Chapter- VI

Evaluation of anti-microbial activity of the synthesized compounds

Abstract

A few synthesized compounds were screened for their *invitro* antimicrobial activities against bacterial species S. *aureus* (MTCC 96) and E.*coli* (MTCC 1687) and fungal species F.*solani* (MTCC 350) and A.*niger* (MTCC 1344) by agar cup method against the standard drugs, streptomycin for bacteria and fluconazol for fungi and the results emanated from the study were discussed in the chapter.

6.1 General introduction

The screening of the newly synthesised derivatives against the microbes has been drawing renewed interest because of the generation of increasing number of multidrug resistant pathogens[1]. As these multidrug resistant strains has been proliferating, the design and synthesis of novel and antimicrobial molecule has become ineviteable to combat the deadly deseases.

s-Triazine has gained attention of synthetic chemists for their wide array of biological activities[2-7]. In view of its adaptable chemistry it was thought of interest to incorporate the bioactive pharmacophores such as azepines, pyrimidines, isoxazoles, pyrazoles, thiadiazoles, benzimidazoles, imidazoles and oxodiazoles into the molecular framework of s-triazine and to study the cummulative bioactivity of the newer homologues containing each of these moieties.

These condensed heterocyclic scaffolds have been identified in the literature as the most active pharmacophores in drug designing[8-12]. The incorporation of bioactive pharmacophores in the existing drug molecule has been known to exert a profound influence on the bioactivity of the molecules.

For the verification of this hypothesis a series of novel compounds were selected for their antimicrobial activity and those compounds which showed promising antimicrobial activity have been presented in this chapter.

The synthesis of these compounds has already been discussed in the **chapter II, III, IV, and V.** The present chapter deals with the results obtained by *in vitro* screening of the synthesised compounds for their antibacterial activity against S. *aureus* (MTCC 96) and E. *coli* (MTCC 1687) and antifungal activity against A. *niger* (MTCC 1344) and F. *solani*. (MTCC 350) The activity index and zone of inhibition were determined in comparison of the standard drugs 'streptomycin' and 'fluconazol' for bacteria and fungi respectively **Table-6.1 and graphs 6.1-6.12.**

6.2 Antibacterial activity:

6.2.1 Bacterial strain:

Pure culture of S. *aureus* (MTCC 96) and E. *coli* (MTCC 1687) bacteria were procured from the Microbilogy Research Laboratory, Department of Bioscience and Biotechnology, Banasthali University, Banasthali, for their *in-vitro* antibacterial activity.

6.2.2 Antibacterial studies:

Antibacterial activity of the newly synthesised compunds **1-12 (Fig.-1)** was determined by agar cup method[13]. DMSO was used as a diluent as it is ineffective to the growth of the microbes. 8mg/ml of the test compounds were dissolved in DMSO so as to make the necessary dilutions of 400μg/ml, 200μg/ml and 100μg/ml to form the stock solutions of the compounds to be tested.

6.3 Methodology:

6.3.1 Preparation of media:

Nutrient agar (28.0 g) was dissolved in a litre of distilled water in a tightly plugged conical flask. The *p*H of the media was adjusted to 6.8-7.4. The media along with the other glasswares were autoclaved at 15 lb pressure and 120 ^{0}C for half an hour. The media was then poured into the petridishes, it gradually solidified, and the holes were made in the plates by cork borer. Each time it was sterilized by heating it over the flame. The bacteria were inoculated with help of a micropipette and were spread by an L-shaped rod evenly over the surface of the agar. The test solutions of different concentrations were then filled in the cavity with the help of a micro-pippette; the plates were then properly sealed with the para film and placed in an incubator at 37 ^{0}C for 24 hr. All the work was done in Laminar air flow.

6.3.2 Analysis and interpretation of data:

After 24 hr it was observed that the test substance had diffused from the cavity through the solidified agar layer to an extent such that the growth of the microorganism was inhibited entirely in a circular zone around the cavity containing a solution of the drug. The plates were then analysed and the diameter of the zones of complete inhibition was measured in mm and was compared with the standard drug streptomycin.

6.4 Antifungal activity:

6.4.1 Fungal strains:

Pure cultures of A. *niger* (MTCC 1344) and F. *solani* (MTCC 350) were procured from the Microbilogy Research Laboratory, Department of Bioscience and Biotechnology, Banasthali University, Banasthali, for their *in-vitro* antifungal activity.

6.4.2 Antifungal studies:

Antifungal activity of the newly synthesised compounds **1-12 (Fig.-1)** was determined by cup-plate method**.** DMSO was used as a diluent as it is ineffective to the growth of the microbes. 8mg/ml of the test compounds were dissolved in DMSO so as to make the necessary dilutions of 400µg/ml, 200µg/ml and 100µg/ml to form the stock solutions of the compounds to be tested.

6.5 Methodology:

6.5.1 Preparation of media:

Potato dextrose agar (40.0 g) was dissolved in a litre of distilled water in a tightly plugged conical flask. The *p*H of the media was adjusted to 6.8-7.4. The media along with the other glasswares were autoclaved at 15 lb pressure and 120 ^{0}C for half an hour. The media was then poured into the petridishes, it gradually solidified, and the holes were made in the plates by cork borer. It was each time sterilized by heating it over the flame. The test solutions of different concentrations were then filled in the cavity with the help of a micro-pippette. The Fungi were inoculated with help of a wire loop on the surface of the agar; the plates were then properly sealed with the para film and covered by aluminium foil and kept in dark at a warm place for 3-4 days. All the work was done in Laminar air flow.

6.5.2 Analysis and interpretation of data:

After 3-4 days, it was observed that the test substance had diffused from the cavity through the solidified agar layer to an extent such that the growth of the microorganism was inhibited entirely in a circular zone around the cavity containing a solution of the drug. The plates were then analysed and the diameter of the zones of complete inhibition was measured in mm and was compared with the standard drug fluconazol.

6.6 Results and discussion:

6.6.1 Antibacterial activity at [MIC] (400-100µg/ml)

Antibacterial screening showed the following order of activity for the compounds **1-12** as compared to the standard drug streptomycin.

The antibacterial screening against S. *aureus* showed that compound **7** (pyrazolo derivative) showed the highest activity (98.5%), compounds **1,5,6,10,11** and **12** were found to be significantly active and compound **2,3,4,8** and **9** were found to be moderately active.

Whereas amongst the compounds **1-12** tested against E. *coli*, most of the compounds showed significant activity. **4,5,10** and **11** showed the highest activity; **8,9** and **12** were found to be significantly active; **2** and **6** were found to be moderately active; rest of the compounds were found to be less active.

Conclusion:

Following conclusion can be drawn on comparing the antibacterial activity of the synthesised compounds:

- No zone of inhibition was observed for DMSO
- For both the strains highest activities were observed at 400µg/ml.
- S. *aureus* showed the following order of antibacterial activity for the compounds 1-12.
  7>6>11>1 =10>12>5>9>4>3>2>8
- E. *coli* showed the following order of antibacterial activity for the compounds 1-12.
  11 > 4 = 10 >5>8>9>12>2>6>1>3>7
- The pyrimido derivative showed the minimum activity and the pyrazolo derivative showed the maximum activity against S. *aureus*.
- The pyrazolo derivative showed the minimum activity and the benzoxazepino derivative showed the maximum activity against E. *coli*.

6.6.2 Antifungal activity at [MIC] (400-100µg/ml)

Antifungal screening showed the following order of activity amongst the compounds **1-12** as compared to the standard drug fluconazol.

The antifungal screening against A. *niger* showed that compound **12** (benzothiazepine derivative) showed the highest activity (98.9%), compounds **1,3** and **11** were found to be significantly active and the remaining compounds were found to be moderately active.

Whereas amongst the compounds 1-12 tested against F. *solani*, most of the compounds showed significant activity. **3,5** and **11** showed the highest activity; compound **7** and **4** were found to be moderately active; compound **12** (benzothiazepine derivative) was found to possess highest activity (98.9%) amongst all. The rest of the compounds were found to be less active.

Conclusion:

Following conclusion can be drawn on comparing the antifungal activity of the synthesised compounds:

- No zone of inhibition was observed for DMSO
- For both the strains highest activities were observed at 400µg/ml.
- Aspergillus *niger* showed the following order of antifungal activity for the compounds 1-12.
  12>1>11=3 >2=4>10>>5>8>7>6>9
- Fusarium *solani* showed the following order of antibacterial activity for the compounds 1-12.
  12 > 11>5>10>7>4= >8>6>9>2>3>1>2
- The pyrimido derivative showed the minimum activity and the benzothiazepino derivative showed the maximum activity against A. *niger*.
- The imidazo derivative showed the minimum activity and the benzothiazepino derivative showed the maximum activity against F. *solani*.

Hence from the above antimicrobial studies it can be concluded that azepine derivatives showed the maximum activity for both antibacterial and antifungal pathogens.

Structures of the synthesized compounds whose antimicrobial screening is described in this chapter:

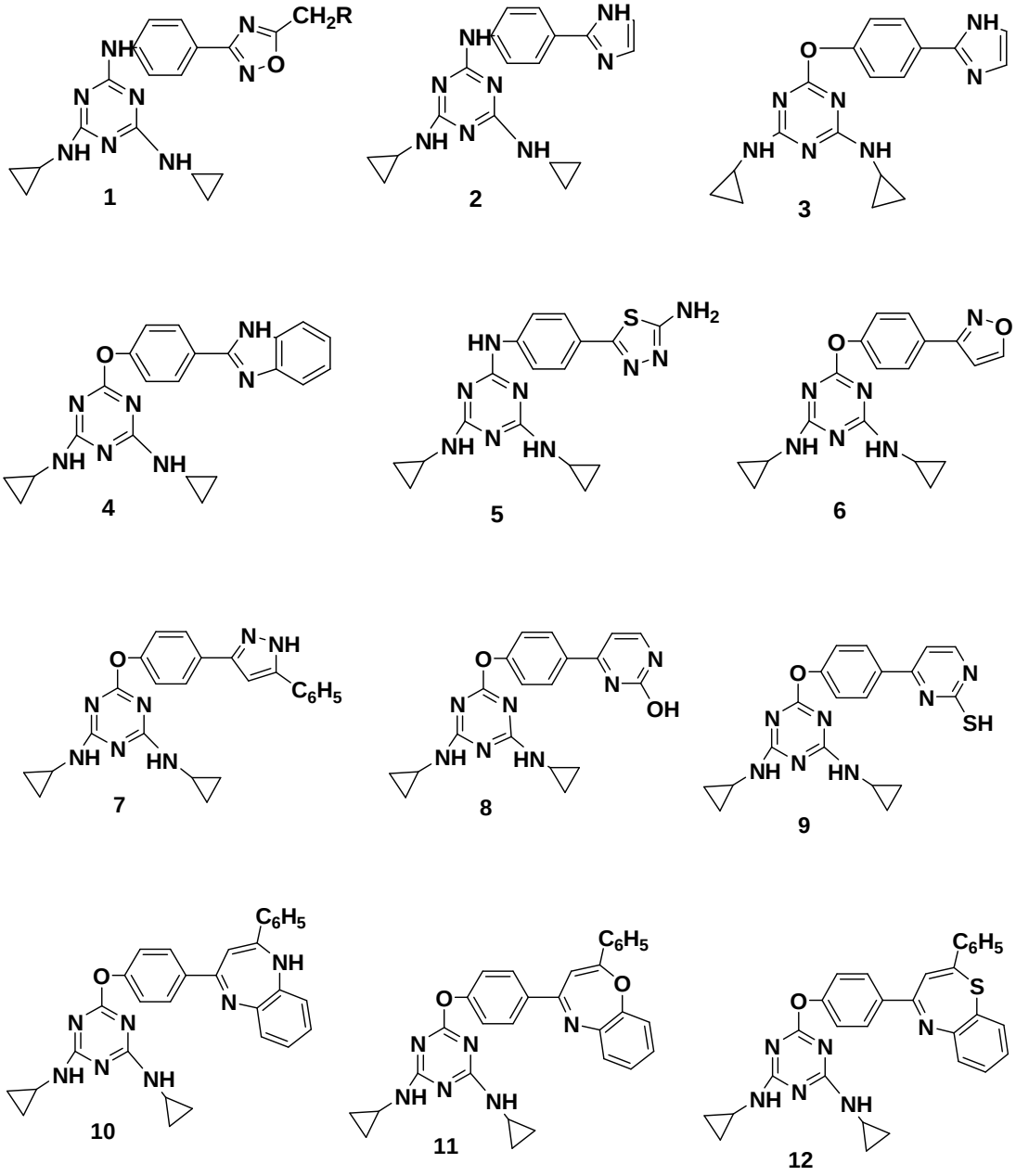
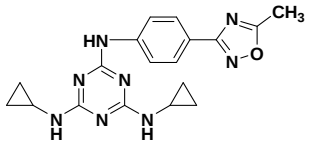
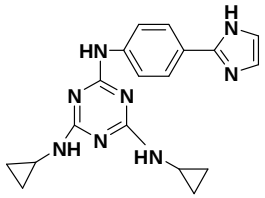
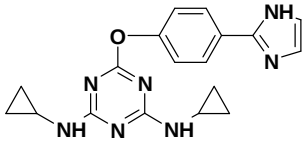
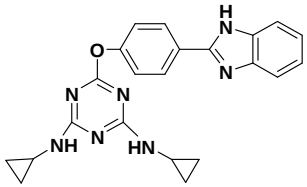
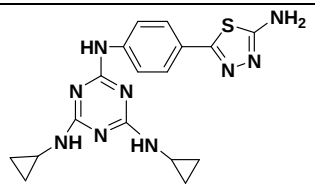
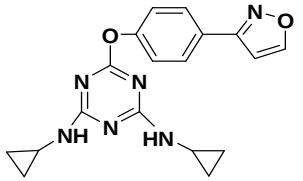
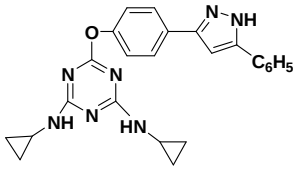
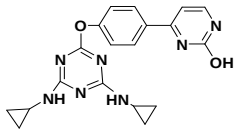
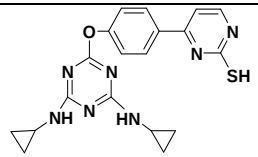
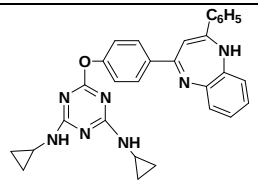
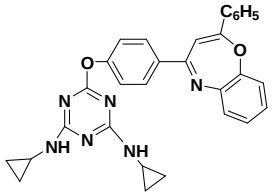


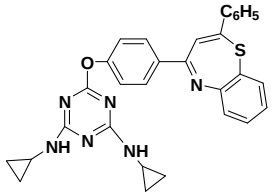
Fig.-6.1

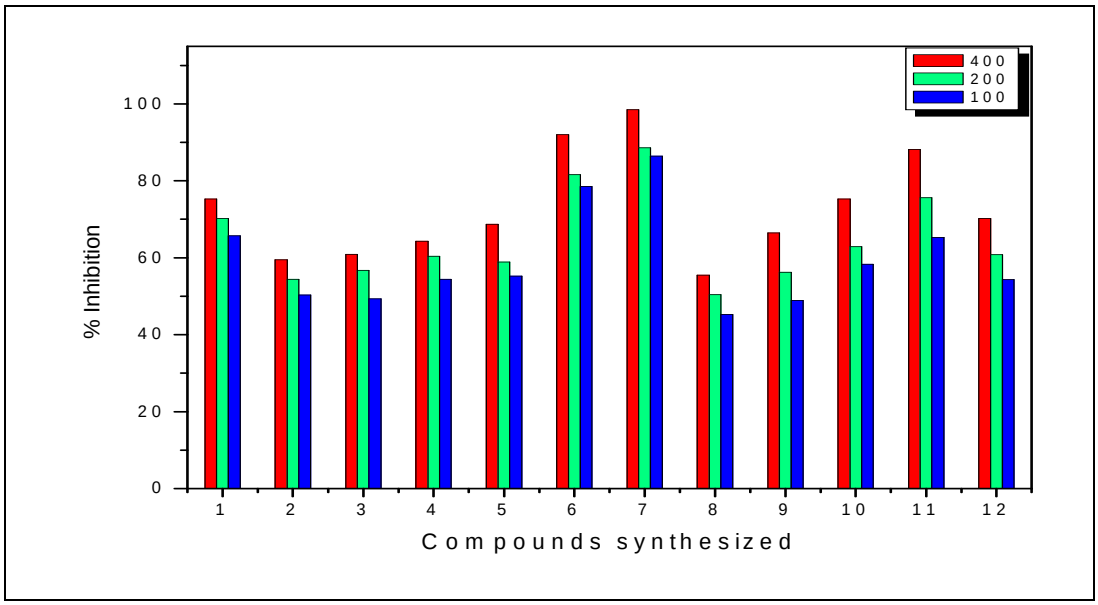
Table: 6.1 Antibacterial and antifungal activity of compounds 1-12

S. No.	Comp. No.	Compd. Str.	Conc. (µg/ml)	*Staphylococcus aureus*		*E. coli*		*Aspergillus niger*		*Fusarium solani*	
				Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard
1.	**1**		400 200 100	18.8 14.0 09.8	75.3 70.2 65.7	14.9 9.4 5.7	62.2 52.4 48.3	22.5 17.6 12.3	90.3 80.2 72.4	12.5 10.6 9.8	50.2 48.3 44.7
2.	**2**		400 200 100	14.8 10.8 07.5	59.5 54.4 50.3	15.6 10.9 6.6	65.2 60.9 55.8	18.0 15.0 10.3	72.3 68.4 60.9	14.8 12.1 11.5	59.5 55.4 52.3
3.	**3**		400 200 100	15.2 11.3 7.3	60.9 56.7 49.3	14.4 10.0 5.8	60.3 55.7 48.6	21.4 17.6 12.7	85.6 80.3 75.2	13.8 11.0 10.6	55.4 50.2 48.3

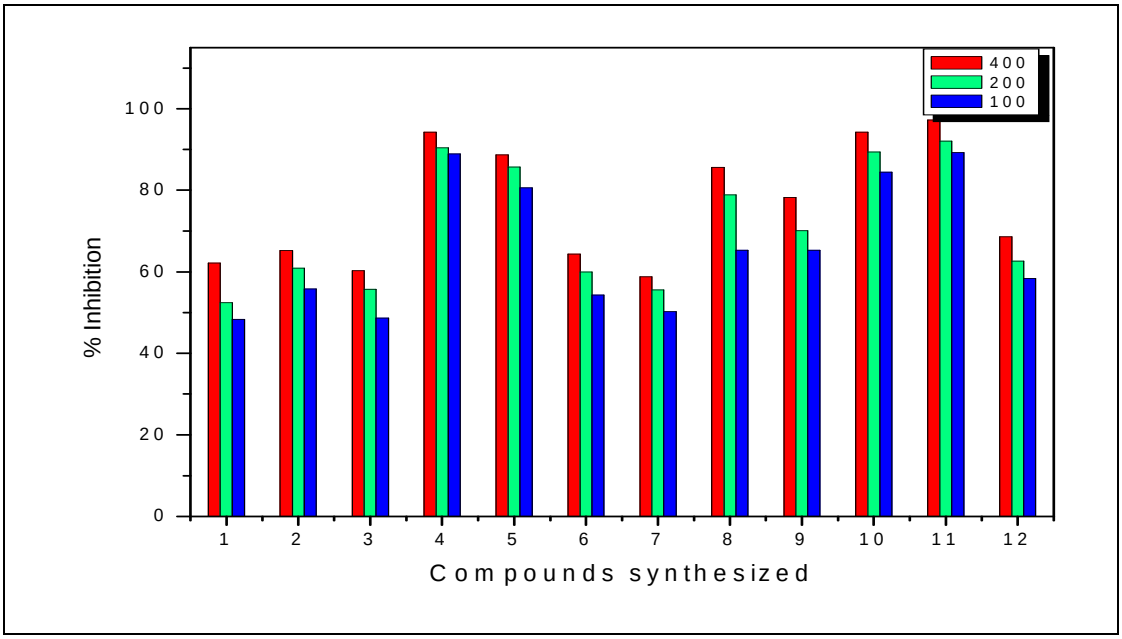
S. No.	Comp. No.	Compd. Str.	Conc. (µg/ml)	*Staphylococcus aureus*		*E. coli*		*Aspergillus niger*		*Fusarium solani*	
				Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard
4.	**4**		400 200 100	16.0 12.0 8.2	64.3 60.4 54.9	22.6 16.2 10.6	94.3 90.4 88.9	18.0 14.8 10.2	72.3 67.5 60.3	17.2 14.2 13.3	68.9 64.8 60.6
5.	**5**		400 200 100	17.1 11.7 8.2	68.7 58.9 55.3	21.2 15.4 9.6	88.7 85.7 80.6	15.8 13.2 9.18	63.4 60.0 54.0	19.6 15.9 15.1	78.5 72.6 68.7
6.	**6**		400 200 100	23.0 16.3 11.7	92.0 81.6 78.6	15.4 10.8 6.5	64.3 60.0 54.3	12.6 10.6 6.9	50.6 48.5 40.6	15.6 13.0 12.2	62.4 59.3 55.5
7.	**7**		400 200 100	24.6 17.7 12.9	98.5 88.6 86.4	14.1 10.0 11.0	58.8 55.6 50.2	13.5 11.0 7.6	54.2 50.3 45.2	17.6 14.3 12.4	70.6 65.3 56.7

S. No.	Comp. No.	Compd. Str.	Conc. (µg/ml)	*Staphylococcus aureus*		*E. coli*		*Aspergillus niger*		*Fusarium solani*	
				Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard
8.	**8**		400 200 100	13.8 10.0 6.7	55.5 50.4 45.3	20.5 14.1 7.8	85.6 78.8 65.3	15.1 12.2 8.6	60.4 55.5 50.6	16.4 13.3 12.8	65.6 60.7 58.2
9.	**9**		400 200 100	16.6 11.2 7.3	66.5 56.2 48.9	18.7 12.6 7.8	78.2 70.1 65.3	12.0 9.7 6.9	48.2 44.4 40.9	15.0 12.4 11.0	60.3 56.4 50.3
10.	**10**		400 200 100	18.8 12.5 8.7	75.3 62.9 58.3	22.6 16.0 10.1	94.3 89.4 84.4	16.3 13.7 9.5	65.4 62.3 56.4	16.1 15.2 14.3	73.4 69.3 65.4
11.	**11**		400 200 100	22.0 15.1 9.7	88.2 75.6 65.3	23.3 16.5 10.7	97.2 92.0 89.2	21.4 17.6 12.8	85.6 80.3 75.4	21.1 17.6 16.6	84.6 80.2 75.9

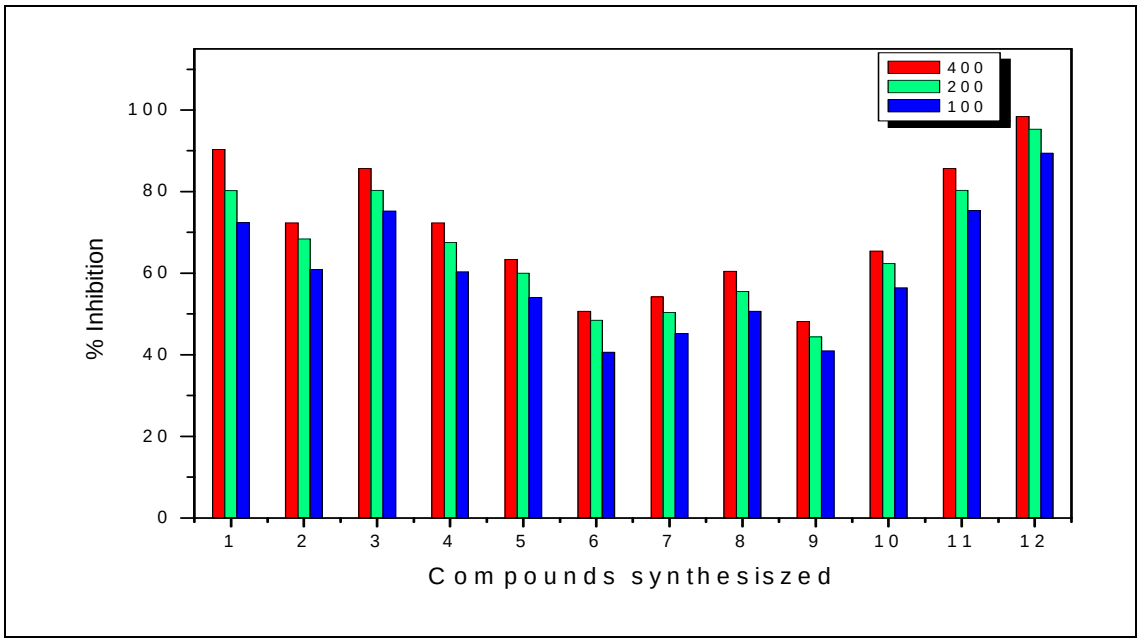
S. No.	Comp. No.	Comp. Str.	Conc. (µg/ml)	*Staphylococcus aureus*		*E. coli*		*Aspergillus niger*		*Fusarium solani*	
				Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard	Zone of inhibition	% activity compared to the standard
12.	**12**		400 200 100	17.5 12.1 8.1	70.2 60.8 54.3	16.4 11.2 7.0	68.6 62.6 58.4	24.6 20.9 15.1	98.4 95.3 89.4	24.7 21.2 19.7	98.9 96.5 89.9
13.		**Streptomycin (std. Antibacterial)**	400 200 100	25 20 15	100 100 100	24 18 12	100 100 100	----	----	----	----
14.		**Fluconazol (std. Antifungal)**	400 200 100	----	----	----	----	25 22 17	100 100 100	25 22 22	100 100 100



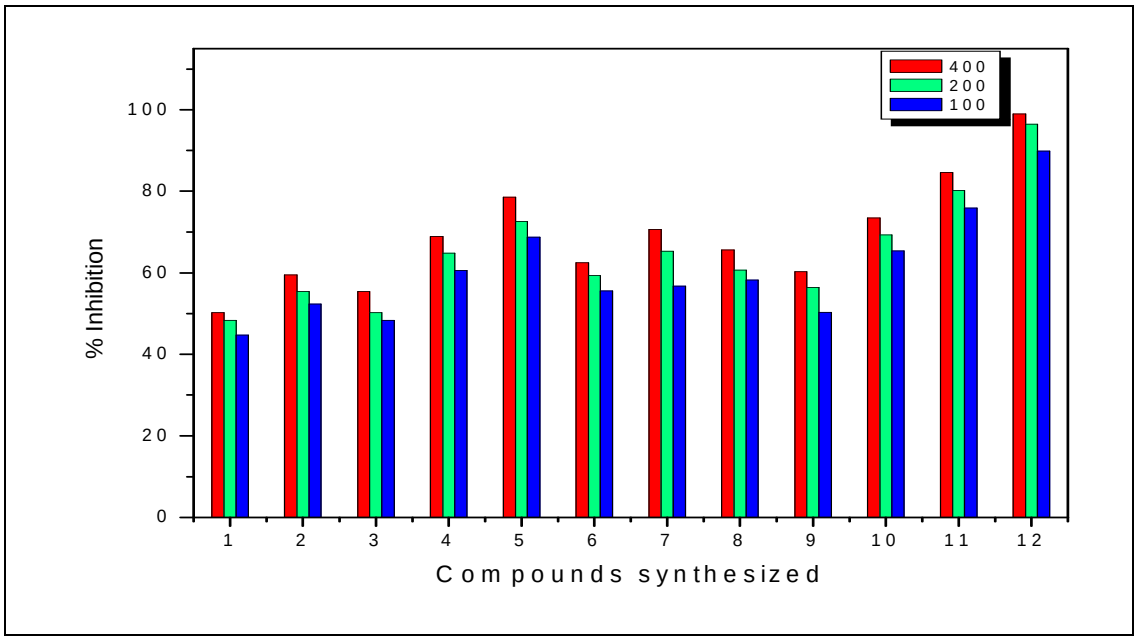
6.1: Antibacterial activity of synthesized compounds against S. *aureus* at 400μg/ml, 200μg/ml and 100μg/ml.



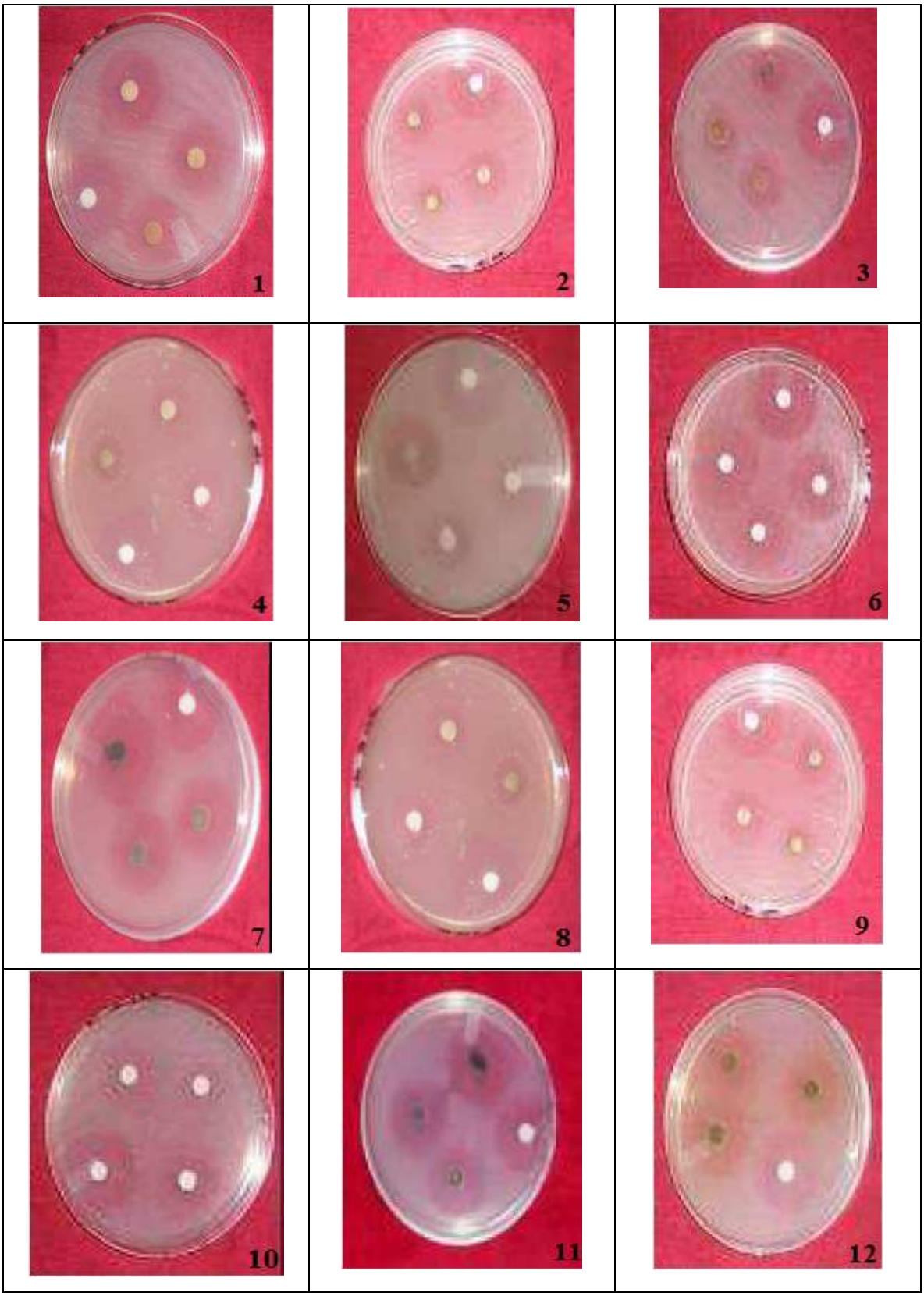
6.1: Antibacterial activity of synthesized compounds against E. *coli* at 400μg/ml, 200μg/ml and 100μg/ml.



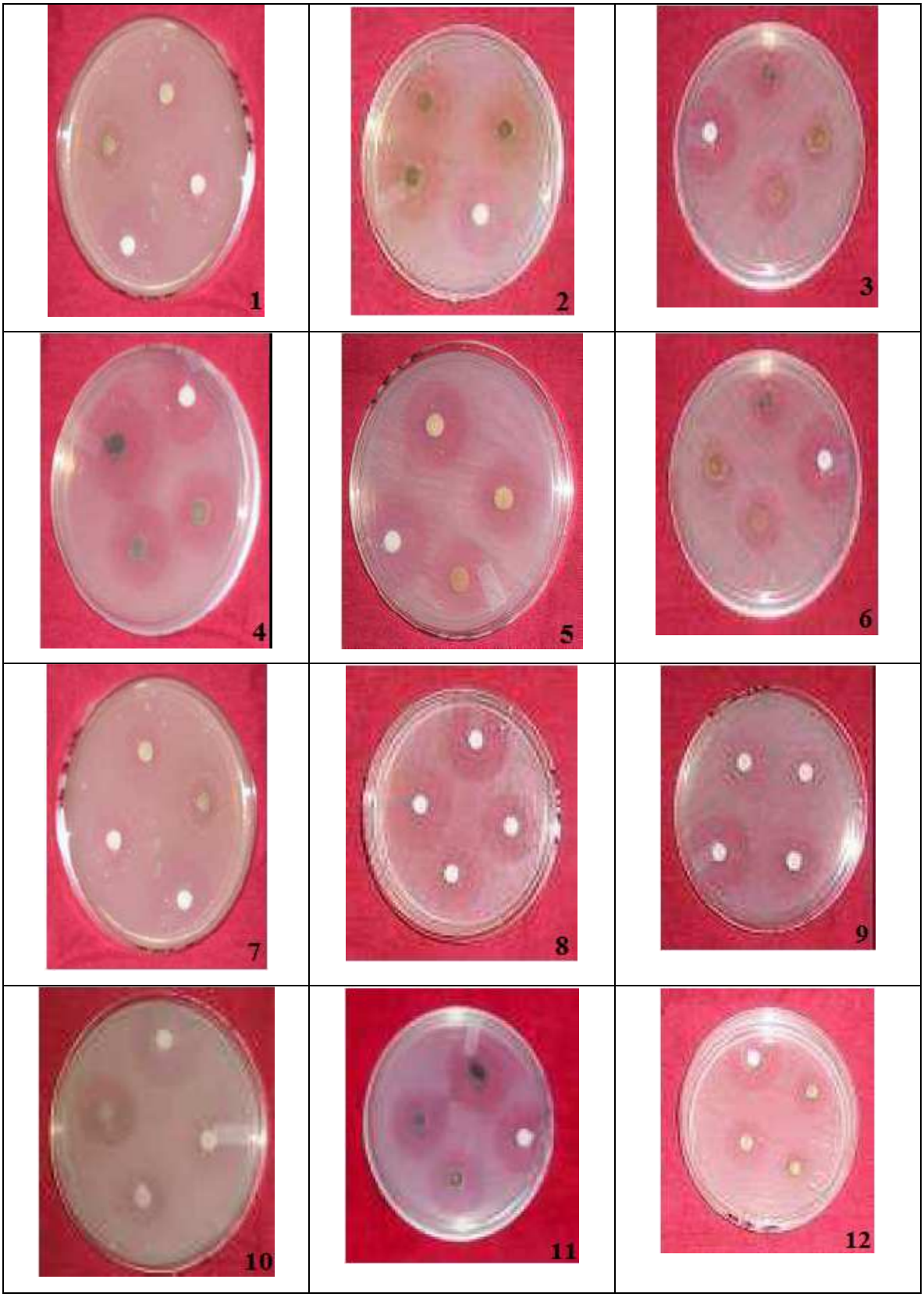
6.1: Antifungal activity of synthesized compounds against A. *niger* at 400μg/ml, 200μg/ml and 100μg/ml.



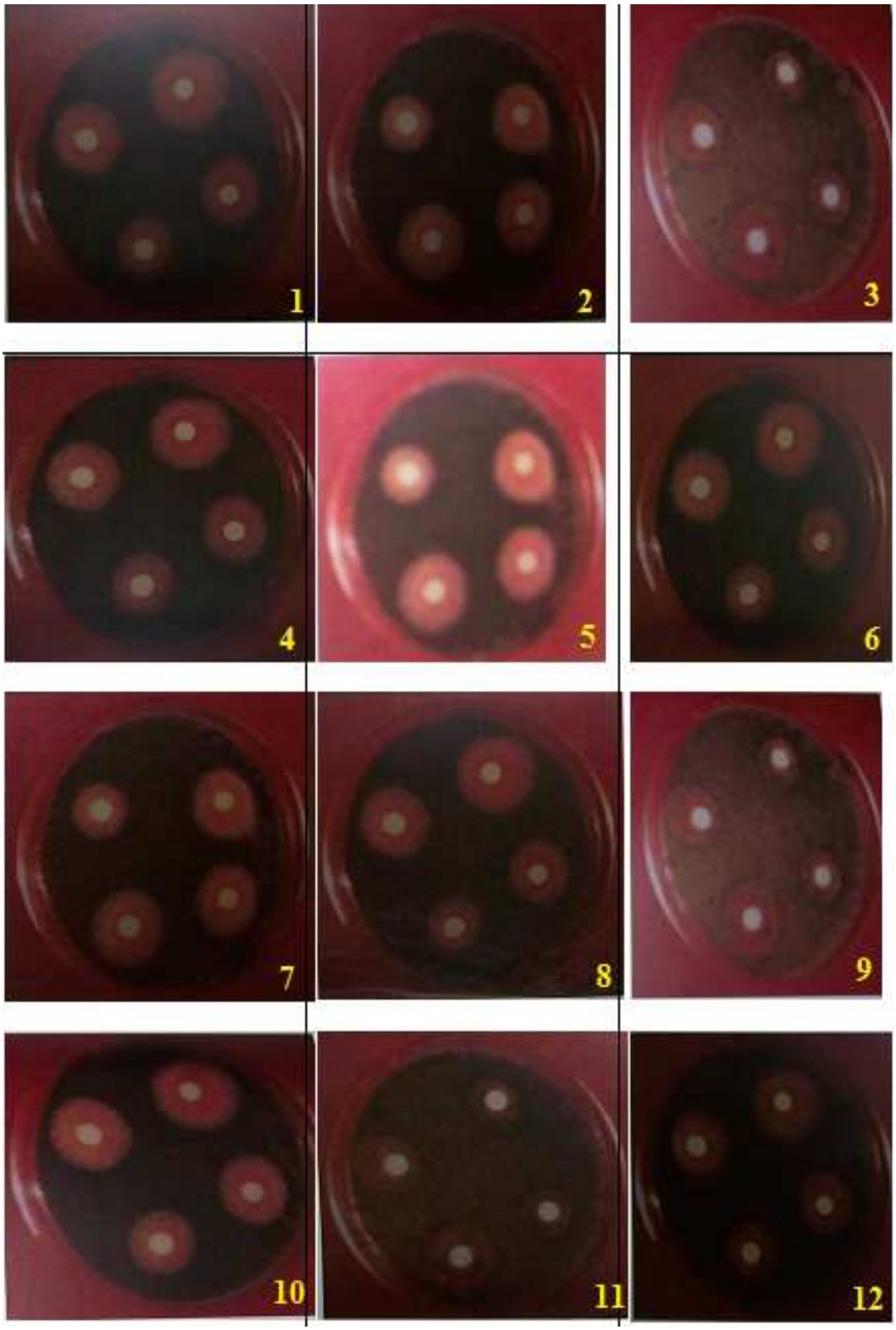
6.1: Antifungal activity of synthesized compounds against F. *solani* at 400μg/ml, 200μg/ml and 100μg/ml.



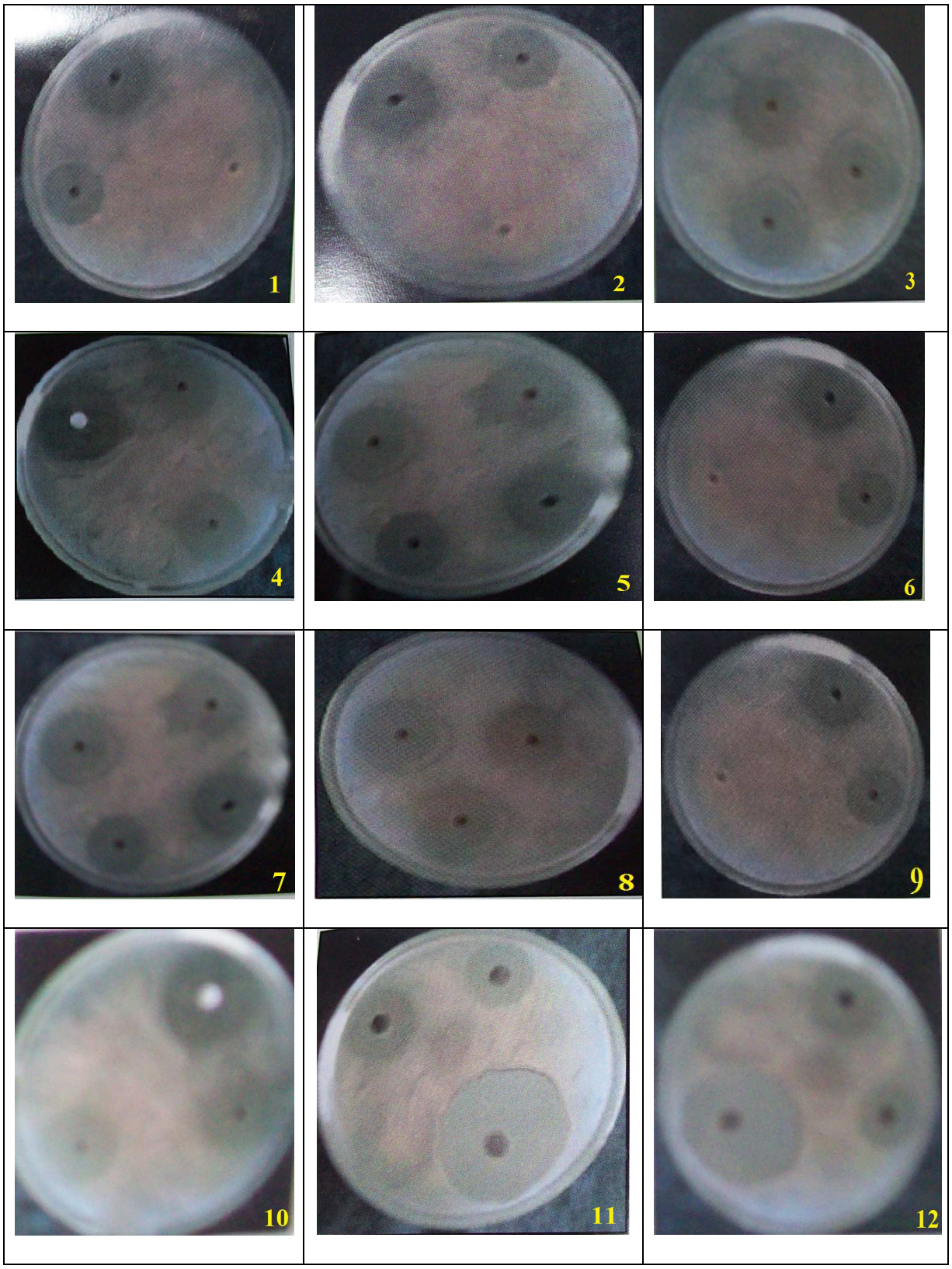
Antibacterial activity of compounds (1-12) against S. *aureus*



Antibacterial activity of compounds (1-12) against E. *coli*



Antifungal activity of compounds (1-12) against A. *niger*



Antibacterial activity of compounds (1-12) against F. *solani*

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