Heterocyclic Letters
Vol. 12| No.3|553-562|May-July|2022
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

SYNTHESIS AND ANTIMICROBIAL ACTIVITY SCREENING OF POLYHYDROQUINOLINE, 4H-PYRAN, THIAZOLIDINEDIONE AND PYRIMIDO [4, 5-D] PYRIMIDINE COMPOUNDS

S. V. Padghan[*1], B. K. Magar[2], M. U. Chopade[3]

[1, 3] *Department of Chemistry, Sant Dnyaneshwar Mahavidyalaya, Soegaon, Dist-Aurangabad*
[2] *Department of Chemistry, Shivaji Arts, Commerce and Science College, Kannad.*

Abstract
In this paper, we present the antimicrobial activity of a series of compounds which contain polyhydroquinoline, 4H-Pyran, thiazolidinedione and pyrimido [4, 5-d] pyrimidine moieties in their molecule. The antimicrobial activity of these compounds was tested against the following microbial strains as a control; Staphylococcus aureus (ATCC 25922), Escherichia coli (ATCC 25922), Candida albicans (ATCC 10231) Aspergillus niger (ATCC 96422) by Agar well diffusion method. Comparing the values of the minimum inhibitory concentrations of the compounds it was noticed that, polyhydroquinoline, 4H-Pyran, thiazolidinedione and pyrimido[4,5-d]pyrimidine had in general the best antibacterial and antifungal activity against standard drug Streptomycin, Flucanazole and Griseofulvin.

Keywords: polyhydroquinoline, 4H-Pyran, thiazolidinedione and pyrimido[4,5-d]pyrimidine, antimicrobial activity.

Introduction

In spite of increasing the availability of drugs and vaccines to treat, many of the infectious diseases are caused by pathogenic micro organisms like bacteria, fungi, viruses etc[i]. In the past decade, due to increase in the rate of infectious diseases there is a demand and need for the development of novel biologically active heterocyclic compounds. The diseases caused due to the pathogenic micro organism may be treated by employing the inhibitory chemicals which kills or prevent the growth of such micro organisms are called antimicrobial agents. Depending upon the spectrum of activity, the antimicrobial agents may be classified as germicides which kill micro organisms and micro-biostatic agents which inhibits the growth of pathogens. The sixteenth century considered the diseases caused due to the use of restored chemical substances. A huge amount of research has been done to overcome the infections caused due to the bacteria and fungi. In attempt to contribute to this type of research, the antimicrobial[ii-v] action of synthesized compounds can be determined by screening them against the pathogenic micro organisms like *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 96422).

In the 20th century the antimicrobial and antifungal drugs plays an important role in medicinal field. In early times, these antimicrobial and antifungal drugs have been used as remedies for many diseases. The biological activity of antimicrobial agents can be measured in different ways. It can be measured in degree or % of inhibition in vitro when the critical site and mechanism of action is known.

The mode of action of antibiotics is different particularly it acts by the inhibition of protein synthesis, DNA synthesis, bacterial cell wall synthesis and folic acid biosynthesis. There are number of clinical drawbacks of antibiotics as it becomes less effective due to the resistance of micro organisms. To overcome the drawbacks of resistance the new antibiotics agents should consists of different chemical chacteristics which differ from existing agents. The heterocyclic compounds containing N, O and S hetero atoms plays vital role in the process of drug designing. The electron rich nitrogen and sulphur heterocycles shows diverse biological activities.

In the last 25 years, the frequency of life threatening infections has been increased with increase in the number of potentially offensive species. Some of the fungal infections are known to show high rate of morbidity and mortality. Most of the infectious diseases have morbidity, mortality, immune compromised and many health related issues. Thus, in order to overcome such type of issues the pharmacological industries may have challenge to develop novel drugs which plays significant role as a therapeutic agent. Microorganisms, often known as microbes, are ubiquitous in nature. Microorganisms that include bacteria, fungi, protozoa, algae and other microbes e.g. viruses. The following classes are used to categorize these groups.

Bacteria are unicellular organisms that live on all living and non living things at temperatures ranging from below zero degrees Celsius (Psychrophiles) to over 100 degree Celsius (thermopiles). Bacteria are classed based on the form of the cell, the temperature at which they develop the number of the cells in a group, a pathogen city and staining. They are further categorized as Gram positive (gram +ve) or Gram negative (gram -ve) based on staining. According to the gram staining procedure developed by C. Gram, the Gram positive bacteria shows violet colour and Gram negative bacteria shows pink colour. Depending upon the pathogenecity, the bacteria are classified as pathogenic i.e. disease or infection causing and non pathogenici. e. non infectious.

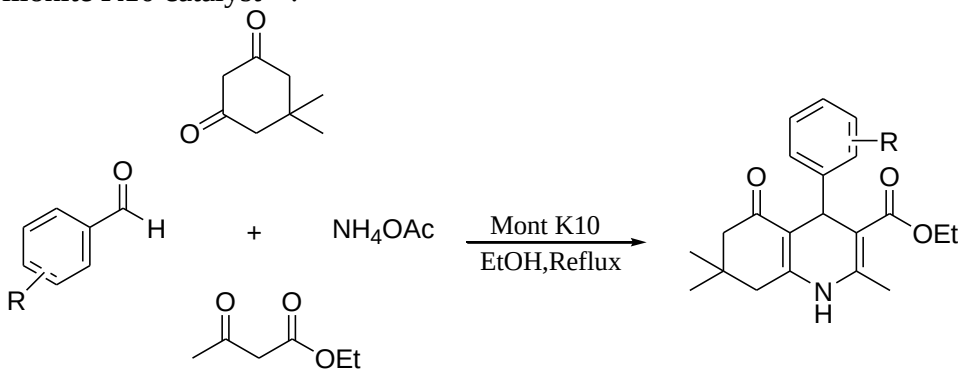
In many nations throughout the world, the frequency of life-threatening infectious diseases caused by multi drug resistant Gram positive and Gram negative pathogen bacteria has increased dramatically in recent years[vi-vii]. In some locations, such as the Indian subcontinent, parts of South America and the tropical portion of Africa, enteric bacterial infection is the leading cause of morbidity and mortality[viii-ix]. Millions of people are killed each year by bacteria of various Gram positive and Gram negative types. Food poisoning, rheumatoid arthritis, salmonellosis and diarrhea are the most common illness caused by these bacteria[x]. Antibiotics are thus cornerstone of microbial (bacterial and fungal) infection treatment. Antibiotic misuse, on the other hand, has become a major contributor in the formation and spread of multi-drug resistance populations of numerous bacteria[xi]. Furthermore, the available pharmacological medicines are either prohibitively expensive or have unfavorable side effects or contradictions[xii]. As a result, given the evidence of rapid global spread of resistant clinical isolates, finding novel antimicrobial medicines is critical. The classification is crucial and valid in medicinal chemistry, as it aids in the design, synthesis and development of medications.

Synthesis of polyhydroquinoline, 4H-Pyran, thiazolidinedione and pyrimido [4, 5-d] pyrimidine compounds.

In present work, we have investigated the greener method for the multi component synthesis of polyhydroquinoline, 4H-Pyran, thiazolidinedione and pyrimido [4, 5-d] pyrimidine compounds.

a) Scheme I

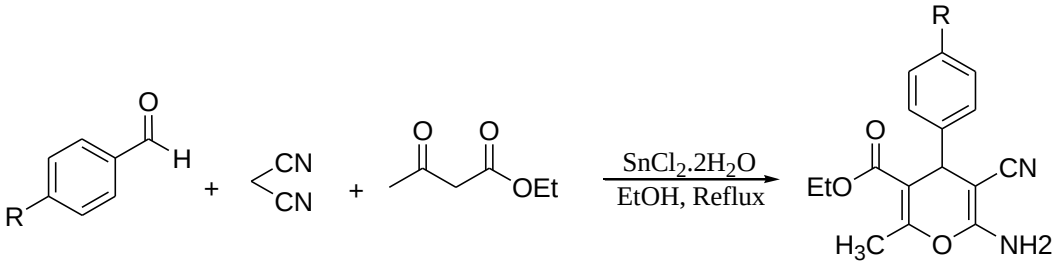
We have synthesized 4-substituted derivatives of 2,7,7-Trimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylic acid ethyl ester by using multi component reaction of substituted aldehyde, ethyl acetoacetate and ammonium acetate in ethanol in presence of Montmorillonite K10 catalyst[xiii].



Scheme I

b)Scheme II

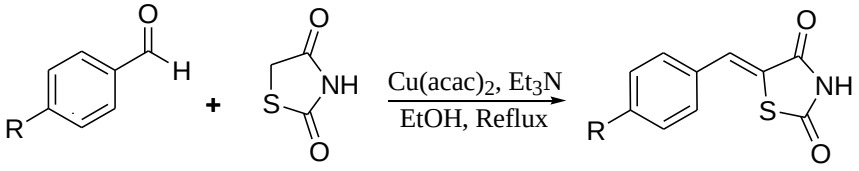
We have carried out the three pot multi component synthesis of 4-substituted derivatives of 6-amino-5-cyano-2-methyl-4-phenyl-4H-Pyran-3-carboxylic acid ethyl ester by the reaction of mixture of substituted aldehyde, malononitrile and ethyl acetoacetate in presence of stannous chloride[xiv].



Scheme-II

c)Scheme III

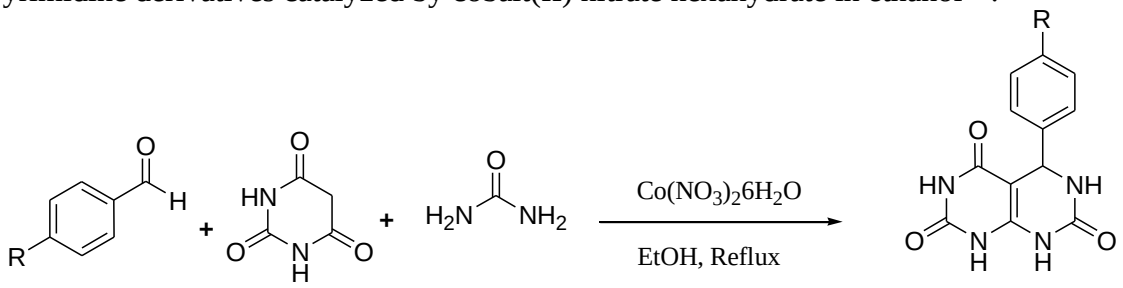
We have synthesized 5-arylidene-2,4-thiazolidinedione by the Knoevenagel condensation of aromatic aldehyde and 2,4-thiazolidinedione catalyzed by copper acetylacetonate[xv].



Scheme-III

d)Scheme IV

We have carried out the three pot multi component synthesis of pyrimido[4,5-d]pyrimidine derivatives catalyzed by cobalt(II) nitrate hexahydrate in ethanol[xvi].



Scheme-IV

Antimicrobial Activity

Materials and Methods

The bacterial pathogenic species or strain Staphylococcus aureus (ATCC 6538), Escherichia coli (ATCC 25922) while fungal species or strain Candida albicans (ATCC 10231) Aspergillus niger (ATCC 96422) Flucanazoles Griseofulvin, Streptomycin were procured from Sigma-Aldrich Co. Muller Hinton ager powder Macfarland standard agar were purchased from (Himedia laboratories Pvt. Ltd, Mumbai).

Preparation of culture media

Panel of test bacteria selected for study included gram negative microorganisms such as Escherichia coli (ATCC25922) and gram positive microorganism included Staphylococcus aureus (ATCC6538). Twenty four hour old culture of bacterial test pathogens was obtained by streaking a loopful of bacterial culture on a fresh nutrient agar slant and incubated for 24 h at 37^{o}C.

Preparation of Inoculums

a) Antibacterial Activity

A loopful of bacterial growth was inoculated into sterile saline so as to obtain sterile suspension. The resulting bacterial suspension (0.1ml) containing approximately 10^6cells/ml, were uniformly spread on sterile Muller Hinton Agar plates with the help of sterile swabs. Antibacterial activity of crude extract was determined by well diffusion method in which wells are prepared using 6mm diameter sterile cork borer. 100 µl of crude extract dissolved in DMSO were loaded in well, streptomycin (50µg/ml) was used as positive control and solvent ethyl acetate was used as negative control. Three replicates were maintained in each case. Plates were kept in refrigerator for 15min to facilitate the diffusion and then transferred to incubator at 37^{0}C for 24 h after incubation, the plates were observed the inhibition of bacterial growth around the wells inoculated with test extracts. The diameter of zone of inhibition around well was measured and compared with the control and standard.

We have used the Agar well diffusion method for assessment of the antimicrobial activity of newly synthesized compounds. On Muller-Hinton Agar medium zone of inhibition were observed and zone diameter was recorded in mm against specific test microorganism.

b) Antifungal Activity

Muller Hinton agar plates were added with 0.1mL of inoculums of respective bacteria and fungi wells (10 mm diameter) were made in each plate using alcohol sterilized cork borer. The newly synthesized compounds were added individually into each well at the concentration of 100 µg/ml in DMSO. The plates were allowed to pre diffuse in refrigerator for 30 minutes and were incubated at 37^{0}C for 24 hrs for bacterial pathogens and 28^{0}C for 48-72 hrs for the fungal

pathogens. The diameter of the zone of inhibition (mm) was measured and the activity was determined.

Result and Discussion:

The synthesized compounds were accessed antimicrobial activity particularly antibacterial and antifungal activity. The antibacterial activity against gram positive Staphylococcus aureus bacteria and gram negative bacteria are Escherichia coli using slandered drug Streptomycin. The antifungal activity screened against Candida albicans and Aspergillus niger against standard drug Flucanazole and Griseofulvin.

The zone of bacterial growth inhibition with these chemicals was close to the slandered. The minimum inhibitory concentration (MIC, g/mL) was determined and compared to the control; the MIC values for the compounds examined are listed in the tables below. The synthesized compounds **A2, A3, A8, A11, B2, B4, B5, B13, C3, C4, C8, C12, C14, D3, D4**, **D8, D12, D14,** and **D17**shows good antibacterial activity against Staphylococcus aureus as compared to standard drug Streptomycin. The compounds **A2, A3, A4, A9, A13, B4, B5, C3, C4,C9, C17, D2, D4, D9, D14** and **D17** showed antibacterial activity against Escherichia coli as compared to standard drug Streptomycin.

The synthesized compounds **A2, A3, A4, A12, B3, B4, B5, B13, C3, C4, C10, C14, C15, D2, D3, D7, D15** and **D16**shows good zone of inhibition against Candida albicans as compared to standard drug Flucanazole and Griseofulvin. The synthesized compounds **A3, A4, A8, A13, B2, B4, B5, C2, C4, C5, C12, C15, C17, D2, D3, D8** and **D17** shows good zone of inhibition against Aspergillus niger as compared to standard drug Flucanazole and Griseofulvin.

Table 6.1: Antimicrobial activity of 4-substituted derivatives of 2,7,7-trimethyl-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydro-quinoline-3-carboxylic acid ester.

Sr. No.	**Com code**	**R_1- R_2-**	**Test Pathogen (Zone of inhibition in mm)**			
			Bacterial Species		**Fungal Species**	
			Staphylococcus aureus	**Escherichia coli**	**Candida albicans**	**Aspergillus niger**
01	A1	Ar-H	14	12	15	10
02	A2	$4OCH_3$	**22**	**18**	**22**	15
03	A3	4Cl	**20**	**15**	**25**	**17**
04	A4	4Br	16	**15**	**24**	**19**
05	A5	$4N(CH_3)_2$	14	08	11	10
06	A6	4NO2	15	13	17	16
07	A7	2Cl	12	12	NA	11
08	A8	4OH	**18**	NA	12	**20**
09	A9	$3NO_2$	NA	**18**	10	12
10	A10	R_1-$3OCH_3$ R_2-$4OCH_3$	12	08	NA	10
11	A11	3Br	**20**	NA	10	08
12	A12	3OH	08	11	**19**	NA
13	A13	R_1-2 Cl R_2-4 Cl	NA	**18**	18	**19**
14	A14	$4CH_3$	12	NA	12	16
15	A15	4F	10	12	NA	15
16	A16	R_1-$3OCH_3$ R_2-4OH	10	12	08	11

Zone of inhibition <15 is non significant; NA: No activity; Standard used for bacteria: Streptomycin; Standard used for fungi: Flucanazole and Griseofulvin.

Table 6.2: Antimicrobial activity of 4-substituted derivatives of 6-amino-5-cyano-2-methyl-4-phenyl-4H-pyran-3-carboxylic acid ethyl ester.

Sr. No.	**Com code**	**R_1- R_2-**	**Test Pathogen (Zone of inhibition in mm)**			
			Bacterial Species		**Fungal Species**	
			Staphylococcus aureus	**Escherichia coli**	**Candida albicans**	**Aspergillus niger**
01	B1	Ar-H	18	15	18	20
02	B2	$4OCH_3$	**20**	16	18	**22**
03	B3	4Cl	20	16	**30**	21
04	B4	4Br	**22**	**25**	**32**	**24**
05	B5	$4NO_2$	**22**	**22**	**25**	**22**
06	B6	4OH	17	13	16	12
07	B7	$3NO_2$	16	17	NA	14
08	B8	3Br	15	NA	14	NA
09	B9	3OH	14	12	16	15
10	B10	R_1-$3OCH_3$ R_2-$4OCH_3$	12	14	NA	12
11	B11	R_1-2 Cl R_2-4 Cl	NA	13	12	NA
12	B12	2Cl	15	16	08	12
13	B13	2OH	**18**	11	**20**	10
14	B14	2NO2	12	08	15	12
15	B15	4N(CH3)2	11	10	08	11

Zone of inhibition <15 is non significant; NA: No activity; Standard used for bacteria: Streptomycin; Standard used for fungi: Flucanazole and Griseofulvin.

Table 6.3: Antimicrobial activity of 5-substituted derivatives of 5-arylidine-2,4-thiazolidinedione.

Sr. No.	**Com code**	**R_1- R_2-**	**Test Pathogen (Zone of inhibition in mm)**			
			Bacterial Species		**Fungal Species**	
			Staphylococcus aureus	**Escherichia coli**	**Candida albicans**	**Aspergillus niger**
01	C1	Ar-H	12	10	14	12
02	C2	$4OCH_3$	13	14	13	**16**
03	C3	4Cl	**18**	**15**	**16**	14
04	C4	4Br	**20**	**15**	**18**	**20**
05	C5	$4NO_2$	11	12	10	**18**
06	C6	4OH	08	10	NA	12
07	C7	$3NO_2$	10	NA	12	10
08	C8	3Br	**15**	13	NA	08
09	C9	3OH	NA	**17**	10	NA
10	C10	R_1-$3OCH_3$ R_2-$4OCH_3$	08	NA	**17**	12
11	C11	R_1-2 Cl R_2-4 Cl	12	10	08	10

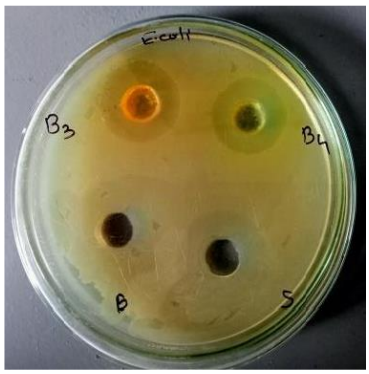
12	C12	4N(CH_3)$_2$	**18**	12	08	**16**
13	C13	4CH_3	11	10	12	08
14	C14	4F	**15**	12	**18**	11
15	C15	2Cl	10	08	**18**	**20**
16	C16	2Br	08	10	11	13
17	C17	2NO_2	12	**18**	12	**16**
18	C18	R_1-3OCH_3 R_2-4OH	10	12	10	08

Zone of inhibition <15 is non significant; NA: No activity; Standard used for bacteria: Streptomycin; Standard used for fungi: Flucanazole and Griseofulvin.

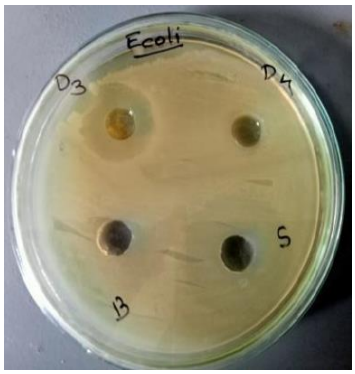
Table 6.4: Antimicrobial activity of pyrimido [4, 5-d] pyrimidine derivatives.

Sr. No.	**Com code**	**R_1- R_2-**	**Test Pathogen (Zone of inhibition in mm)**			
			Bacterial Species		**Fungal Species**	
			Staphylococcus aureus	**Escherichia coli**	**Candida albicans**	**Aspergillus niger**
01	D1	Ar-H	12	14	12	13
02	D2	4OCH_3	14	**15**	**22**	**20**
03	D3	4Cl	**22**	12	**25**	**18**
04	D4	4Br	**15**	**22**	14	12
05	D5	4NO_2	10	12	08	NA
06	D6	4OH	08	NA	12	14
07	D7	3NO_2	NA	10	**18**	12
08	D8	3Br	**16**	08	10	**16**
09	D9	3OH	11	**18**	NA	12
10	D10	R_1-3OCH_3 R_2-4OCH_3	10	NA	08	10
11	D11	R_1-2 Cl R_2-4 Cl	12	10	10	12
12	D12	4F	**15**	10	11	12
13	D13	4CH_3	11	08	12	14
14	D14	4N(CH_3)$_2$	**16**	**18**	10	08
15	D15	2OH	10	11	**18**	10
16	D16	2OCH_3	12	12	**16**	11
17	D17	R_1-3 Cl R_2-4 Cl	**16**	**16**	12	**18**

Zone of inhibition <15 is non significant; NA: No activity; Standard used for bacteria: Streptomycin; Standard used for fungi: Flucanazole and Griseofulvin.



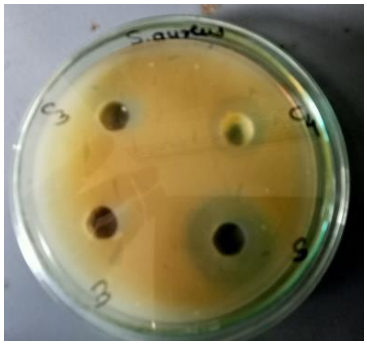
VI-01



VI-02

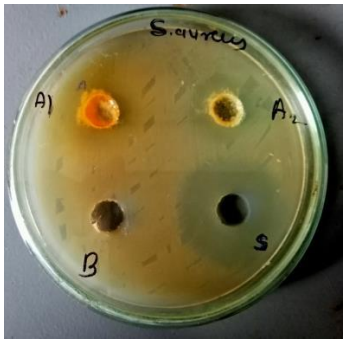
VI-03

Figure showing the antibacterial activity of E. coli species.



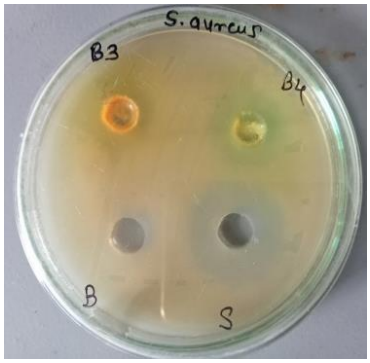
VI-04

VI-05



VI-06

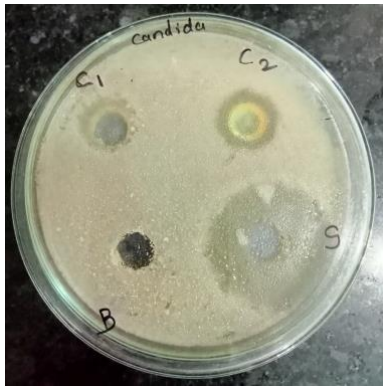
VI-07



VI-08

Figure showing the antibacterial activity of S. aureus species.

VI-09

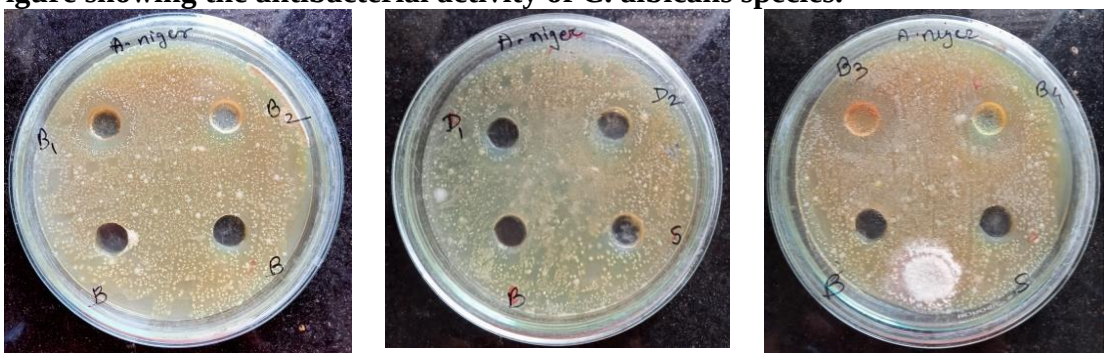


VI-10

VI-11

VI-12 **VI-13** **VI-14**

Figure showing the antibacterial activity of C. albicans species.



VI-15 **VI-16** **VI-17**

Figure showing the antibacterial activity of A. niger species.

Conclusion

Antimicrobial activity was tested on all of the produced compounds in Scheme I, II, III, and IV. The majority of the compounds evaluated showed antibacterial and antifungal activity comparable to that of the standard medication tested. The majority of synthesized compound shows antibacterial and antifungal activity throughout a broad spectrum.

Acknowledgement

Authors are thankful to Central instrumental facility,Savitribai Phule University Pune for structural characterization, Department of Microbiology and Biotechnology, R. C. Patel Arts, Science and Commerce College, Shirpur (MS) for providing biological activities and Department of Chemistry Shivaji Arts, Science and Commerce College Kannad for infrastructural facility.

References

i. Okeke, I. N., Laxmaninarayan, R., Bhutta, Z. A., Duse, A. G., Jenkins, P., O'Brien, T. F., Rablos-Mendez, A., Klugman, K. P., *Lancet Infect*. *Dis*. **2005**, 5, 481.
ii. Sirockin, G., Cullimore, S., Practical Microbiology, *Mc Graw Hill Publishing C. Ltd*., London, **1969**.
iii. Lorian, V., Antibiotics in Laboratory Medicine, William and Wilkins, Baltimore, **1986**.
iv. Pelczer, M., Reid, R., Chan., E., Microbiology, *Tata Mc Graw Hill Publishing*, C. Ltd., London, **1978**.
v. Indian Pharmacopoeia, Vol-I and II, Government of India publication, **1996**.
vi. Berber, I., Cokmus, C., Atalan, E., *Mikiobiologia*, **2013**, 72, 54.

vii. Mitscher, L. A., Pillai, S. P., Gentry, E. J., Shankel, D. M., *Med. Res. Rev*. **1999**, 19, 477.
viii. Quadri, F., Svennerholm, A, M., Faruque, A. S. G., Sack, R. B., Clin. Microbiol. Rev. 2005, 18, 465.
ix. Devasia, R. A., Jones, T. F., Ward, J., Stafford, L., Hardin, H., Bopp, C., Beatty, M., Mintz, E., Schaffner, W., Am. J. Med. 2006, 119, 168.
x. Khan, S. A., Kumar, P., Joshi, R., Iqbal, P. F., Saleem, K., Eur. J. Med. Chem. 2008, 43, 2029.
xi. Harbottle, H., Thakur, S., Zhao, S., White, D. G., Anim.Biotechnol. 2006, 17, 111.
xii. Berger, S., Horm. Metab. Res. 1985, 17, 111.
xiii. Padghan, S. V., Magar, B. K., Chopade, M. U.,*Heterocyclic Letter*, May-July/**2021**, Vol. 11, 03, 477-484.
xiv. Padghan, S. V., Magar, B. K., Chopade, M. U.,*Gis Science Journal*, **2021**, Vol. 08, 08, 1379-1386.
xv. Padghan, S. V., Magar, B. K., Chopade, M. U., *Journal of Research and Development*, **2020**, Vol. 10, 10, 235-243.
xvi. Padghan, S. V., Magar, B. K., Chopade, M. U., *Our Heritage*,**2020**, Vol. 68, 38.

Received on May 17, 2022.