CHAPTER-V

Antibaterial Studies of Aminopsoralens, Psora-Schiff bases and Psora-azetidinones

Antibacterial Studies of Aminopsoralens, Psora-Schiff bases and Psora-azetidinones

V.1 Introdution:

The term *bacterium* has been described by a German scientist C. E. Ehrenberg in 1928. Bacteria are small microscopic prokaryotic organisms with a relatively simple and primitive form of the cellular organization. A Danish physician Gram discovered 'a dark purple stain' which has great importance for differentiation and identification of the bacteria. The bacteria which retain dark purple stain are regarded as 'Gram positive bacteria' whereas which decolorize dark purple colour are regarded as 'Gram negative bacteria'. These tiny micro organisms are responsible for spreading fatal diseases such as tuberculosis, typhoid, syphilis, gonorrhea etc. Therefore, their population or growth must be controlled or stopped. The chemical agents or chemotherapeutic agents which control or stop the population of these disease causing bacteria are referred to as 'Antibacterial substances'.

Antibacterial substances form a family of wide range of chemicals including alcohols and related compounds, acids and their derivatives, iodine and chlorine containing compounds, oxidizing agents, dyes, metal ions, oxine derivatives and antibiotics.

Medical significance of the bacteriostatic dyes was first recognized by Churchman.[1] Antibacterial properties of yellow acridine dyes, which led to their wide clinical use, has been studied in detail by Browning.[2] Albert reported that Metal chelate of 8-hydroxy quinoline (oxine) exhibits antibacterial properties.[3] A number of metallic salt solutions including Zn, Cu, Au, Ag have been found to show antibacterial properties.[4]

Victory of antibiotics over the disease causing bacteria is one of the modern medicinal greatest successful stories. Antibiotics saved innumerable lives and blunted serious complications of many infectious diseases and were widely used in the World War-II. Antibiotics, through their functional character, show chemical interaction with the proteins of microorganism as well as the structural features of the microorganism and causes mutation in them which results either growth inhibition or killing of the cells and thus controls the growth or stops the growth of microorganism. Widely used, over more than half a century, antibiotic Penicillin (a product of the soil mould Penicillium fungi)

was proved to be an efficient medicine for curing diseases. But soon after, as a consequence of overuse, the microbes began to resist it. Therefore, scientists began to fight with other analog cousins of Penicillin such as Oxacillin, Methicillin etc. By the half of the twentieth century various antibiotics viz. Chloramphenicol, Tetracyclin, Terramycin, Neomycin etc. started spreading their effects. However, for the antibiotic, a global spread of 'Methicilline-Resistant Stephylococus Aurens (MRSA)' became one of the most contemporary challenges to the treatment of hospital-acquired infections worldwide and these are known to carry a uniquely effective drug resistant mechanism that can protect the pathogens against all members of β-lactam family of the antibiotics. The ability of infectious organisms to adapt quickly to the new environmental conditions has become a key factor in development of antibiotic resistance for microorganisms.

Microorganisms have very few numbers of genes and they are generally single celled. Hence, any change in their DNA sequence, affect their ability to cause diseases. But as the microorganisms grow rapidly, a change that help them survive against antibiotics, quickly dominates their populations having such mutations. Hence, the microorganisms acquire genes having 'code of resistance' to the antibiotics. This has made tuberculosis, typhoid, syphilis, gonorrhea etc. diseases more difficult to be treated than they earlier had. All these have left new challenges for the scientists to prepare new antibiotics so that they can fight effectively against the resistant-growing microorganisms.

V.2 Evaluation of antibacterial activity

Both *in vivo* as well as *in vitro* methods have been screened for evaluating the microbial activity[5] and two common *in vitro* methods are described as follows.

Serial dilutions in Broth:

Several drug dilutions have been prepared in uniform amounts of standard broth in culture tubes and then the solutions are inoculated with a uniform number of cells to test the organism. After incubation, turbidity or its absence is measured by turbidimetry. Comparison of the turbidity obtained with standard drug reference solutions show the extent of antibacterial properties.

Streak Assay in Agar:

Prepared different dilutions of the drug are placed in a series of Petri dishes in which agar is spread. On cooling the agar is solidified then it is subjected to growth environment of bacteria. The change in the spreading is observed and the antibacterial properties are rationalized.

Many other methods such as agar strip diffusion test and diffusion tests with filter paper-disks for determining sensitivity have been reported in literature.[6-7]

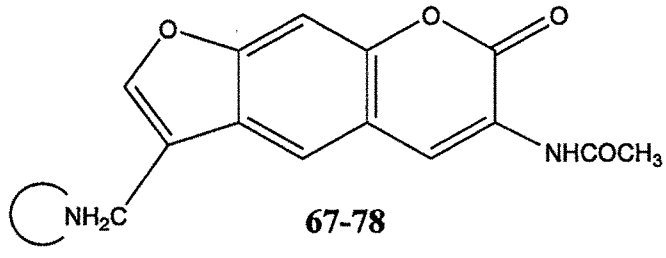
A well known *in vitro* method 'use of the controlled single disk' has been employed commonly in the laboratory.[8] In this method, nutrient agar of appropriate composition is heavily inoculated with the desired organism all over the surface of the solidified agar or mixed with agar while still fluid before pouring into the plate. Measured concentrations of the antibiotic or drug solutions are applied to the inoculated agar in the disks of uniform thickness or sterile filter paper are placed on the surface of the agar plate. The width of zone indicates the sensitivity of the organism being tested through the presence or absence of zones. It is found that the factors such as culture media, pH, size of inoculums, composition of nutrient agar, concentration of drug, storage of disks, incubation time etc. affect the zones of inhibition and therefore these factors should be taken into account while studying antibacterial properties.

Antibacterial activities of n-butoxypsora-Schiff bases has been studied by Desai and observed that many n-butoxy Schiff bases show mild antibacterial activities.[9]

Keeping this view in mind, a strategy to prepare 'Psora-azetidinones' having new lactam ring was developed so as to generate compounds with effective antibiotic properties and their antibacterial activities have been studied.

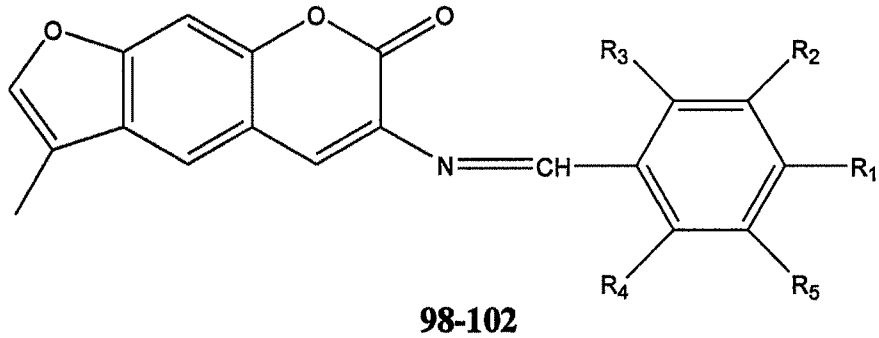
V.3 Results and Discussion:

Compounds, described below, have been screened for antibacterial activity and are tabulated in the **table I-III.**



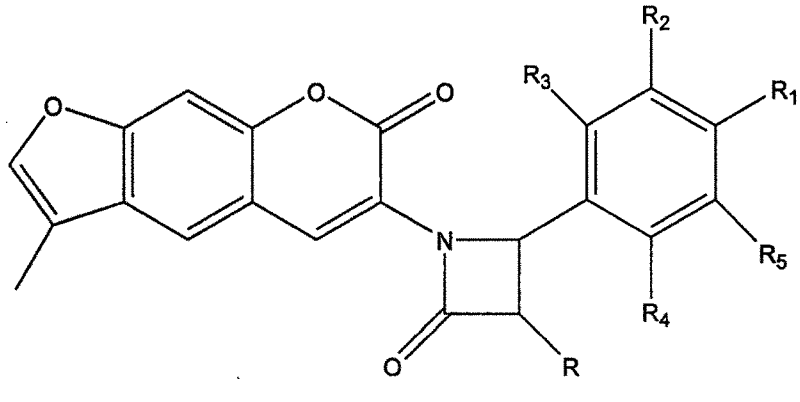
67-78

Aminopsoralens



98-102

Psora-Schiff bases



113-117

Psora-azetidinones

Table-I: Antibacterial activity of Aminopsoralens **67-78.**

Compound no.		Interpretation of zone of inhibition at 100 and 500 ppm concentration				**Relative % of activity with respect to benzyl penicillin for *E. coli.***
		E coli.		***S. aurens***		
		100	500	100	500	
67	2,5-dihydropyrrolinyl	-	+	-	-	1%
68	piperazinyl	-	+	-	-	5%
69	2-methylpiperadinyl	-	+	-	-	1%
70	N-(methylcyclohexyl)piperazinyl	-	+	-	-	2%
71	N,N-diethanolamino	-	+	-	-	3%
72	morpholinyl	-	+	-	-	2%
73	pyrrolidinyl	-	+	-	-	1%
74	4-(1-piperidinyl)piperidinyl	-	-	-	-	0%
75	N-methylpiperazinyl	-	+	-	-	2%
76	N-phenylpiperazinyl	-	+	-	-	2%
77	N-allylpiperazinyl	-	+	-	-	2%
78	piperidinyl	-	+	-	-	1%

The activities were observed in stationary phase in which the compounds inhibit growth of *E. coli.* and *S. aurens* bacteria at the growing stages. The pictures show the zone of inhibition of growth of the bacteria.

From **table-I** it is observed that most of aminopsoralens show antibacterial activity towards *E. coli.* at 500 ppm concentration except **74** while the activity was not observed with *S. aurens* even at 500 ppm. At low concentration of 100 ppm aminopsoralens did not show antibacterial activity.

Table-II: Antibacterial activity of Psora-Schiff bases **94-102.**

Compound no.	Interpretation of zone of inhibition at 100 and 500 ppm concentration				**Relative % of activity with respect to benzyl penicillin for *E. coli.***
	E coli.		***S. aurens***		
	100	500	100	500	
94	-	-	-	-	0%
95	+	+	+	+	28%
96	-	+	-	-	1%
97	-	+	-	-	8%
98	+	+	-	-	11%
99	+	+	-	+	19%
100	+	+	-	-	11%
101	+	+	-	+	18%
102	+	+	+	+	28%

Table-III: Antibacterial activity of Psora-azetidinones **113-117.**

Compound no.	Interpretation of zone of inhibition at 100 and 500 ppm concentration				**Relative % of activity with respect to benzyl penicillin 100ppm for *E. coli.***
	E coli.		***S. aurens***		
	100	500	100	500	
113	+	+	-	-	14%
114	+	+	+	+	21%
115	+	+	-	-	14%
116	+	+	+	+	29%
117	+	+	+	+	31%

From **table-II** it is observed that most of Psora-Schiff bases show mild activity towards *E. coli.* at 500 ppm concentration except **94.** At 100 ppm concentration poor activity was observed for psora-Schiff bases except **94, 96** and **97.**

Psora-Schiff bases did not show antibacterial activity towards *S. aurens* except chloro compounds. At 500 ppm concentration chloro compounds **95, 99, 101** and **102** showed mild activity towards *S. aurens* whereas other Schiff bases did not show activity at all.

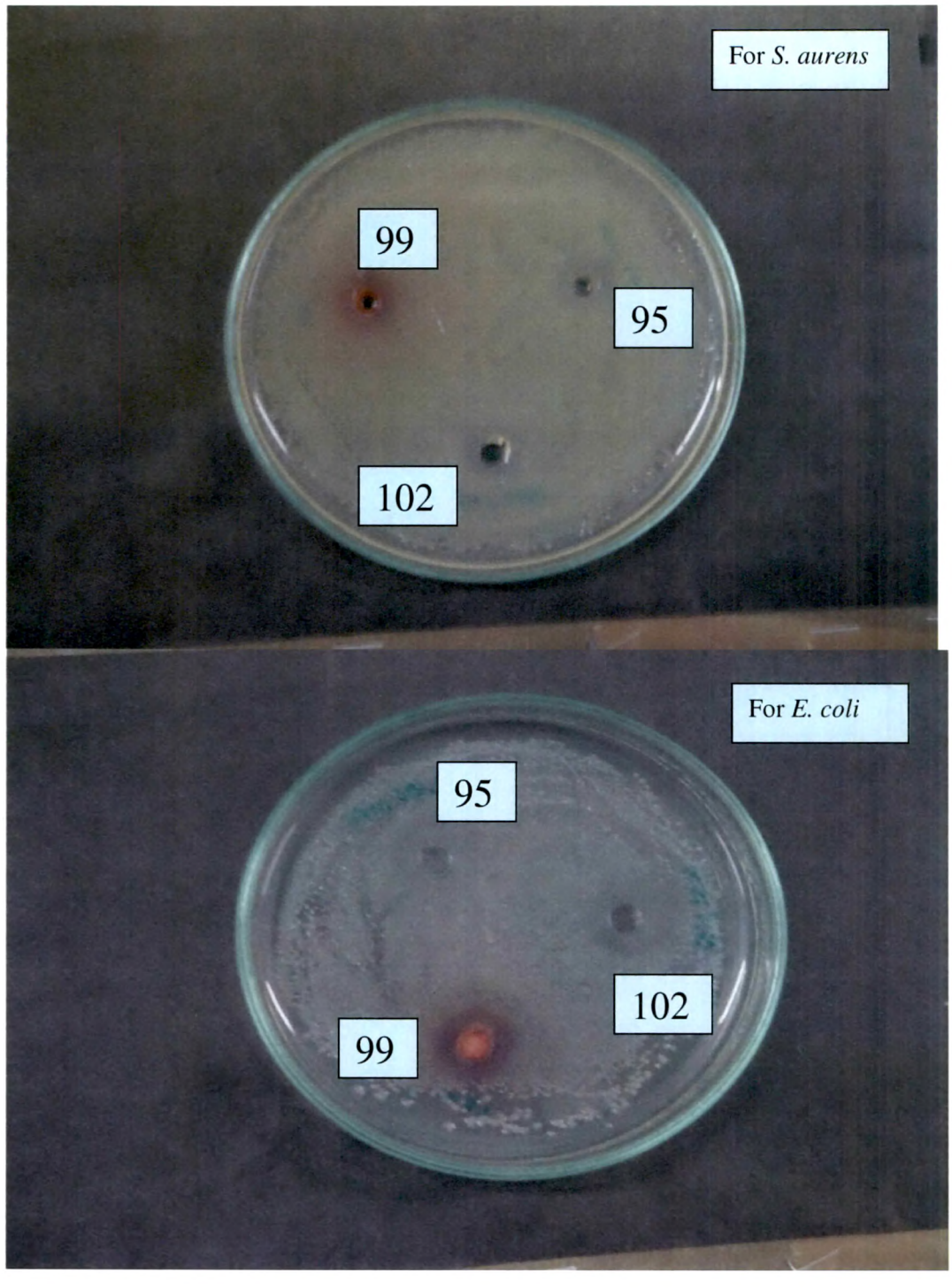
From table-III it is observed that all azetidinones showed good antibacterial activity towards *E. coli.* as well as *S. aurens*. However, azetidinones **113** and **115** did not show any activity towards *S. aurens.*

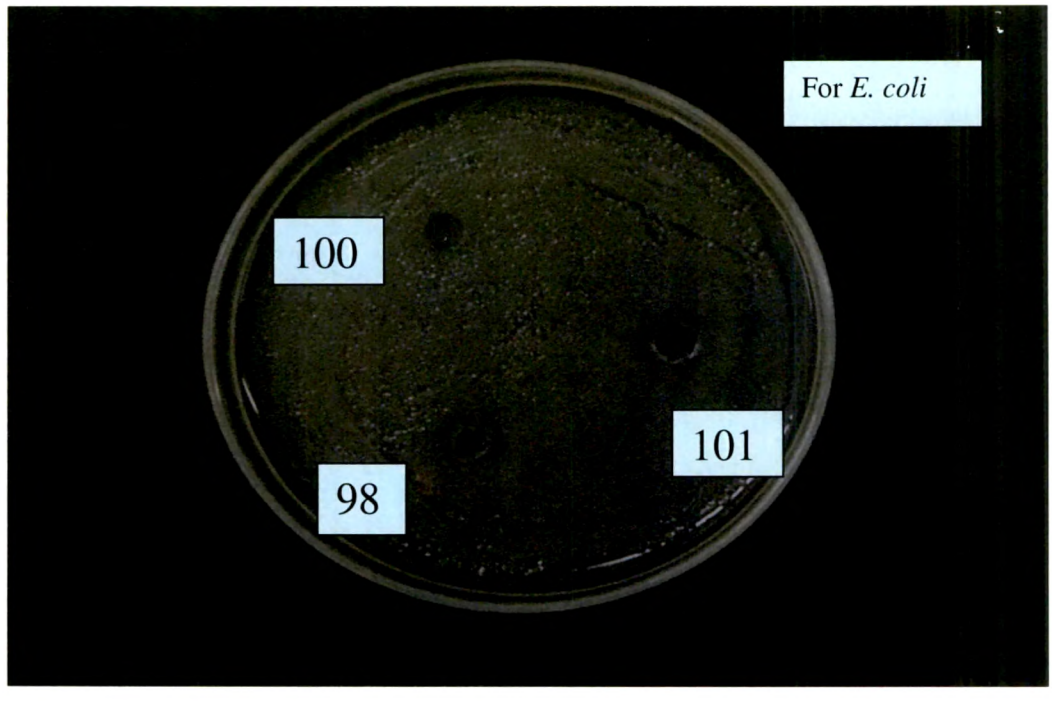
V.4 Experimental:

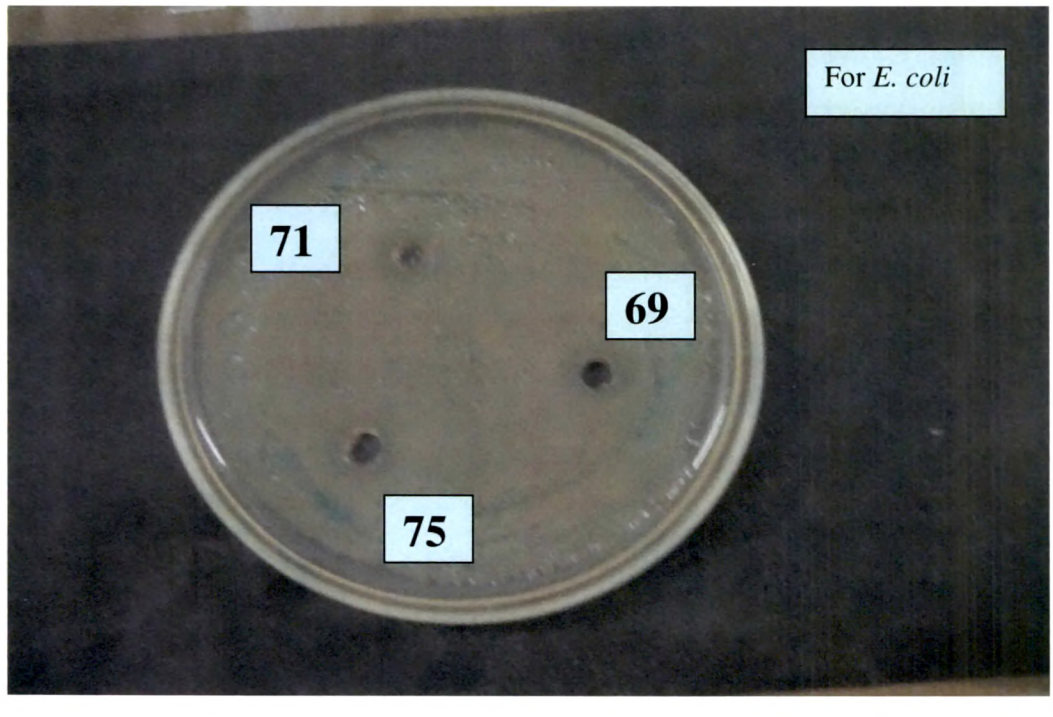
First of all, Luria Agar was subjected to autoclave followed by 20mL of its plating in Petri plate. To this 20mL of *E. Coli.* (DH 5 α) was spread above the Petri plate in stationary phase. The Agar with cup borer was cut to make the 'Wells' in the Petri plate and then 50mL solution of each of Aminopsoralens **67-78**, Schiff bases **94-102** as well as azetidinones **113-117** in concentrations of 100ppm, 500ppm were added to the 'Wells' and incubated at 37°C for 12 hours. The 'Zones of Inhibition' were analysed for each solutions.

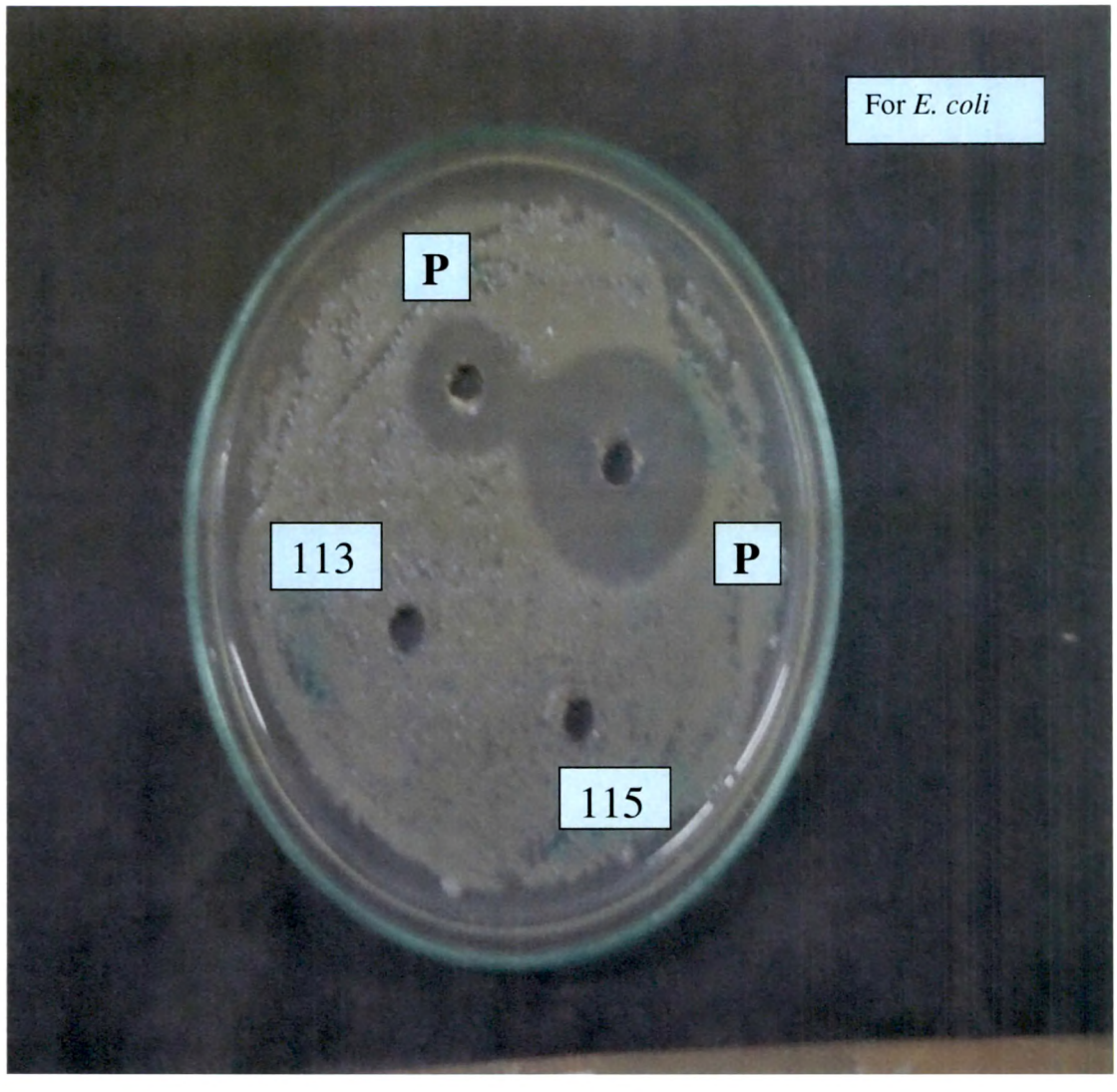
V.5 Conclusion:

The study of antibacterial activity of aminopsolens, psora-Schiff bases and psora-azetidinones reveals that these compounds show moderate activity towards E. coli (DH 5 α) micro organisms whereas they show poor activity towards S. aurens micro organisms. Psora-azetidinones showed good antibacterial activity which may prove fulfillment of new effective analogues of the drug history.


For *S. aurens*
99
95
102
For *E. coli*
95
102
99


For *E. coli*
100
101
98


For *E. coli*
71
69
75


For *E. coli*
P
113
P
115


For *E. coli*
113
116
117

V.6 References

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