Chapter - V

ANTIMICROBIAL ACTIVITY
Introduction -

Any chemical substance inhibiting the growth or causing the death of a microorganism is known as 'antimicrobial agent'. A wide range of chemicals exhibit these properties when used in a sufficiently high concentration. However, the term is usually restricted to those substance that are effective at concentrations suitable for practical applications. The chemical, at a low concentration should have a broad spectrum of antimicrobial activity. Chemical agents not only provide the structural basis and energy supply of living organism but also regulate their functional activities. The interaction between potent chemicals and living system contribute to the understanding of life processes and provide effective methods for the treatment, prevention and diagnosis of many diseases. Chemical compounds used for this purpose are called 'drugs' and their action on living system are referred to as 'drug effect'. The subject of drug is as old as disease. Sickness has been human being's heritage from the beginning of his existence and search for remedies to combat is perhaps equally old. Fighting disease with drug is the timeless struggle. It's beginning echoed out of premeval jungle. Mankind's survival on this planet is dependent upon its success.

The 'papyri' were the first written account of medical experiences from Egypt. The papyrus discovered by Eder in 1872 was prepared in 1500 B.C. and mentions about 700 herbal medicines. A Babylonian clay-tablet (700 B.C.) has been discovered, which mentions about 300 drugs.

The concept of 'Homeopathy' was first introduced in the early 19th century by 'Hanneman' who thought that 'like cure like' and dilution potentiates the action of drugs. Homeopathy outlines the therapy for various ailments with drugs in very high dilution.
Modern medicine is considered to date from 'Hippocrates' a Greek physician (450 B.C.) who for the first time introduced the concept of disease as a pathologic process and tried to organise the science of medicine on the basis of observations, analysis and deduction.

Till the beginning of 19th century, the treatment of disease consisted of obnoxious remedies such as flesh, excreta and metallic and plant preparations. James Gregory (1753-1821) was responsible for popularizing heroic symptomatic treatment consisting of blood letting large doses of emetics and drastic purgatives often with disastrous results. Such treatment without any rational basis was called 'Allopathy' (meaning the other suffering).

'Antibiotics' are a special kind of chemotherapeutic agents usually obtained from living organisms. The word antibiotic has come to refer to metabolic product of one organism that is detrimental or inhibitory to other microorganisms in very small amounts. According to Waksman (1945) the term antibiotics applies to those chemical substances of microbial which in small amounts exert antimicrobial activity. Antibiotics were known by their activities long before they were given the same by which we know them. Many years ago the Chinese used moldy soyabean curd for the treatment of boils and controlled foot infection by wearing sandals furry with mold.

Subdivision of the antimicrobial agent into different groups is possible on the basis of the action and purpose for which they are employed. Subdivision can be based upon the group of microorganisms affected like antibacterial, antifungal, antiprotozoal, antiviral and antineoplastic chemotherapeutic agents, which are more or less specific for treatment of disease
caused by specific pathogenic agents.\textsuperscript{1,2} For example, the antibacterial agents acting on bacteria are called bacteriostatic/bacteriocidal, bacteriostatic are those having the property of inhabiting bacterial multiplication. Multiplication resumes upon removal of the agent while bacteriocidal are those having the property of killing bacteria. Antibacterial agents include disinfectants and the antimicrobial drugs i.e. chemotherapeutic agents and antibiotics. Many different disinfectant compounds are in use, some with a wide range of activity and others more specific in their effects.

The term 'chemotherapy' was first used by Paul Ehrlik, who proposed that infectious disease might be cured by using chemicals that inhibit or kill the infectious agents but do not harm the host at the concentrations used. He discovered the famous organoarsenical compound 'salvarsan' which was active against the causative organisms of 'syphillus'. According to his theory of drug action, cell possesses chemical receptors to which the drug binds. He recognized the importance of quantitative measurement to determine the drug dose, that would be effective against the causative agent and not have any toxic effect on the host. He also pioneered method for screening a large number of compounds for biological activity in relation to chemical structure. Chemical variants of effective compounds were then synthesized and tested to see whether they have improved antimicrobial activity and reduced toxicity.

Alexander Flemming (1929), noticed that an agar plate inoculated with \textit{Staphylococcus aureus} has become contaminated with a mold and that the mold colony was surrounded by a clear zone, indicating inhibition of bacterial growth or lysis of the bacteria. The importance of Flemming's observation was realized when it proved to be effective in preventing death from infection of war
wounds. With the aid of many investigators in England and the United States, and at the expenditure of a great deal of money, the inhibitory substances from Flemming's contaminant mold became a 'miraculous drug'. Because the mold was identified as a *Penicillium sp.*, Flemming was knighted and shared the Nobel Prize in physiology and medicine for 1945 with Ernst B. Chain, a chemist and Sir Howard W. Flarey, a physician.

The credit for creating a popular interest in antibiotics must be shared by Flemming with Dubos (1939). He isolated from New Jersey soil a culture of *Bacillus brevis* which produced a substance that killed many gram negative bacteria. The cell-free extract produced from *B. brevus* by Dubos was found to contain two active principles now known as gramicidin and tyrocidine. These successes were followed closely by the discovery of streptomycin by Selman Waksman and associates.

Several thousand antibiotic substances have been isolated and identified since 1940. Many of them are of no practical importance as yet, but a few have changed an entire concept of chemotherapy. There is no doubt that many more and possibly better, antibiotics will be found. The popularity of antibiotics is due to their ability to destroy many kinds of pathogens and to their relatively non-toxic properties when given systematically.

The point of attack of chemotherapeutic agents on microorganisms varies. It may be at the molecular level, e.g. interference with enzyme synthesis, or at the cellular level, e.g. inhibition of cell-wall synthesis. There are, no doubt, many vulnerable sites for attack between these extremes. There are four ways in which antibiotics can inhibit or kill microorganisms.
1. Inhibit cell-wall formation  
2. Damage the cell membrane  
3. Interfere with protein synthesis  
4. Inhibit nucleic acid metabolism.

In general, it may be said that agents that damage the cell wall or the cell membrane are bactericidal while those, which interfere with an enzyme action, are bacteriostatic.

Review of Literature -

A survey of literature reveals that extensive work has been done on many heterocyclic compounds for their antimicrobial activities including both gram positive and gram negatives pathogens. Chalcones and their derivatives are reported to have antibacterial, antifungal, antiparasitic, antitubercular, anti-inflammatory and insect repellent properties. The other heterocycles like pyrazoles, pyrimidines, thiazoles, thiazolines, triazines etc. also show good antimicrobial activity. Benzofuran nucleus containing heterocyclic compounds have their own identity and importance since the plant extracts containing these are used in traditional medicine and exhibit various physiological activities such as antihistaminic, anti-inflammatory, estrogenic and anti-implantation etc.

Raghuwanshi and Doshi have studied antimicrobial activity of nitropyrazolines against S. typhi, S. paratyphi, P. vulgaris, Xanthomonas Spp., T. solanii and B. cinerea by serial dilution method and found that the derivatives of nitropyrazolines are more active than simple pyrazolines.

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Utale et al\textsuperscript{14} have synthesized a series of pyrimidine-2-thiols and arylthiazolines and screened the antimicrobial activity against \textit{S. aureus}, \textit{S. pyogens}, \textit{S. agalactiae}, \textit{S. faecolis}, \textit{C. ulcerans}, \textit{C. septicum}, \textit{C. tetani}, \textit{E. coli}.

Raut et al\textsuperscript{15} reported that the compounds containing bromo and nitro groups together are more active than simple benzisoxazoline while studying the synthesis and antimicrobial activities of substituted benzisoxazolines against \textit{S. typhi}, \textit{S. paratyphi}, \textit{P. vulgaris}, \textit{Xanthomonas Spp.}, \textit{T. solanii} and \textit{B. cinerae}.

The activity against bacteria like \textit{E. coli}, \textit{P. vulgaris}, \textit{B. megaterium}, \textit{S. aureus} and fungi \textit{A. niger} was tested with cyanopyrans and cyanopyridines by Popat et al\textsuperscript{16}.

Recently, similar studies have been carried out against \textit{B. anthrhexis}, \textit{S. aureus}, \textit{S. typhi}, \textit{A. fumigatus}, \textit{A. niger}, \textit{C. albicans} etc.\textsuperscript{17-18} The antimicrobial activity of Schiff’s base ligands derived from acetoacetanilide and \textit{o}-phenylene-diamine against \textit{S. aureus}, \textit{S. typhi}, \textit{P. aeruginosa} etc. have been tested using nutrient agar as medium, and compared with the standard ampicillin.\textsuperscript{19} Similar activities were found out by cup-plate method against \textit{E. coli}, \textit{S. aureus}, \textit{P. vulgaris} etc.\textsuperscript{20-22}

A perusal of literature has revealed that very few attempts have been made for testing antimicrobial activity of cumaran-3-ones or \textit{\alpha}-bromo-acetophenones, but benzodipyran derivatives are found to exhibit anti-allergic, anti-asthmatic, insecticidal and anti-feedant activities. D. Ashok et al\textsuperscript{23} envisaged that incorporation of cyclopropyl groups in benzodipyrans might have enhanced the above mentioned activities against \textit{E. coli} and \textit{S. aureus}. Recently, dialkylthiophosphate derivative of macrocyclic complexes of Ni(II) were...
studied against fungus *Trichoderma*. Kadu and Doshi reported antimicrobial activities of 2-(substituted benzylidene)-6,7-bromocoumaran-3-ones and its dibromides against *S. aureus*, *S. typhi*, *E. coli*, *P. mirabilis*, etc. Activity of these compounds was compared with standard drug chloramphenicol. MIC values were determined by serial dilution method and a significant increase in activity of aurone-dibromides was observed than aurones.

**Present Work** -

The literature survey shows that α-bromoacetophenones are used as intermediates in the synthesis of drugs. Most of the pharmaceuticals contain electron donor groups likely to bind metal ions occurring naturally. Therefore, we intended to synthesize and explore antimicrobial activities of 2-hydroxy-3-H/substituted-5-methyl-α-bromoacetophenones which are multifunctional molecules containing bromine atom at α-position while –OH group in the ring at ortho position. It also contains reactive methylene group, which is situated in between two strong electron withdrawing carbonyl and bromo groups.

The review of literature survey clearly mentioned that when there are chlorosubstituted heterocyclic drugs they have high antibacterial, antifungal, antiparasitic and insecticidal activity. Very less work has been carried out for determining antimicrobial activities in case of nitrosubstituted heterocycles.

Hence, it was thought worthwhile to synthesize heterocyclic coumaran-3-ones by cyclization of α-bromoacetophenones and to find out their antimicrobial activity. Since chloro and nitro compounds may have higher activity we have synthesize some coumaran-3-ones having –Cl or –NO₂ substitutions.
The work presented in this chapter deals specially with the study of antimicrobial activity of \( \alpha \)-bromoacetophenones and coumaran-3-ones synthesized in Chapter-II against the following test organisms.

1. *Escherichia coli* :- A gram positive bacteria, causative agent of diarrhea.
2. *Bacillus cereus* :- A gram positive bacteria, causative agent of diarrhea and abdominal pain.
3. *Salmonella typhi* :- A gram negative bacteria, causative agent of typhoid.
4. *Staphylococcus aureus* :- A gram positive bacteria, causative agent of wound infection.

The following compounds were tested,

1. 2-Hydroxy-5-methyl-\( \alpha \)-bromoacetophenone (III)
2. 2-Hydroxy-3-bromo-5-methyl-\( \alpha \)-bromoacetophenone (IV)
3. 2-Hydroxy-3-nitro-5-methyl-\( \alpha \)-bromoacetophenone (VI)
4. 2-Benzylidene-5-methylcoumaran-3-one (VIIa)
5. 2-(2'-Hydroxy)benzylidene-5-methylcoumaran-3-one (VIIb)
6. 2-(4'-Methoxy)benzylidene-5-methylcoumaran-3-one (VIIc)
7. 2-(4'-Chloro)benzylidene-5-methylcoumaran-3-one (VIIId)
8. 2-(4'-Nitro)benzylidene-5-methylcoumaran-3-one (VIIIf)
9. 2-(4'-N-dimethylamino)benzylidene-5-methylcoumaran-3-one (VIIIf)
10. 2-Benzylidene-5-methyl-7-bromocoumaran-3-one (IXa)
11. 2-(2'-Hydroxy)benzylidene-5-methyl-7-bromocoumaran-3-one (IXb)
12. 2-(4'-Methoxy)benzylidene-5-methyl-7-bromocoumaran-3-one (IXc)
13. 2-(4'-Chloro)benzylidene-5-methyl-7-bromocoumaran-3-one (IXd)
14. 2-(4'-Nitro)benzylidene-5-methyl-7-bromocoumaran-3-one (IXe)
15. 2-(4'-N-dimethylamino)benzylidene-5-methyl-7-bromocoumaran-3-one (IXf)
16. 2-Benzylidene-5-methyl-7-nitrocoumaran-3-one (Xa)
17. 2-(2'-Hydroxy)benzylidene-5-methyl-7-nitrocoumaran-3-one (Xb)
18. 2-(4'-Methoxy)benzylidene-5-methyl-7-nitrocoumaran-3-one (Xc)
19. 2-(4'-Chloro)benzylidene-5-methyl-7-nitrocoumaran-3-one (Xd)
20. 2-(4'-Nitro)benzylidene-5-methyl-7-nitrocoumaran-3-one (Xe)
21. 2-(4'-N-dimethylamino)benzylidene-5-methyl-7-nitrocoumaran-3-one (Xf)

**Experimental -**

The antibacterial and antifungal activities of various compounds synthesized in Chapter-II were tested to evaluate their efficiencies against animal and plant pathogenic organisms. All the chemical and media were purchased from M/s. Hi-Media Pvt. Ltd., Mumbai, India. The Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati was kind enough to carry out the antimicrobial activity studies. The organisms used were *E. coli, B. cereus, S. typhi, S. aureus* and *Trichoderma*.

For the evaluation of in-vitro antimicrobial activity, the following three conditions must be fulfilled,

i) First the substance to be evaluated must be brought in an intimate contact with the test organisms against which activity is to be estimated.

ii) Secondly, favourable conditions (nutritional, environmental etc.) must be provided to offer a maximum opportunity for optimum growth of the organisms in absence of antimicrobial agent, and

iii) Thirdly, there should be a method for measuring antibacterial response obtained by antimicrobial agent.28
Various methods have been proposed and adopted for the measurement of antibacterial activity, these are -

1. Agar streak dilution method
2. Agar diffusion (cup, paper, disc, cylinder) method
3. Turbidometric method
4. Serial dilution method
5. Specific method (specific for measuring the action of specific substance).

In the present study, we used agar diffusion method to find out the activity of all synthesized compounds against the microbes. Then the minimum inhibitory concentrations were measured by serial dilution method for those compounds only which were found to be active.

A) Media Used:

1. Nutrient Agar Medium -

Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>1.5 gms</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.5 gms</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0 gms</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0 gms</td>
</tr>
<tr>
<td>Agar powder</td>
<td>20.0 gms</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.2 (at 25°C)</td>
</tr>
</tbody>
</table>
2. Nutrient Broth Medium -

Composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast extract</td>
<td>1.5 gms</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.5 gms</td>
</tr>
<tr>
<td>Peptone</td>
<td>5.0 gms</td>
</tr>
<tr>
<td>NaCl</td>
<td>5.0 gms</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.2 (at 25°C)</td>
</tr>
</tbody>
</table>

Both the above cited media used were of bacteriostatic grade. Above media were found to be suitable for the growth of all four organisms used in the present work.

B) Slant Preparation -

Nutrient agar medium was dissolved in distilled water and was sterilized by auto claving. About 5 ml of molten media was transferred aseptically in previously sterilized test tubes. The test tubes were then plugged tightly and placed in a slanting position to cool and solidify.

C) Stock Culture -

Culture was grown on nutrient agar slants by incubating them for 24 hrs at 37°C.

D) Culture Dilution (Sub-culturing) -

One loopful of stock culture was added to 5 ml of nutrient broth medium for inoculation. The inoculated broth was incubated for 24 hrs at 37°C. For all experimental purposes 24 hrs fresh diluted culture of both the organisms were used.
E) Preparation of Sample Solution -

An antibacterial activity is usually tested by making aqueous solution samples. However, compounds used in the present study are insoluble in water. Hence, to study antimicrobial activity their dilutions were prepared by using 70% methanol. Thus, 70% methanol was taken and tested as control.

To check the potency of compounds, the solutions were prepared with 50 μ gm/ml concentration. 1 ml of this solution was added to 5 ml of nutrient broth solution containing organism to be tested. Tubes with organism and medium with solvent, were used as controls. These tubes were kept for incubation at 37°C for 24 hrs. Most of the compounds under study exhibited total inhibition of the test cultures within 24 hrs of incubation. The tube containing compounds showing inhibition (antimicrobial activity) was clear and the tube which was kept as control where no compound was added showed growth. Therefore, for all the antibacterial screenings, the concentrations of 50 μ gm/ml were used, which is in the range of the substance to be used as antibiotic.

F) Disc Diffusion Method\(^\text{30,31}\) -

Every time fresh sterile nutrient agar medium was prepared. The proceedings were carried out aseptically. All the glassware and apparatus required were sterilized.

In each sterile petridish 15-20 ml of molten medium was added. Simultaneously 0.05-0.1 ml (approx. 2-3 drops) of 24 hrs fresh diluted culture of organism under study was added to each petriplate. The nutrient broth culture and nutrient agar media were mixed thoroughly by rotatory motion of agar plate on a plane surface. It was allowed to solidify at room temperature. Then
sterilized Whatmann filter paper No. 1 discs (6 mm diameter) thoroughly moistened with the same concentration of each of the compound were placed on the surface of the plate. Disc moistened with 70% methanol were used as control. They were allowed to diffuse in the media and then the plates were incubated at 37°C for 24 hrs. The diameter of the zones of inhibition were observed.

The same procedure was followed for determining antifungal activity, only the potato dextrose plate was used.

3. **Potato Dextrose Agar**

**Composition**

- Potato infusion form -- 200 gms
- Dextrose -- 20 gms
- Agar -- 15 gms
- Distilled water -- 1000 ml
- pH -- 5.6 ± 0.2 (at 25°C)

The compounds, which showed antimicrobial activity, were further tested for their minimum inhibitory concentration by Serial Dilution Method.

**G) Serial Dilution Method**

To determine the MIC of various compounds the following procedure (Serial Dilution Method) was followed.

Nutrient broth was prepared by dissolving 13 gms of dehydrated medium in 1 litre of distilled water. The pH of the medium was adjusted to 7.4. 5 ml of the medium was distributed in each tube. All the tubes were sterilized at 121°C for 20 minutes.
The appropriate amount of test compound was dissolved in the solvent 70% methanol to give final concentration of $1 \times 10^{-2}$ M. Various amounts of the above stock solution was aseptically added to the various nutrient broth tubes (viz. 0.5, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 .......5.8, 6.0 ml). Fresh culture of the test bacterium was inoculated in each tube (0.2 ml culture). The inoculum size of the test bacterium was adjusted to give approx. $10^7$ CFU. All the tubes were incubated at 37°C for 24 hrs. Uninoculated tube was kept as a control in which nutrient broth and 5 ml of the solvent was taken.

After 24 hrs. of incubation, all the tubes were observed for MIC against test bacterium.

This was observed by the absence of visual turbidity in the tube receiving the highest dilution of the test compounds.

To determine MIC of various test compounds against moulds (fungus) the following procedure was adopted.

Potato dextrose broth was prepared as follows.

200 gms. of potato (Peeled) was added to 1 litre of distilled water. It was steamed for 20 mins and volume adjusted to 1 litre. 20 gms of dextrose was added to this.

Appropriate amount of test compounds was dissolved in 70% dioxane methanol to give final concentration of $1 \times 10^{-2}$ M. Various amounts of the above stock solution was added aseptically to the potato dextrose broth tubes (viz. 0.5, 1.0, 1.2, ...., 6 ml). Fresh fungal culture was inoculated aseptically in each tube (0.2 ml of culture). All the tubes were incubated at 28°C for 96 hrs. After 48 hrs. of incubation all the tubes were observed for the MIC of test compounds.
Results and Discussion -

Total 21 synthesized compounds were studied for their antimicrobial activities. All the pathogens tested during analysis are human pathogens. The activities of compounds were tested against all the pathogens by disc diffusion method. It was found that all the compounds are active against bacteria except (IV), (VIIIc), (VIIe) and (Xe) against *S. aureus*. Whereas, only (VIIe), (IXd), (IXe) and (Xd) were found to be active against fungus *Trichoderma* (Table-1). MIC values were measured for the active compounds only, and given in Table-2, (Fig. 1).

Activity against *E. coli* –

*E. coli* is a gram negative parasite living only in human or animal intestine. Voided in faeces it remains viable in environment only for some days. The clinical infections which are caused by *E. coli* are urinary tract infection, diarrhea, pathogenic infection and septicemia. In the last two decades, throughout the world the patients of diarrhea in February to July are generally observed. Medical practitioners generally use sulphonamides, cotrimoxazole, quinolones, ampicillin, cloxacillin, ciperacillin, carbenicillin, ciphalosporin, gentamycine, chloramphenicol, tetracycline etc. for the treatment of these patients. These drugs directly affect on digestive system and ultimately circulatory system and finally on kidneys. Also the above drugs once used for the treatment of *E. coli* infection should not be used upto six months.

The antimicrobial activity of the synthesized compounds against *E. coli* is highly remarkable, total 11 compounds are highly active, (VIIIf) is moderately active, (Xb) and (Xc) are weakly active while (VIIa-c) and (IXa-c)
### Table 1 - Antibacterial and antifungal activity of compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>E. coli</th>
<th>B. cereus</th>
<th>S. typhi</th>
<th>S. aureus</th>
<th>Trichoderma</th>
</tr>
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<tbody>
<tr>
<td>(III)</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Inactive</td>
</tr>
<tr>
<td>(IV)</td>
<td>Active</td>
<td>Active</td>
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<td>Inactive</td>
</tr>
<tr>
<td>(VI)</td>
<td>Active</td>
<td>Active</td>
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<td>Inactive</td>
</tr>
<tr>
<td>(VIII a)</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Inactive</td>
</tr>
<tr>
<td>(VIII b)</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Inactive</td>
</tr>
<tr>
<td>(VIII c)</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>(VIII d)</td>
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<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Inactive</td>
</tr>
<tr>
<td>(VIII e)</td>
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<td>Active</td>
<td>Inactive</td>
<td>Active</td>
</tr>
<tr>
<td>(VIII f)</td>
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<td>Active</td>
<td>Active</td>
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</tr>
<tr>
<td>(IX a)</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
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</tr>
<tr>
<td>(IX b)</td>
<td>Active</td>
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<td>Active</td>
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<tr>
<td>(IX c)</td>
<td>Active</td>
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<tr>
<td>(IX d)</td>
<td>Active</td>
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<td>Active</td>
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<td>(IX e)</td>
<td>Active</td>
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<td>Active</td>
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<tr>
<td>(IX f)</td>
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<tr>
<td>(X a)</td>
<td>Active</td>
<td>Active</td>
<td>Active</td>
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<td>(X e)</td>
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<td>Inactive</td>
<td>Inactive</td>
</tr>
<tr>
<td>(X f)</td>
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</table>
### Table: 2 - Minimum Inhibitory Concentration (MIC) values of active compounds in µg ml⁻¹

<table>
<thead>
<tr>
<th>Compound</th>
<th>E. coli</th>
<th>B. cereus</th>
<th>S. typhi</th>
<th>S. aureus</th>
<th>Trichoderma</th>
</tr>
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<tbody>
<tr>
<td>(III)</td>
<td>800</td>
<td>950</td>
<td>1200</td>
<td>1200</td>
<td>--</td>
</tr>
<tr>
<td>(IV)</td>
<td>600</td>
<td>680</td>
<td>900</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(VI)</td>
<td>320</td>
<td>470</td>
<td>320</td>
<td>800</td>
<td>--</td>
</tr>
<tr>
<td>(VIII a)</td>
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<td>1540</td>
<td>2200</td>
<td>3000</td>
<td>--</td>
</tr>
<tr>
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<td>3000</td>
<td>--</td>
</tr>
<tr>
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<td>2460</td>
<td>1320</td>
<td>1500</td>
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<td>--</td>
</tr>
<tr>
<td>(VIII d)</td>
<td>560</td>
<td>370</td>
<td>490</td>
<td>620</td>
<td>--</td>
</tr>
<tr>
<td>(VIII e)</td>
<td>800</td>
<td>540</td>
<td>340</td>
<td>--</td>
<td>920</td>
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<tr>
<td>(VIII f)</td>
<td>1200</td>
<td>880</td>
<td>1150</td>
<td>1440</td>
<td>--</td>
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<tr>
<td>(IX a)</td>
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<td>1540</td>
<td>1800</td>
<td>2320</td>
<td>--</td>
</tr>
<tr>
<td>(IX b)</td>
<td>2100</td>
<td>1780</td>
<td>1520</td>
<td>1900</td>
<td>--</td>
</tr>
<tr>
<td>(IX c)</td>
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<td>2100</td>
<td>1430</td>
<td>2120</td>
<td>--</td>
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<tr>
<td>(IX d)</td>
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<td>360</td>
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<tr>
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<td>490</td>
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<td>750</td>
</tr>
<tr>
<td>(IX f)</td>
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<td>640</td>
<td>550</td>
<td>1000</td>
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</tr>
<tr>
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<td>1370</td>
<td>1540</td>
<td>1800</td>
<td>--</td>
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<tr>
<td>(X b)</td>
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<td>980</td>
<td>1200</td>
<td>1620</td>
<td>--</td>
</tr>
<tr>
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<td>1600</td>
<td>1100</td>
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<td>1420</td>
<td>--</td>
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<tr>
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<td>200</td>
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<tr>
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<td>340</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>(X f)</td>
<td>760</td>
<td>510</td>
<td>570</td>
<td>820</td>
<td>--</td>
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</table>

**MIC value =**
- 3500-1900 Inactive;
- 1800-1500 Weakly active;
- 1400-1000 Moderately active;
- < 1000 Highly active.
Figure 1 - Minimum Inhibitory Concentration (MIC) values of active compounds

- E. coli
- B. cereus
- S. typhi
- S. aureus
- Trichoderma
are inactive against *E. coli*. From the Table-2 it can be easily seen that the compounds (VI), (IXd), (Xd) and (Xe) show very high activity in minimum concentration. All these compounds have a nitro group in common which may be responsible for the higher activity. The still higher activity of (IXd) and (Xd) may be attributed to the presence of chloro group at para position of benzylidene nucleus and heterocyclic coumaran-3-one nucleus. The other seven compounds also show high activity most of which are heterocyclic coumaran-3-ones and have nitro/chloro groups, whereas, (VIII) is moderately active. Thus in all 12 compounds are having good activity against *E. coli*. So these synthesized drugs can be used as the best alternative drugs for the treatment of diseases caused by *E. coli*, only after the pharmaceutical, biochemical and medicinal significance, if these drugs do not have toxic and other side effects.

**Activity against *B. cereus* –**

*B. cereus*\(^{31}\) is a gram positive food poisoning bacterium widely distributed in nature and may be readily isolated from soil, vegetables and wide variety of foods including milk, cereals, spices, meat and poultry etc. It produces two patterns of food borne diseases, first is associated with wide range of food including cooked food and vegetables. It is characterized by diarrhea and abdominal pain in 8-16 hrs. after intake of contaminated food, vomiting is rare. The second type is associated almost exclusively with the consumption of cooked /fried rice. The illness is characterized by acute nautia and vomiting, 1-5 hrs. after meals, diarrhea not common. The treatment of *B. cereus* is similar to that of *E. coli*.

Out of 21 compounds for which activity was measured against *B. cereus* a total of 13 compounds are found to be highly active, 4 compounds...
moderately active, 3 weakly active and compound (IXc) is inactive. It is evident from Table-2 that the higher activity is generally associated with compounds containing nitro and/or chloro groups, and cyclization to 5 membered heterocyclic compound increases the activity.

**Activity against S. typhi**

*S. typhi* again is a gram negative bacterium causative agent of typhoid ranging from 7-14 days. The patient shows mild pyrexia which may become fatal fulminating disease. As bile is good culture medium for the bacteria, it is multiplied abundantly in gall bladder and is discharged continuously into intestine where it involves the Teyer’s patches and lymphoid follicles of the ilium. This becomes inflamed and undergoes necrosis and slough off, leaving behind ulcer complications, intestinal perforation and haemorrhage.

The common drugs used against *S. typhi* are chloramphenicol, streptomycin, tetracycline etc. which are now found to be inactive in vivo. Therefore, nowadays more powerful antibiotics like ampicillin, amoxycillin, furazolidone, totrimoxazole are administered for the treatment, which have their own side effects. Moreover, by continuous administration of these drugs the bacteria develops resistance making the drugs ineffective.

The antimicrobial activity of the synthesized compounds is again remarkable and considerable. Ten out of 21 compounds show higher activity whereas, 4 are moderately and 6 weakly active. Only compound (VIIIa) is inactive against *S. typhi*. As the synthesized compounds show remarkable antimicrobial activity against *S. typhi* at minimum concentration, so these synthesized drugs can be used as an alternative drug for the treatment of disease.
caused by *S. typhi* only after their detailed study in pharmaceutical, biochemical, medicinal sciences. These drugs may replace the traditional drugs if they do not have toxic and other side effects.

It is observed that antimicrobial activity of coumaran-3-one is more than of α-bromoacetophenones. This higher activity may be due to the presence of five member heterocyclic ring in the compound, the ketonic group as well as cyclic oxygen, which may enhance the potentiality of that compound.

It can also be noted that when −NO₂ group and −Cl group are present in the molecule the reactivity enhances, which increases the potency of the drug. This probably may be due to higher resonance stability provided by these groups and hence prolonged activity of the compound.

**Activity against *S. aureus* –**

*S. aureus*[^1] is a gram positive bacterium found in wounds and is a causative agent of wound infection. It occurs in clusters like grapes. Its ability to develop resistance to penicillin and other antibiotics enhances its importance as human pathogen. It produces two types of diseases – infection and intoxication. In the former, the cocci gain access to damage skin, mucosal or tissue sites. In intoxication, the disease is caused by infected host. Staphylococcal infections are among the most common bacterial infections ranging from trivial to fatal.

Since such type of bacteria develop resistance to common antibiotics[^2], newer and newer types of drugs will have to be always synthesized and tested against them.

It is observed from Table-2 that, 21 compounds are tested against *S. aureus* pathogen from which 7 compounds are highly active, one is moderately

[^1]: Aswale, Ph.D. Thesis, 186
[^2]: Aswale, Ph.D. Thesis, 186
active while 4 are weakly active and remaining 9 compounds are inactive. So active compounds can be used for treatment of wound infection after biological, pharmaceutical, medical study and if these do not have any toxic side effects.

**Activity against Trichoderma –**

*Trichoderma* is a soil fungus and some species of which cause alimentary toxic aleukia.\(^{35}\) *Trichoderma* causes a particular problem in the mushroom cultivation industry, where it can parasitize the mycelium and fruiting bodies of mushroom. This is known as green mold disease of edible mushroom. When the mushroom is parasitized, it develops a green mold over the surface making the mushroom ugly and deformed.\(^{36,37}\)

All the 21 compounds were tested for antifungal activity against *Trichoderma*. (VIIe), (IXd), (IXe) and (Xd) are found to be highly active while all others are inactive against *Trichoderma*.

From Table-2 and Fig. 1, it is observed that MIC values for *E. coli* ranges from 200-3600 microgram/ml. MIC values for *S. aureus*, *S. typhi*, *B. cereus* and *Trichoderma* are found to be ranging from 800-3000, 200-2200, 270-1540 and 240-920 microgram/ml respectively. It was observed that all the above compounds are having good antimicrobial activity but are less active against fungus. Out of 21 compounds only 4 compounds show antifungal activity but these have high antifungal activity, which is shown from less MIC values (240-920 microgram/ml). This antifungal activity may be due to presence of chloro and nitro groups in coumarins-3-ones. All the \(\alpha\)-bromoacetophenones are inactive against *Trichoderma*.
Antimicrobial Activity

It is seen that compounds are having good activity against microbes when the structure contains nitro group. It is also observed that in all the above compounds, antimicrobial activity is the highest in those compounds which contain –Cl and –NO$_2$ groups. It is proved from MIC values, ranging from 180-310 microgram/ml. Activity is also increased when heterocyclic nucleus is present in addition to benzenoid nucleus.
REFERENCES


S. S. Aswale, Ph.D. Thesis...


37. website : [http://mushgrowinfo.cos.psu.edu/Trichoderma%20Green%20mold.html](http://mushgrowinfo.cos.psu.edu/Trichoderma%20Green%20mold.html).
LIST OF PAPERS

Published :


2. "Synthesis and characterization of Cr(III), Mn(III), Fe(III), Ti(III), VO(IV), Th(IV), Zr(IV) and UO₂(VI) polychelates derived from bis-bidentate salicylaldemine Schiff base", S.R. Aswale, P.R. Mandlik, S.S. Aswale and A.S. Aswar, Indian J. Chem., 42(A), pp. 322-326 (Feb. 2003).


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