CHAPTER - VI
CHAPTER VI

ANTIMICROBIAL AND ANTIFUNGAL ACTIVITIES

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6.1 Introduction

Literature survey reveals that many coumarins are naturally occurring compounds and known to have biological activities. A well-known compound, dicoumarol is best known for its anticoagulant effect on blood.

Dicoumarol

Dicoumarol has been found in many investigations to exert a number of biological effects in addition to its blood coagulation and important among these is its action as an uncoupling agent. Martius and Nitzlitzous reported that it is a potent uncoupler of NAD-linked oxidative phosphorylation, and a large number of studies on inhibitory effects of diacoumarol on a variety of enzymes have been reported in addition to its antimicrobial action against a variety of bacteria.

Novobiocin
Novobiocin is one of the best known coumarin antibiotics, has been recognized the larger and has been the more intensively studied. Novobiocin binds to albumin, a plasma protein. Finland and Nichols\textsuperscript{10} reported its binding to human and bovine serum albumins. Many effects of Novobiocin on metabolic process have been observed. \textit{Staphylococcus aureus} treated with Novobiocin accumulates a nucleotide, UDPAG – Lact-Ala-Glu-Lys-Ala-Ala, a precursor of the cell wall glycopeptide.\textsuperscript{11} It inhibits oxidative phosphorylation in \textit{Mycobacterium phlei}.\textsuperscript{12}

Another coumarin antibiotic reported coumermycin A, which has also been the subject of a number of studies on its mode of action, which has proved to be very similar to that of Novobicin. Michaeli et al.\textsuperscript{13} reported a rapid and marked inhibition by this drug of nucleic acid synthesis in \textit{Staphylococcus aureus}.

7-hydroxycoumarin is known for its antibiotic and antifungal activities,\textsuperscript{14,15} having substituent like acetyl at position – 8 and methyl at position –4 has been found to exhibit anticoagulant and plant growth regulant properties.\textsuperscript{16,17} Some coumarin derivatives possessing carboxamide moiety are found to have diuretic, analgesic, myorelaxant,\textsuperscript{18} antifungal,\textsuperscript{19,20} anthelmintic,\textsuperscript{21,22} antimicrobial,\textsuperscript{23,24,25} antiulcer\textsuperscript{26} and antiulcer\textsuperscript{27} activities. Many coumarin derivatives have also been used extensively in designing various pesticidal agents including some well-known rodenticides and insecticides.\textsuperscript{28}

The compound bearing azo, azomethine group and 2-amino-thiazole derivatives are known to exhibit bacteriostatic, anticancerous, inhibitor of tumor growth\textsuperscript{29,33} and other biochemically important activities. The chemical compounds having reactive center which can combine to enzyme, co-enzyme and
apoenzyme are able to exhibit a biological activity and in many cases this combining with enzyme is nothing but a formation of metaloenzyme complex.\textsuperscript{34,35}

Many drugs contains the common electron donor groups\textsuperscript{36,37} like

\[
= \text{N} \equiv, \quad \text{-NH}_2, \quad \text{-NH}, \quad \text{-N}, \quad \text{-C}=\text{N}-, \quad \text{-N}=\text{N}, \quad \text{-N-N}-, 
\]

\[
= \text{-N}=\text{O}, \quad =\text{C}=\text{N}=\text{O}, \quad =\text{C}-\text{O}-, \quad =\text{C}-\text{S}, \quad =\text{C}=\text{S} \text{ etc.}
\]

The actual donor groups to be reacted with metalo-enzyme depend upon many factors. These factors include the character of the metal, nature of donor group and type of dissolving solvent. Some carcinogens are capable of acting directly as powerful organic compounds, either in an aqueous or in non-aqueous environment and other can react directly by undergoing metabolic reactions.

It is also reported that certain \(\omega\)-aminophenols are carcinogenic owing to their ability to combine with metal ions in the body (as metalo-enzymes). Bonser, Clayson and Jull\textsuperscript{36} found that a number of \(\omega\)-aminophenol derivatives in addition to \(2\)-amino-\(1\)-napthol produces cancer of the bladder. They have suggested that aromatic amines may be active after conversion to \(\omega\)-aminophenols.

Weinman et al.\textsuperscript{37} have investigated the effect of azoesters and some heterocyclic compounds on the germination of fungus \textit{Trichoderma viride} and noted that the germination is either inhibited or severely delayed by the reagent.

Chichibabin and Zeide\textsuperscript{38} observed that certain diazo dyes having negligible toxicity exhibit a high bacteriostatic index (this term designate here maximum
Among many biologically active compounds, those derived from thiazole amine are biologically more active due to the presence of thiazole nucleus. These have been found to possess herbicidal properties and antitumor activity. Richmond and Somars have observed that germination of the fungus *Neurospora crassa* accompanied an increase in the \(-\text{SH}\) content and the rate of spore germination decreases on treatment with the irreversible alkylating agent like iodoacetic acid which diminishes the soluble \(-\text{SH}\) of conidia.

### 6.2 Antifungal Activity

In present study, compounds 1 to 8 have been tested for the inhibition of the growth of fungi *Aspergillus niger* and *Rhizopus* and results are summarized in table 6.1.

The fungi *Aspergillus niger* and *Rhizopus* used in the present investigation were produced by the Nikhil Analytical and Microbiological Laboratory, SANGLI.

#### 6.2.1 Method of Testing the Antifungal Activity

The fungus inoculum was prepared by using the fungus sabouraud’s broth to which 100 \(\mu\text{g/ml}\) of streptomycin was added to prevent bacterial contamination. After sporulation the spores were harvested in the same media by gentle stirring using a magnetic stirrer and further spore suspension was decanted into another sterile flask.
Into 5 ml of subouraud’s broth contained in 15 ml corning test tubes, 0.1 ml of the test solution (10 mg/ml in DMF) was added and autoclaved at 15 lb pressure for 15 min. The tubes were then cooled and were inoculated with 0.1 ml of spore suspension. Salicylic acid was used as standard antifungal compound. The tubes were then kept on a rotary shaker and incubated at room temperature for 24 h. The percent inhibition growth of the fungus was calculated after determining the optical density of the solution with a spectrophotometer Elico – Model CL-24 at 530 nm with inoculated sabouraud’s broth as blank.

The growth of fungus in the tube which contained none of antifungal agent was assumed as 100 percent. Some of the compounds were coloured and hence an equal amount of compound was added to the subouraud’s broth which was to use as blank. Subouraud’s broth contained glucose 40 g/lit, peptone 10 g/lit and distilled water. It was autoclaved at 15 lb pressure for 15 min.

6.2.2 Results and Discussion

From Table 6.1, it can be seen that all compounds have shown inhibition in growth for both species studied i.e. Aspergillus niger and Rhizopus. The parent compound 7-hydroxy-4-methyl coumarin shown 12% inhibition against Aspergillus niger while 14% for Rhizopus. In compound 2, where there is a substitution of 2- azothiazole on parent compound the inhibition almost remained unaltered for both species. However, further addition of methyl and phenyl groups at 4-position of thiazole ring (compounds 3 and 4 respectively) shown a considerable enhancement in growth of inhibition and it is reached up to 18% and 30% for both the species tested. The highest inhibition i.e. 34% and 32% is observed for compound 5 against Aspergillus niger and Rhizopus respectively. This enhancement in growth inhibition can be correlated to the presence of chloro...
group on the $p$-position of the phenyl ring. Similar type of observation is also reported by Audus and Quastel.\textsuperscript{41} Two derivatives of coumarin viz. monochlorocoumarin and coumarin carboxylic acid were tested on the carrot, the former has about one twenty fifth the inhibitive effect of coumarin on the growth rate and one quarter the inhibitive effect coumarin on germination. The later carboxylic acid has about one half the inhibitive activities of mono chloro coumarin. These results make it clear that the presence of chloro substituent generally enhances the photocidal action.

In compound 6, where chloride group is replaced by methyl, the growth inhibition of \textit{Aspergillus niger} and \textit{Rhizopus} is found to be 28\%, similar to that of compound 4. Further additions of methoxy and nitro groups instead of chloride, at $p$-position of phenyl ring (compound 7 and 8), there appeared a lowering in inhibition and it was found to be 10 to 16\%. Overall growth inhibition for all compounds is found to be slightly higher in \textit{Rhizopus} than \textit{Aspergillus niger}.

\section*{6.3 Antibacterial Activity}

All compounds have been tested for gram positive organisms like \textit{Bacillus subtilis} and \textit{Staphylococcus aureus} as well as gram negative organisms like \textit{Escherichia coli}, \textit{Klebsiella pneumoniae}, \textit{Salmonella paratyphi B}, \textit{Pseudomonas aerogens} and \textit{Proteus vulgaris}. The corresponding results are described in Table 6.2.

\subsection*{6.3.1 Preparation of Media}

Nutrient agar was prepared by dissolving bacteriological peptone (1\%), meat extract (0.5\%), sodium chloride (0.5\%) in distilled water and the pH of solution was adjusted to 7.4 by using sodium hydroxide solution (40\%). This
solution was filtered, agar was added (2%) and then sterilized for 30 min at 15 lb pressure.

6.3.2 Preparation of Subcultures

A day before the test, the bacterial types were inoculated into sterilized nutrient broth tubes and incubated at 37 °C for 18-24 h. The petridishes were wrapped with brown paper and sterilized for 30 min at 15 lb pressure. These were further dried in oven. Tuberculin syringe and needles were sterilized by boiling them in water for 5-10 min. The test tubes were sterilized in an autoclave at 15 lb pressure for 20 min.

6.3.3 Method of Testing

The nutrient agar was melted in hot water bath and was cooled at 45 °C with gentle shaking to bring about uniform cooling. This was poured into the petridishes 20-25 ml each and then allowed to solidify. To this, 0.5 – 0.6 ml of 18-24 h old culture was added ascetically and the inoculum was spread evenly by using a spreader. There after, the sterilized filter paper discs 6 mm in diameter, were dipped into the test solution (10^{-3} M) and were placed on seeded medium. The plates were incubated at 37 °C. The extent of inhibition was measured by zone of inhibition produced in millimeters after 24 h.

Aqueous phenol was used as positive control and solvent dimethyl formamide (DMF) was also run to know its activity.

6.3.4 Results and Discussion

7-Hydroxy – 4-methyl coumarin (compound 1) was found to be less active against Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Salmonella
paratyphi B, Staphylococcus aureus, Proteus vulgaris and moderately active against Pseudomonas aerogens. Upon substitution of 2-amino azothiazoie on the parent coumarin (compound 2), the activity remained unaffected against Escherichia coli, Salmonella paratyphi B, Staphylococcus aureus and Proteus vulgaris. However, against Bacillus subtilis, Klebsiella pneumoniae and Pseudomonas aerogens compound 2 is found to be moderately active.

In compound 3, where there is addition of methyl group at the 4- position of thiazole ring, the antibacterial activity is found to be enhanced in all the species tested. For Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Salmonella paratyphi B, Staphylococcus aureus and Proteus vulgaris, the compound have shown moderate activity. Highest activity by this compound (3) is found against Pseudomonas aerogens. In compound 4, it has been found that the replacement of methyl group by a phenyl ring has enhanced the activity against all the species tested except Staphylococcus aureus where it is totally inactive. In case of Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Salmonella paratyphi B and Proteus vulgaris compound 4 is found to be highly active while against Pseudomonas aerogens it has shown moderate activity. Amongst all compounds (1 to 8), compound 4 is found to posses high activity against maximum species tested.

Further, substitution on phenyl ring, at 4- position of thiazole ring have lowered the bacterial activities than unsubstituted phenyl ring, however, the activities are still remained at higher side as compared to parent coumarin molecule. Compound 5 was moderately active against Klebsiella pneumoniae, Pseudomonas aerogens, and Proteus vulgaris and less active against salmonella para typhi B while totally inactive against Escherichia coli, Bacillus subtilis and staphylococcus aureus. Least activity against all species is observed for
compound 6, it is found to be totally inactive against Escherichia coli, Bacillus subtilis, and Staphylococcus aureus, less active against Klebsiella pneumoniae, Pseudomonas aerogens and Salmonella paratyphi B while only moderately active against Proteus vulgaris.

Compound 7 has shown to possess high activity against Staphylococcus aureus and Proteus vulgaris, moderate activity against Escherichia coli and Bacillus subtilis, very less active against Klebsiella pneumoniae and Salmonella paratyphi B and totally inactive against Pseudomonas aerogens.

Antimicrobial activity of compound 8 is just comparable with compound 7. It is moderately active against Staphylococcus aureus, Proteus vulgaris, Escherichia coli and Bacillus subtilis, less active against Klebsiella pneumoniae, and Salmonella paratyphi B while totally inactive against Pseudomonas aerogens.

In conclusion, substitution of azothiazole moieties on parent 7-hydroxy - 4-methyl coumarin enhances antibacterial activity. All compounds are active against Proteus vulgaris, Salmonella paratyphi B and Klebsiella pneumoniae. Maximum activity is observed for compounds 3 and 4, medium activity is shown by compounds 2, 7, 8 and 5 and least activity is shown by compound 6.
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<td>++ Positive activity (moderately active)</td>
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Table 6.2 Bacteriological results of compound

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6. References:


