 hrs for comparison and control of the solvent respectively.

Zones of inhibitions of growth were measured in mm and are compared with zone of standard compound. i.e. Oxytetracycline. On this basis the percentage inhibition is calculated.

Antimicrobial screening results have been reported in the Table 13.Growth inhibition zone of Oxytetracycline is considered as 100% inhibition for gram (+ve) and gram(-ve) bacteria.

TABLE 13

ANTIMICROBIAL SCREENING DATA OF N[1]-SUBSTITUTED PYRAZOLE DERIVATIVES (V) AND (VI)

Compound	Name of the compound	Antimicrobial activity in % Inhibition	
		S.aureus	E.coli
V_a	3,6-Dimethyl-1(8-chloro-4-methyl-quinolin-2-one-1-yl methyloxo)-4-oxopyrano[4,3-c]pyrazole	62	67
V_b	3,6-Dimethyl-1(7-chloro-4-methyl-quinolin-2-one-1-ylmethyloxo)-4-oxopyrano[4,3-c]pyrazole	59	59
V_c	3,6-Dimethyl-1(6-chloro-4-methyl-quinolin-2-one-ylmethyloxo)-4-oxopyrano[4,3-c]pyrazole	58	65
VI_a	3,5-Dimethyl-1(8-chloro-4-methyl-quinolin-2-one-1-ylmethyloxo) pyrazole.	78	65
VI_b	3,5-Dimethyl-1(7-chloro-4-methyl-quinolin-2-one-1-ylmethyloxo) pyrazole.	68	62
VI_c	3,6-Dimethyl-1-(6-chloro-4-methyl quinolin-2-one-1-ylmethyloxo) pyrazole	70	53

TABLE 14

ANTIMICROBIAL SCREENING DATA OF N-SUBSTITUTED PYRAZOLE DERIVATIVES (XI) AND (XII)

Compound No.	Name of the compound	Antimicrobial activities (% inhibition) S.aureus (gram +ve)	E.coli (gram - ve
XI_a	3,6-Dimethyl-1(8-chloro-2-methyl-quinolin-4-one-1-ylmethyloxo) -4-oxopyrano[4,3-c]pyrazole	65	63
XI_b	3,6-Dimethyl-1-(7-chloro-2-methyl-quinolin-4-one-1-ylmethyloxo) -4-oxopyrano[4,3-c]pyrazole	60	68
XI_c	3,6-Dimethyl-1-(6-chloro-2-methyl-quinolin-4-one-1-ylmethyloxo) -4-oxopyrano[4,3-c]pyrazole	33.3	59
XII_a	3,5-Dimethyl-1-(8-chloro-2-methyl-quinolin-4-one-1-ylmethyloxo) pyrazole	40.7	75
XII_b	3,5-Dimethyl-1-(7-chloro-2-methyl-quinolin-4-one-1-ylmethyloxo) pyrazole	29.6	70
XII	3,5-Dimethyl-1-(6-chloro-2-methyl-quinolin-4-one-1-ylmethyloxo) pyrazole	37	56

RESULTS AND DISCUSSION :

All the compounds V_{a-c} were tested in vitro for their antimicrobial activity against Staphylococus aureus (Gram +ve) and Escherichia coli (Gram -ve) bacteria by paper disc method and 100 ppm concentration in acetone. Most of them exhibited moderate antibacterial activity against gram(-ve) and gram(+ve) (Table -13 and Table-14) and are found almost equally active against the bacterial species under study. Further the compounds VI_{a-c} exhibited better antimicrobial activity against gram +ve bacteria than gram-ve bacteria.

The compound with 8-chloro substituent in the benzene ring of quinoline nucleus enhances the antibacterial activity in the pyrazoles. The compounds XI_{a-c} and XII_{a-c} exhibited better activity against E.coli (gram-ve) bacteria except compounds XI_c and XII_c . Here also 8-chloro substituted compound XI_a and XI_b were found to be more active against S.aureus than rest of the compounds.

ANTIFUNGAL SCREENING :

The antifungal activity of one synthesised compounds was measured by comparing the zones of inhibition with standard compound Dithane M-45; at 100 ppm concentration, it showed

85% inhibition where as at 500 ppm it showed 100% inhibition. On the basis of this average percentage inhibition is calculated. Antifungal screening data of the compounds V,VI and XI,XII have been incorporated in Table 15.

TABLE 15
ANTIFUNGAL SCREENING DATA OF THE COMPOUNDS V,VI AND XI, XII.

Scheme-I compound No.	Antifungal Screening C.Sacchari		Scheme-II compound No	Antifungal Screening C.Sacchari	
	100 ppm	500 ppm		100 ppm	500 ppm
V_a	50	60	XI_a	55	75
V_b	40	50	XI_b	50	70
V_c	55	65	XI_c	35	65
VI_a	58	75	XII_a	50	69
VI_b	35	70	XII_b	30	67
VI_c	46	60	XII_c	50	60

The antifungal screening of the compounds V_{a-c}, VI_{a-c}, XI_{a-c}, XII_{a-c} was carried out against the fungal species C.Sacchari at 100 & 500 ppm concentrations. Most of the tested compounds were found toxic to C.Sacchari at higher concentrations (Table 15) and not so spectacular at lower concentrations. The compound V_a & XI_a with 8-chloro substitent showed better antifungal activity.

B) ANTIMICROBIAL ACTIVITY OF PYRANOPYRAZOLES :

The pyranopyrazole derivatives (III_{a-z})were tested for their antimicrobial activity against gram +ve baceria. Most of

the derivatives are observed to be more active against S.aureus than E.coil gram (-ve) bacteria. The promising antimicrobial activity of the some compounds of this series is attributed to tthe presence of an electron withdrawing group in the benzene ring of 'R'.However, the presence of electron donating group in 'R' decreases the antimicrobial activity (Table 16).

TABLE 16

ANTIMICROBIAL SCREENING DATA OF THE COMPOUNDS, PYRANOPYRAZOLES(III'_{a-z})

Sr.No.	Name of the compound	Antimicrobial activities in % inhibition	
		S.aureus	E.coli
III'_a	3,6-Dimethyl-1-(phenylacetyl)-4-oxopyrano[4,3-c]Pyrazole	70.0	45.0
III'_b	3,6-Dimethyl-1(P-nitro phenoxyacetyl)-4-oxo-pyrano [4,3-c] pyrazole	74.1	41.1
III'_c	3,6-Dimethyl-1-(3,5-dinitro-benzoyl)-4-oxopyrano [4,3-c] pyrazole	77.8	55.5
III'_d	3,6-Dimethyl-1-(m-methylphenoxy) -4-oxopyrano [4,3-c]pyrazole	75.0	50.0
III'_e	3,6-Dimethyl-1-(p-nitrobenzoyl) oxopyrano[4,3-c]pyrazole	71.0	52.0
III'_f	3,6-Dimethyl-1-(o-chlorophenoxyacetyl) 4-oxopyrano[4,3-c]pyrazole	77.8	55.0
III'_g	3,6-Dimethyl-1-(p-methyl-phenoxyacetyl)-4-oxopyrano [4,3-c]pyrazole	78.0	57.0

(Contd...Table 16

Sr.No.	Name of the compound	Antimicrobial activities in % inhibition	
		S.aureus	E.coli
III'_h	3,6-Dimethyl-1-(p-chloro,m-cresol phenoxyacetyl) -4-oxopyrano[4,3-c]pyrazole	66.7	48.0
III'_i	3,6-Dimethyl-1-(p-chloro-phenoxypropionyl) -4-oxopyrano[4,3-c]pyrazole	65.0	43.0
III'_j	3,6-Dimethyl-1-(o-hydroxy,m-chloro benzoyl)-4-oxopyrano[4,3-c] pyrazole	84.6	45.0
III'_k	3,6-Dimethyl-1-(o-acetoxybenzoyl) -4-oxopyrano[4,3-c]pyrazole	73.0	55.0
III'_l	3,6-Dimethyl-1-(o-chlorobenzoyl) -4-oxopyrano[4,3-c]pyrazole	78.0	59.0
III'_m	3,6-Dimethyl-1-(nicotinoyl)-4- -oxopyrano[4,3-c]pyrazole	79.0	60.0
III'_n	3,6-Dimethyl-1-(cinaamoyl)-4- oxopyrano[4,3-c]pyrazole	73.0	61.0
III'_o	3,6-Dimethyl-1-(thymoxyacetyl)- -4-oxopyrano[4,3-c]pyrazole	55.6	40.7
III'_p	3,6-Dimethyl-1-(benzoyl)-4- oxopyrano[4,3-c]pyrazole	72.0	42.0
III'_q	3,6-Dimethyl-1-(phenylacetyl)-4- oxopyrano[4,3-c]pyrazole	74.0	43.0
III'_r	3,6-Dimethyl-1-(p-methoxybenziyl)-4 -oxopyrano[4,3-c]pyrazole	100.0	72.6
III'_s	3,6-Dimethyl(2-thymoxy propionyl) -4-oxopyrano[4,3-c]pyrazole	68.0	45.0

(Contd...Table 16)

Sr.No	Name of the compound	Antimicroobial activities in % inhibition	
		S.aureus	E.coli
III'_t	3,6-Dimethyl(2-phenoxypropionyl) -4-oxopyrano[4,3-c] pyrazole	65.0	48.0
III'_u	3,6-Dimethyl-1-(p-nitrophenoxyacetyl) -4-oxopyrano[4,3-c] pyrazole	55.6	37.0
III'_v	3,6-Dimethyl-1-(2m4-dimethyl-phenoxy)-4-oxopyranol [4,3-c] pyrazole	69.0	49.0
III'_w	3,6-Dimethyl-1-(o-methyl-phenoxyacetyl)-4-oxopyrano [4,3-c]pyrazole	68.0	52.0
III'_x	3,6-Dimethyl-1-(2,4-dibromo-phenoxyacetyl)-4-oxopyrano [4,3-c]pyrazole	66.7	55.6
III'_y	3,6-Dimethyl-1-(2,6-dibromo,4-chloro phenoxyacetyl)-4-oxopyrano[4,3-c]pyrazole	68.0	33.3
III'_z	3,6-Dimethyl-1-(p-chloro-phenoxyacetyl)-4-oxopyrano[4,3-c]pyrazole	75.0	54.0

The results of the antimicrobial screening indicated that the compounds III'_c,III_g,III_j,III'_l ,III'_m ,III'_n ,III'_q , ,and III'_r , have exhibited good antibacterial activity against both type of bacterial species. The compound III'_r was found to be the most active compound of this series having considerable medicinal value.

ANTIFUNGAL ACTIVITY :

Some compounds were tested for their antifungal activity against C.sacchari and the antifungal data have been furnished in Table 17.

TABLE 17
ANTIFUNGAL SCREENING DATA OF THE COMPOUNDS

Compound No	Antifungal screeing (% inhibition) C.sacchari	
	100 ppm	500 ppm
III'_b	11.5	17.8
III'_c	42.0	42.0
III'_f	42.0	91.0
III'_h	37.0	78.0
III'_j	13.5	49.0
III'_o	20.5	17.0
III'_r	18.0	14.0
III'_u	34.0	39.0
III'_x	15.0	54.0
III'_y	36.0	38.0

By careful scrutiny of the antifungal data. It is observed that most the compounds showed weak antifungal activity except III'_f and III'_h which are found toxic to C. sacchari at higher concentrations and not so spectacular at lower concentrations.

A generalisation can be made from these results i.e. pyronopyrazoles III'_{a-z} are of considerable importance as antibactorial compounds having medicinal value as drug.