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# STUDIES ON ANTIBACTERIAL ACTIVITY OF MONOAZO DISPERSE DYES BASED ON 2,4 – DIHYDROXY – 6 – METHYL QUINOLINE

**Dina Y. Champaneri[1], A.G.Mehta[2]**

[1]*Chemistry Department, B. P. Baria Science Institute, Navsari, (India)*
[2]*Retired Principal, Sir P.T. Sarvajanik College of Science, Surat, (India )*

**ABSTRACT**

*A large number of quinoline derivatives possess wide therapeutic activity. Realizing the medicinal importance of azo compounds and quinoline derivatives, it was considered worthwhile to incorporate these two compounds. In the present paper, disperse dyes synthesized by coupling of diazotized components with 2,4-dihydroxy-6-methyl quinoline were tested for antibacterial activity using "Disc Diffusion Technique" and the results are presented.*

***Key words: 2,4- dihydroxy-6-methyl quinoline, antibacterial activity, B. Subtilis, S. aureus, E. Coli, Salmonella paratyphi B, Zone of inhabition.***

## I INTRODUCTION

Organic compounds which has the ability to impart colour to the material to be dyed are called "dyes". The art and science of dyes began more than 10,000 years ago but the method of extraction of these dyes from natural sources and their application on textile materials were cumbersome and secondly these dyes being natural products, it could not be obtained in enough quantity. In the modern period, these dyes are prepared on a large scale from aromatic compounds. Picric acid was the first synthetic dye prepared by Woulfe by the action of nitric acid on indigo. Disperse dyes came into existence after the introduction of cellulose acetate fibres. These fibres, being hydrophobic in nature, could not be dyed with those meant for hydrophilic fibres like cotton, wool and silk. Hence, the need for a new dye arose, one which could dye cellulose acetate. The disperse dyes gave the most satisfactory results due to their simple application properties and ability to cover regularities in yarn [ 1 ]. The dyes based on heterocyclic ring system are known to possess high tinctorial power and good fastness properties. Heterocyclic coupling components give heterocyclic azo disperse dyes with colour ranging from yellow to deep red. The synthesis and application of azo dyes derived from quinoline [ 2 – 5 ] and quinolinoquinazoline [ 6 ] systems have been reported.

Bacteriology is the science that deals with the study of bacteria, the microscopic organisms of plant and animal kingdom. The microbiologists and clinical personnel overlooked the possibility that the bacteriostatic compounds would inhibit the reproduction of pathogenic bacteria and enable the leucocytes and other defence mechanism of the host to cope up with few static invaders.

Paul Ehrlich, the father of chemotherapy used chemotherapeutic agents for curing the infectious disease without injury to the host's tissue such as antibacterial, antiprotozoal, antiviral, antineoplastic, antitubercular and antifungal agents. Antibacterial substances and preparation are classified as disinfectants, antiseptics and chemotherapeutic agents. The term "disinfectants" is used to eliminate or destroy infections and should be capable of killing a wide range of bacteria and not the bacterial spores. An antiseptic is used to control or to eliminate bacterial infection and as much it should have antibacterial properties similar to those of infectants. A chemotherapeutic agent is an antibacterial substance administered systematically for the treatment of infection; its main function is to prevent the multiplication of infective organisms.

In the present paper, we report on the antibacterial activity using the " Disc Diffusion Technique " described by Bauer et al., [ 7 ] of some new azo disperse dyes [ DINA Y. CHAMPANERI and A. G. MEHTA, Multi Disciplinary Edu Global Quest (Qtly), Volume 2, Issue 1≠5, January 2013 ]. The bacteria selected for this study were Escherchia coli, Salmonella paratyphi B, Bacillus subtilis and Staphylococcus aureus.

## II EXPERIMENTAL

The bacteriostatic property of the compound was tested by disc diffusion method as described by Bauer.

[A] Preparation of Mueller – Hinton agar

| | | | |
|---|---|---|---|
| (1) | Beef infusion | : | 300 g. |
| (2) | Acid hydrolysate of Casein | : | 17.5 g. |
| (3) | Starch | : | 1.5 g. |
| (4) | Agar | : | 17 g. |
| (5) | Distilled water | : | 1 litre. |

The above constituents were weighed and dissolved in water. The mixture was warmed on water bath till agar dissolved. This was then sterilized in an autoclave at 15 lbs pressure and 121°C for fifteen minutes. The sterilized medium (20.0 ml) was poured in sterilized Petridishes under aseptic conditions, allowing them to solidify on a plane table.

[B] Preparation of antibacterial solution

All the compounds were dissolved in dimethylformamide (DMF). Proper drug controls were used. Fig. 1 and Fig. 2 represent the control and the compound.

2,4-Dihydroxy-6-methyl quinoline derivatives and monoazo disperse dyes based on 2,4-Dihydroxy-6-methyl quinoline derivatives were taken at the concentration 50 µg per ml for testing antibacterial activity. The compound diffused into the medium produced a concentration gradient. After the incubation period, the zone of inhibition was measured in mm. The tabulated results represent the actual readings control.

[C] Test cultures

The test bacterial cultures used were grown over night in Mueller – Hinton broth. These test cultures were as follows;

| | |
|---|---|
| *Bacillus subtilis* | gram-positive large rods |
| *Staphylococcus aureus* | gram-positive cocci |
| *Escherichia coli* | gram-negative small rods |
| *Salmonell para typhi – B* | gram-negative small rods |

[D] Inoculum preparation

The inoculum was standardized at $1.5 \times 10^8$ CFU/ml by comparing with turbidity standard (0.5 MacFarland tube).

[E] Swabs preparation

A supply of cotton wool swabs on wooden applicator sticks was prepared. They were sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat.

The following experimental procedure was followed:

[F] Procedure

(1) The plates were inoculated by dipping a sterile swab into the inoculum. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid.

(2) The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60° after each application. Finally the swab was passed round the edge of the agar surface. The inoculum was dried for a few minutes, at room temperature, with the lid closed.

(3) The antibiotic discs were placed on the inoculated plates using a pair of sterile forceps.

(4) A sterile needle tip was used to place the antibiotic discs on the plate.

(5) The plates were placed in an incubator at 35°C within 30 minutes of preparation.

(6) After overnight (16 to 18 hours) incubation, the diameter of each zone (including the diameter of disc) was measured and recorded in mm. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid.

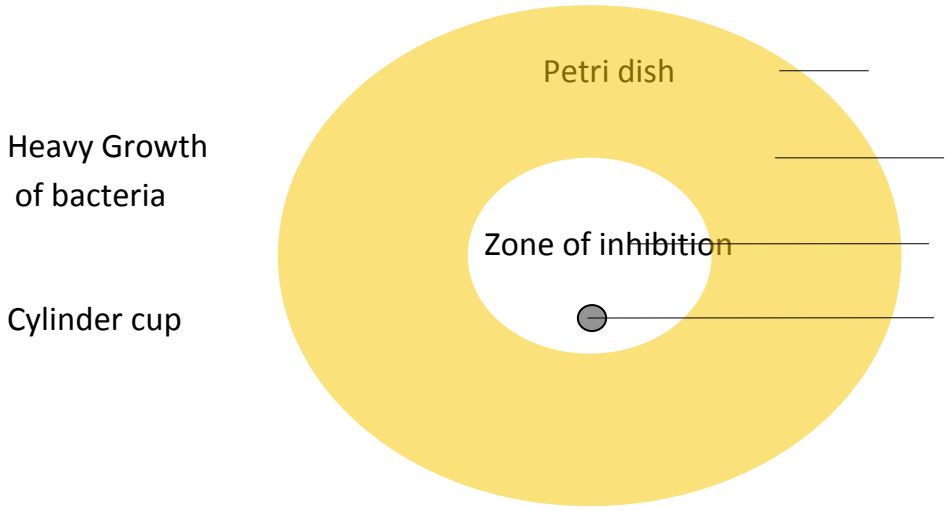



**Fig. 1 Cylinder cup method (Agar Diffusion Technique) with essential arrangement (diagrammatic).**

**Fig. 2 Zone of inhibition with antimicrobial agents**

## III RESULT AND DISCUSSION

The rationale behind the synthesis of these compounds is as follows.

The antibacterial activity of various quinoline derivatives has been reported. Azo compounds are known for their antibacterial properties. Antibacterial activity of 2,4- dihydroxy-6-methyl quinoline and mono azo disperse dyes based on 2,4- dihydroxy-6-methyl quinolone were tested.

The organisms selected were :

1. *Bacillus subtilis* gram-positive large rods
2. *Staphylococcus aureus* gram-positive cocci
3. *Escherichia coli* gram-negative small rods
4. *Salmonella paratyphi B* gram-negative small rods

The results of these compounds showing antibacterial activities are given in tables 1 and 2.

**Table -1**

| Sr. No. | Name of the compound | Zone of inhibition in mm | | | |
|---|---|---|---|---|---|
| | | *Bacillus subtilis* | *Stayphylococcus aureus* | *Escherichia coli* | *Salmonella paratyphi B* |

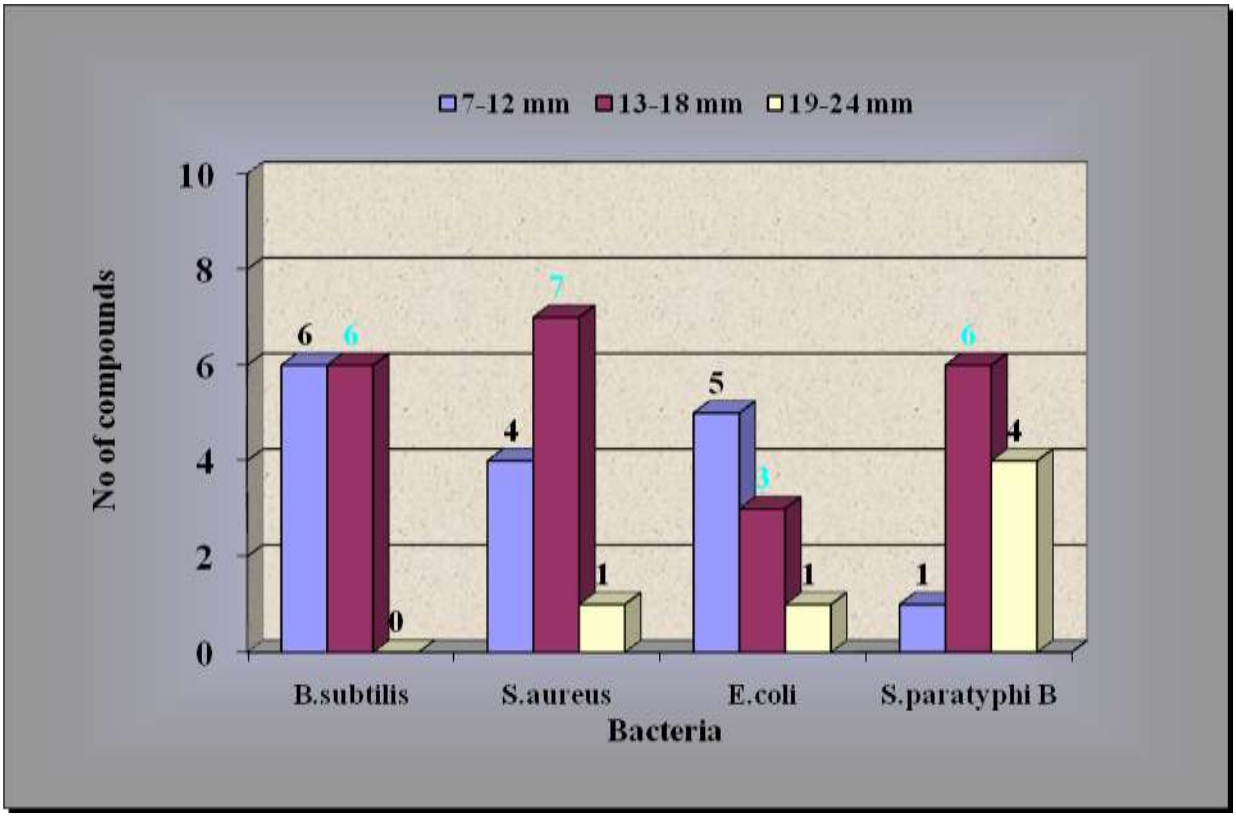
| | | | | | |
|---|---|---|---|---|---|
| 1 | 2,4 Dihydroxy - 6-methylquinoline | 11 | 11 | Nil | 20 |
| 2 | Solvent ( DMF ) | Nil | Nil | Nil | Nil |

**Table – 2**

| Dye No. | Coupling Component | Molecular Formula | Zone of inhibition in mm | | | |
|---|---|---|---|---|---|---|
| | | | *Bacillus subtilis* | *Staphylococcus aureus* | *Escherichia coli* | *Salmonella paratyphi B* |
| D-1 | 2-Amino-5,6-dichlorobenzothiazole | $C_{17}H_{10}O_2N_4Cl_2S$ | Nil | 16 | Nil | 21 |
| D-2 | 2-Amino-6-nitrobenzothiazole | $C_{17}H_{11}O_4N_5S$ | 10 | Nil | 17 | Nil |
| D-3 | 3-Chloro-4-fluoroaniline | $C_{16}H_{11}O_2N_3ClF$ | 14 | 15 | 20 | Nil |
| D-4 | 4-Aminoacetanilide | $C_{18}H_{16}O_3N_4$ | 12 | 13 | Nil | Nil |
| D-5 | 4-Nitroaniline | $C_{16}H_{12}O_4N_4$ | 15 | Nil | 18 | 15 |
| D-6 | p-Toluidine | $C_{17}H_{15}O_2N_3$ | 12 | 20 | 12 | 20 |
| D-7 | 4-Chloroaniline | $C_{16}H_{12}O_2N_3Cl$ | Nil | Nil | Nil | 15 |
| D-8 | 3-Aminoacetanilide | $C_{18}H_{16}O_3N_4$ | Nil | 10 | Nil | 22 |
| D-9 | 4-Chloro-2-nitroaniline | $C_{16}H_{11}O_4N_4Cl$ | Nil | 16 | 12 | Nil |
| D-10 | 2,6-Dibromo-p-toluidine | $C_{17}H_{13}O_2N_3Br_2$ | 13 | 8 | 12 | 13 |
| D-11 | 6-Chloro-2,4- dinitroaniline | $C_{16}H_{10}O_6N_5Cl$ | 14 | 11 | 14 | 19 |
| D-12 | 6-Bromo-2-cyano-4-nitroaniline | $C_{17}H_{10}O_4N_5Br$ | 10 | Nil | Nil | Nil |
| D-13 | 4-Acetylamino-2-aminoanisole | $C_{19}H_{18}O_4N_4$ | 11 | 11 | 10 | 15 |
| D-14 | 2-Cyano-4-Nitroaniline | $C_{17}H_{11}O_4N_5$ | 14 | 16 | Nil | 10 |
| D-15 | 2,6-Dibromo-4-Nitroaniline | $C_{16}H_{10}O_4N_4Br_2$ | 15 | 14 | Nil | 14 |

| | | | | | | |
|---|---|---|---|---|---|---|
| D-16 | 3-Amino-o-sulphophenylphenol | $C_{22}H_{17}O_5N_3S$ | Nil | 13 | Nil | 14 |
| D-17 | 5-Nitro-2-methoxyaniline(SCRbase) | $C_{17}H_{14}O_5N_4$ | 12 | Nil | 12 | Nil |

### 3.1 Graphical Representation of Results




2,4-dihydroxy-6-methyl quinoline is active against gram positive organisms (B. subtilis and S. aureus) and gram negative bacteria S. paratyphi B.

It would be seen from table – 2 that highest antibacterial activity is exhibited by compounds $D_5$ and $D_{15}$ (B. subtilis),$D_6$ (S. aureus), $D_3$ (E. coli) and $D_8$ (S. paratyphi B). Compounds $D_6$ , $D_{10}$, $D_{11}$, $D_{13}$ show fairly good antibacterial activity towards all the test organisms. The compound $D_7$ does not show any antibacterial activity towards gram positive bacteria while compounds $D_4$, $D_{12}$ are not effective against gram negative bacteria.

Comparing the antibacterial activity of these compounds with the parent 2,4-dihydroxy-6-methyl quinoline, it seen that compounds $D_3$ to $D_6$, $D_{10}$, $D_{11}$, $D_{14}$, $D_{15}$ and $D_{17}$ show increased antibacterial activity against B. subtilis and compounds $D_1$, $D_3$, $D_4$, $D_6$, $D_9$, $D_{14}$ to $D_{16}$ against S.aureus .The compounds $D_2$, $D_3$, $D_5$, $D_6$, $D_9$ to $D_{11}$, $D_{13}$ and $D_{17}$ show pronounced antibacterial activity against E. coli while compounds $D_1$, $D_8$ show moderate antibacterial activity against s. paratyphi B.

The compound $D_3$ having fluoroand chloro group in the diazo components show maximum additive antibacterial activity against gram negative bacteria E. coli. The compound $D_6$ having methyl group in the parent 2,4-diahydroxy-6-methyl quinoline and the diazo compound show highest antibacterial activity against gram positive bacteria S.aureus.

The compounds $D_5$ and $D_{15}$ having either nitro,fluoro-chloro and bromo groups in the diazo component show maximum antibacterial activity against gram positive bacteria B. subtilis.

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