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Synthesis, Identification and Antibacterial Potency of Azo Dyes having Quinolin-8-ol and Active Methylene Moiety

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ABSTRACT: In the present study we have reacted the five aryl diazonium salts prepared from nitro substituted anilines and amino substituted benzoic acids and coupled with Quinolin-8-ol and Active methylene group of pent-1,4-dione respectively. Synthesized compounds are then identified by FTIR Spectroscopical method. The final product, I to V formed has potential to use as azo dyes and as an intermediate in other synthetic procedures or transformations along with this they exhibits good biological activities.

Keywords: Active methylene group; diazonium salt; antibacterial activity; nitroanilines and azo dye.

INTRODUCTION: Azo compounds were under study from the long time for their remarkable partaking in pharmaceuticals, cosmetics and textiles industries. They have immense important biological activities and high therapeutic values for all mammals reported by Sisley and Porsche, 1911[1]. reductive cleavage of Azo group gives great therapeutic properties for treatment of serious disorders in human beings[1,2,3].Biological outcomes from an enzymatic metabolism (occurs in *vivo)*[4, 5] for example in skin bacteria such as *Staphylococcus aureus*[6] which involves reduction of azo group (-N=N-) to produce toxic or nontoxic resultant amines[7] which may produce carcinogenic effects[8, 9]. Despite of having negative role for environment and human health azo groups have attracted medical attentions. Later studies showed health hazards from these azo families in the form of carcinogenic and mutagenic properties, even though many of the synthetic strategies have been developed to produce these classes of compounds for their specific physico-chemical and biological activities. The azo coupling reaction with AMG results into formation of bioactive compounds like cinnolines which is one of the important class of natural products having remarkable pharmaceutical and biological importance[10]. Due to simple process in aqueous media we can produce wide varieties of azo dyes and can determined biological actions. In our study we synthesized novel azo compound in two different schemes forming diazonium salt of o and m substituted nitro anilines and substituted amino benzoic acids followed by coupling reaction with quinolin-8-ol and active methylene group of acetyl acetone respectively, so here in we reported some novel azo compounds and testing out their antibacterial potency on selected strains of bacterias like *Staphylococcus aureus* and *Bacillus subtilis* with discussion of their infections.

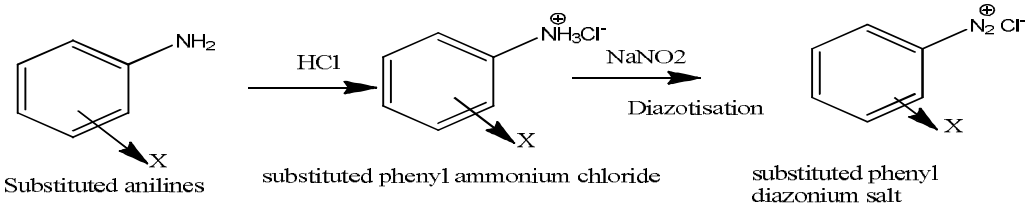
Many authors have reported about the infections registered through *S. aureus* and *B. subtilis* bacterias in human body. Logan et al. 1988 stated the infections with bacilli species in impotent patients that is who are immunologically compromised. Similarly Kiss et al.1988 also reported the *B. subtilis* infections in enervate patients. Donzis et al. 1988 reported *B. subtilis* eye infections due to contaminated lenses. Penington et al.1976 described infections in patients having blood cancer.

Staphylococcus aureus is also one of the dangerous pathogen found in human. Rates of *S.aureus* infections are high among patients with type-1 diabetes,[12] intravenous drug users,[13] patients facing hemodialysis,[14] patients having done with major surgery,[15,16] and who are HIV positive[17]. Patients with leukaemia are also at increased risk for staphylococcal disease[18]. So therefore many studies have been done for preparing antibiotics for these organisms.

MATERIALS AND METHODS: All the chemicals were of standard grade and used without further puri-

fications. Silica gel was used to monitor the progress of reactions by TLC and visualized under Iodine chamber. The colours of resultant dyes are recorded visually and melting point range were recorded by one end open capillary tube method. The purity of compounds was noted by melting point determination and silica gel-G TLC, elemental analysis (C,H,N), FTIR spectral data. The bacterial strains, *Staphylococcus aureus* and *Bacillus subtilis* are purchased from National Centre for Cell Science (NCCS), Pune, India and maintained at Smt. G. G. Khadse College, to determine the antibacterial activity of synthesized all five azo compounds (I-V).

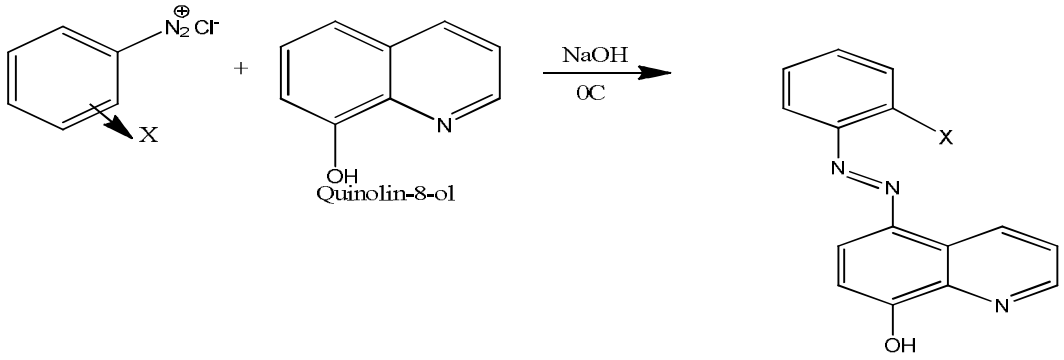
Scheme 1: General procedure for synthesis of azo dyes[20] (I-III)



X= -H (I), 2-NO_2 (II), 3-NO_2 (III)

Stage 1: Preparation of Diazonium Salt - In 100 ml Capacity beaker add 2 ml aniline (or its derivative) and to this mixture of 5ml conc. HCl and 10 ml water were added and stirred with the glass rod to get clear solution. Cool, the solution upto 0º C by keeping in freezing mixture. Dissolving (1gm) sodium nitrite in 10 ml water. Allowed to cool the solution in ice bath to 0º C, after attaining 0º C we added $NaNO_2$ solution into aniline hydro chloride (or derivatives) solution drop wise with constant stirring (kept maintained temperature below 50ºC during addition). After decompose of excess of nitrous acid by adding pinch of urea filtered the solution and collect the filtrate which was diazonium salt of aniline and its derivatives.

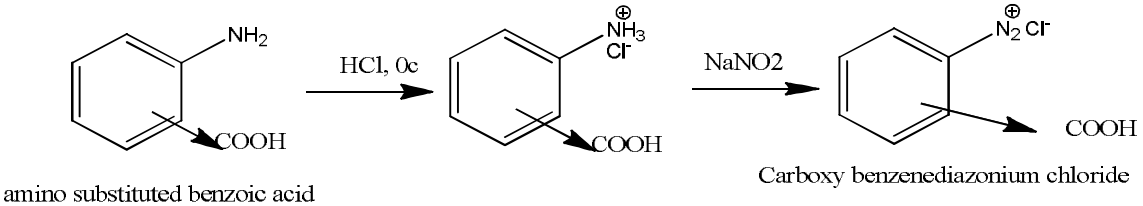
Stage 2: Diazonium coupling reaction with quinolin-8-ol - Prepared a mixture of solution of 1.5gm quinolin-8-ol in 10 ml 10% NaOH and allowed to cool up to 0ºC, after attaining 0ºC ,we added solution of diazonium salt dropwise in to quinolone-8-ol in NaOH solution with constant stirring, after complete addition allowed reaction mixture to stand for 10 min in ice bath, filtered the azo colourant and washed it with cold water, dry, weight and noted the yield of clear crude azo product then recrystallized by using solvent ethanol. Recorded the dried weight and the color , melting point range of compounds.



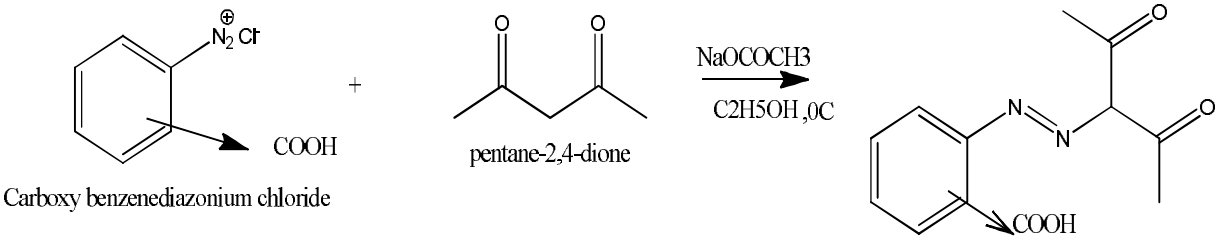
(E)-5-[(nitro substituted phenyl) diazenyl] quinolin-8-ol

Scheme 2: General procedure for synthesis of azo dyes (IV & V)

Stage 1: preparation of diazonium salts of amino substituted benzoic acids - Same as discussed in scheme-1.



Stage 2: Diazonium coupling reaction with AMG of Acetyl acetone (pent-2,4-dione) - Add (-o/-p) carboxy aryl diazonium salt solution drop wise, to the well cooled mixture of, Pentane-2,4-dione (1.8ml) which is dissolved in 5 ml ethanol and sodium acetate , 8-10 gm in 10-15ml of water and maintained the 0^0C temperature , a coloured precipitate is separated, then adding 20 ml of con. HCl the product is filtered, then by checking the absence of ester, the product obtained is recrystallized by using ethanol, dried it. Record the dried weight (in gms) and then melting point range of the compounds VI & V.



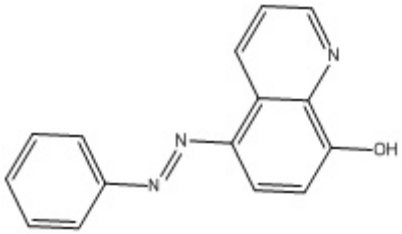
2, 4-dioxopentan-3-yl) diazenyl) Substituted benzoic acid (IV & V)

Substitution on benzoic acid: 2-NH_2 (IV) & 4-NH_2 (V)

The synthesized azo compounds (I-V) were tested for their anti-bacterial activity against two strains of bacterias named *Staphylococcus aureus* and *Bacillus subtilis* by disc diffusion assay using solutions of azo compounds at two different concentrations of 500 & 1000 µg/ml.

RESULTS AND DISCUSSION: In the present study diazonium salts of aniline (and nitro derivatives) in scheme-1 is coupled with Quinolin-8-ol which results into formation of compounds I[19] (B.E. Ezema et,al. 2014) II & III 5-[(nitro substituted phenyl) diazenyl] quinolin-8-ol. Similarly in scheme-2, carboxy diazonium salt is coupled with AMG of Pent-2, 4-dione to form products IV & V (2,4-dioxopentan-3-yl)diazenyl) substituted benzoic acids. The synthesised compounds then screened for antibacterial activity at two antibiotic concentrations of 500 and 1000 µg/ml. All the compounds were of high purity and ascertained by melting point determinations as well as by silica gel TLC. The probable structural of the compounds (I-V) was assigned by FTIR spectroscopical method. The I.R frequencies of the presents functional groups are shown in Table 1 The name of the compound, practical yield, melting point range and color are shown in Table 2 .The photographical view of antibacterial action (zone of inhibition shown in Table 3) of azo compounds on two strains *Staphylococcus aureus* and *Bacillus subtilis* has been figured as a, b, c, d and e. The antibacterial activity test shown that the synthesised compounds I and II is active against both of the bacterial strains , and compound IV is active towards only *S. aureus* at both of the concentrations while the azo compounds III and V has no action towards any of the tested strains. Results outcomes has also confirms that the compound II is most active against both of the bacterial strains forming growth inhibition zone upto 18mm at 1000 µg/ml while at lower concentration of 500 µg/ml there is occurrence of smaller inhibition zone of 11mm and 15mm in *S.aureus* and *B.subtilis* respectively. This concludes that azo colorant II can exhibit better antibiotic properties against *S.aureus* as well as *B. subtilis* with increasing concentration.

Table 1: FTIR spectral data for the synthesized azo compounds (I-V).

Compound ID	IR cm^{-1}	Assigned structure
I	$\gamma_{N=N}$ =1311-1193 γ_{Ar-H} =1502-1407 γ_{CH} =2992-2825 γ_{C-O} =1193-1072 $\gamma_{Para-sub}$ = 898-668 γ_{NO2} =1669-1598	

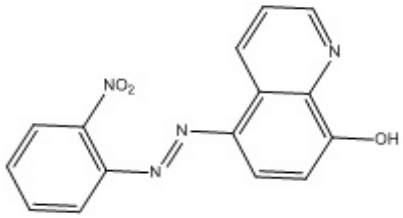
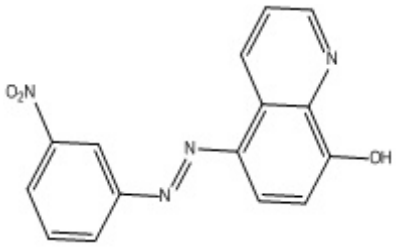
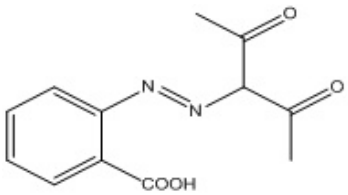
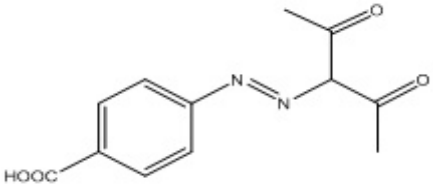
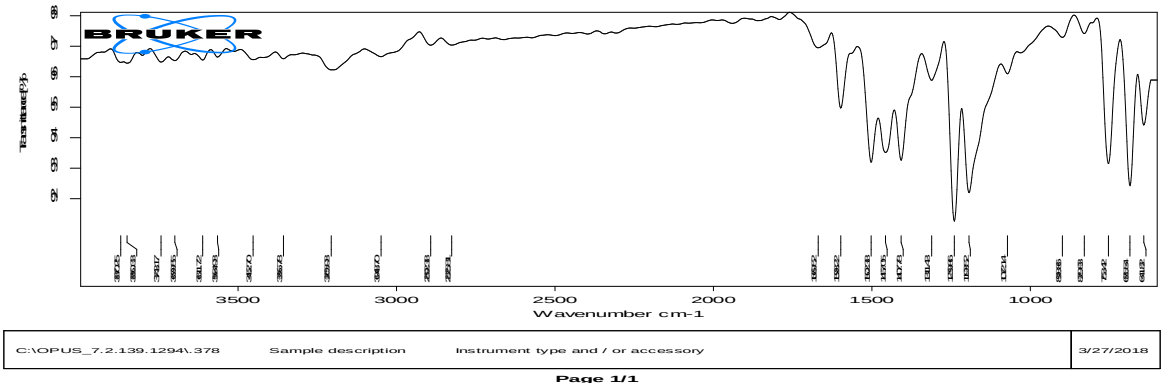
II	$\gamma_{N=N}$ = 1277-1164 γ_{Ar-H} = 1574-1405 γ_{C-H} = 2892 γ_{C-O} = 1093-1024 γ_{p-Sub} = 816-638 γ_{NO2}= 1735-1668 γ_{Ar-OH} = 3601-3505	
III	$\gamma_{N=N}$ – 1346-1289 γ_{Ar-H}=1572-1462 γ_{C-H} -2887-2823 γ_{C-O} = 1228-1071 $\gamma_{Para\ sub\ Ar}$ 786-672 γ_{NO2}=1735 $\gamma_{Ar—OH}$ =3616-3523	
IV	$\gamma_{Ar-c=c-}$ = 1503 &1602cm^{-1} $\gamma_{-N=N-}$ =1282 cm^{-1} $\gamma_{ortho\ di\ sub.\ Ring}$ =723 &748 cm^{-1} γ_{-COCH3} = 1362 cm^{-1}	
V	$\gamma_{Ar-c=c-}$ = 1487&1577cm^{-1} $\gamma_{-N=N-}$ = 1259cm^{-1} γ_{-COCH3} = 1362&1316cm^{-1} $\gamma_{p-sub.}$ = 851 cm^{-1}	

Table 2: Analytical and physical data of azo colourants I-V.

Azo compounds	Practical Yield	Melting point range	Colour
(E)-5-(phenyldiazenyl)quinolin-8-ol (I)	81.73%	222- 226º C	Glittering red
(E)-5-((2-nitrophenyl)diazenyl)quinolin-8-ol (II)	87.16%	259- 261º C	Reddish brown
(E)-5-((3-nitrophenyl)diazenyl)quinolin-8-ol (III)	79.49%	243 -246º C	Yellowish
(E)-2-((2,4-dioxopentan-3-yl)diazenyl) benzoic acid (IV)	68.84%	259-260º C	Bright yellow
(E)-4-((2,4-dioxopentan-3-yl)diazenyl) benzoic acid (V)	81.52%	240-242º C	Greenish yellow

Table 3: Showing antibacterial actions of the compounds in mm of zone of inhibition.

Compound → / Bacterial strain ↓	I		II		III		IV		V	
	Concentration		Concentration		Concentration		Concentration		concentration	
	500 µg/ml	1000 µg/ml	500 µg/ml	1000 µg/ml	500 µg/ml	1000 µg/ml	500 µg/ml	1000 µg/ml	500 µg/ml	1000 µg/ml
	zone diameter (mm)		zone diameter (mm)		zone diameter (mm)		zone diameter (mm)		zone diameter (mm)	
B. subtilis	06	08	11	18	-	-	-	-	-	-
S. aureus.	06	08	15	18	-	-	08	08	-	-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Wavenumber cm-1
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Sample description
Instrument type and / or accessory
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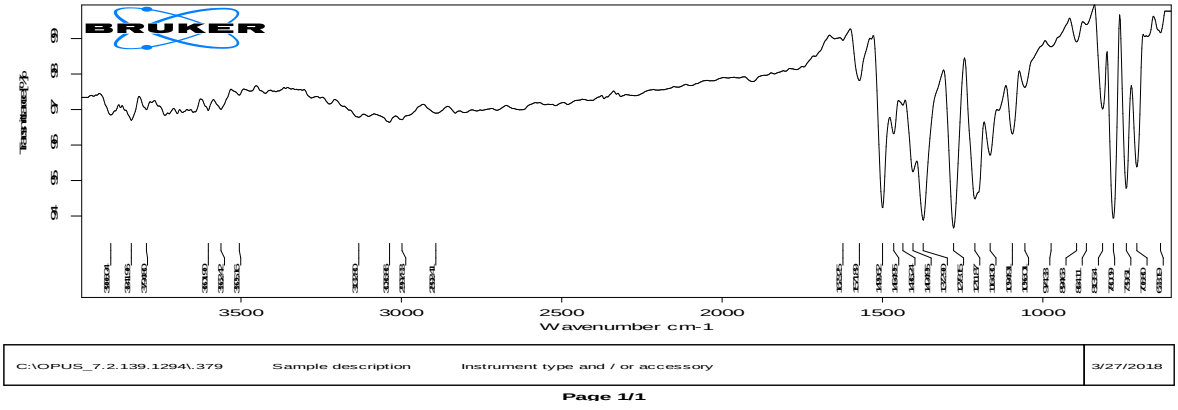
(a)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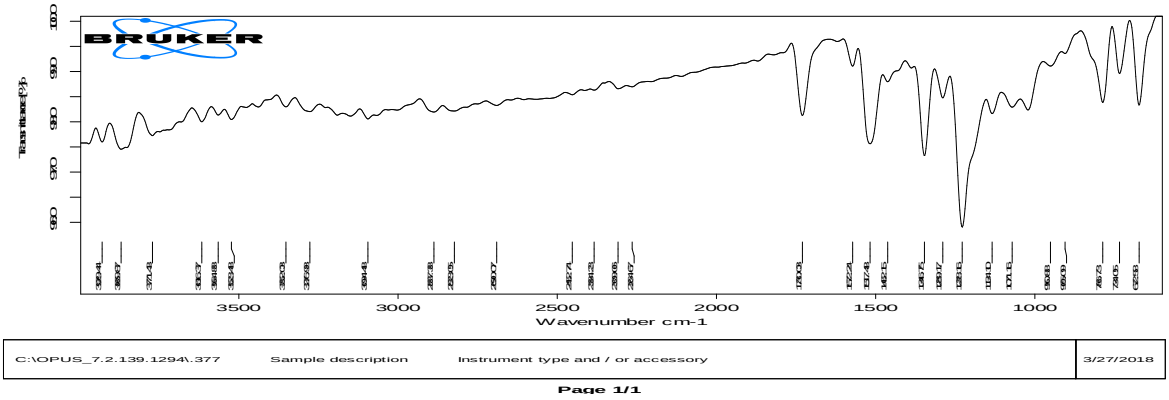
(b)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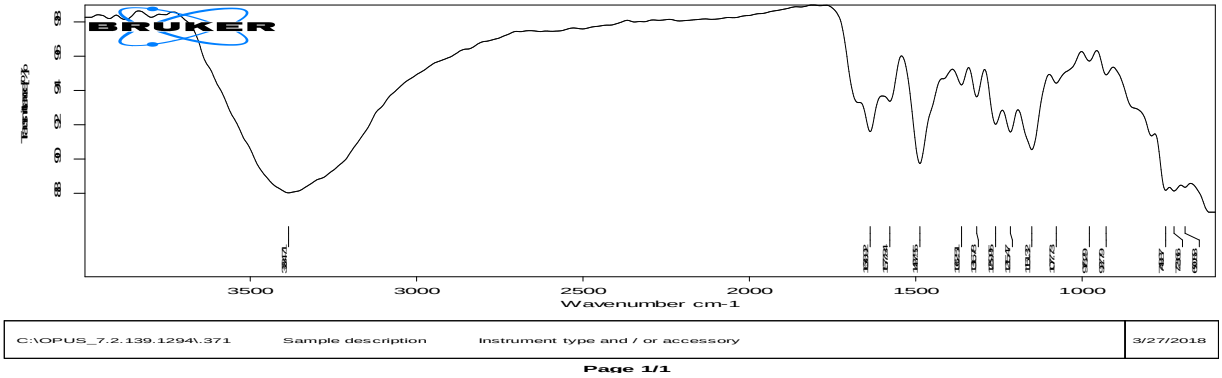
(c)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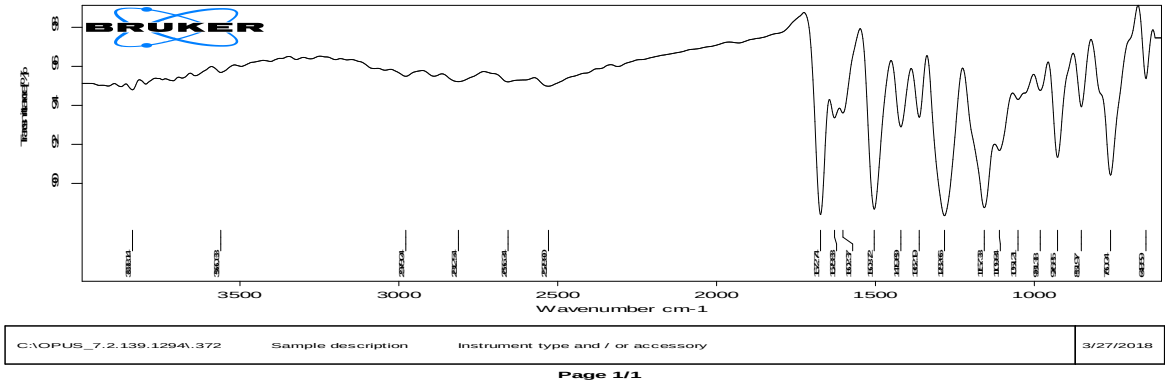
(d)



(e)

Figure 1: FTIR spectrum for azo dyes I-V shown in a-e.

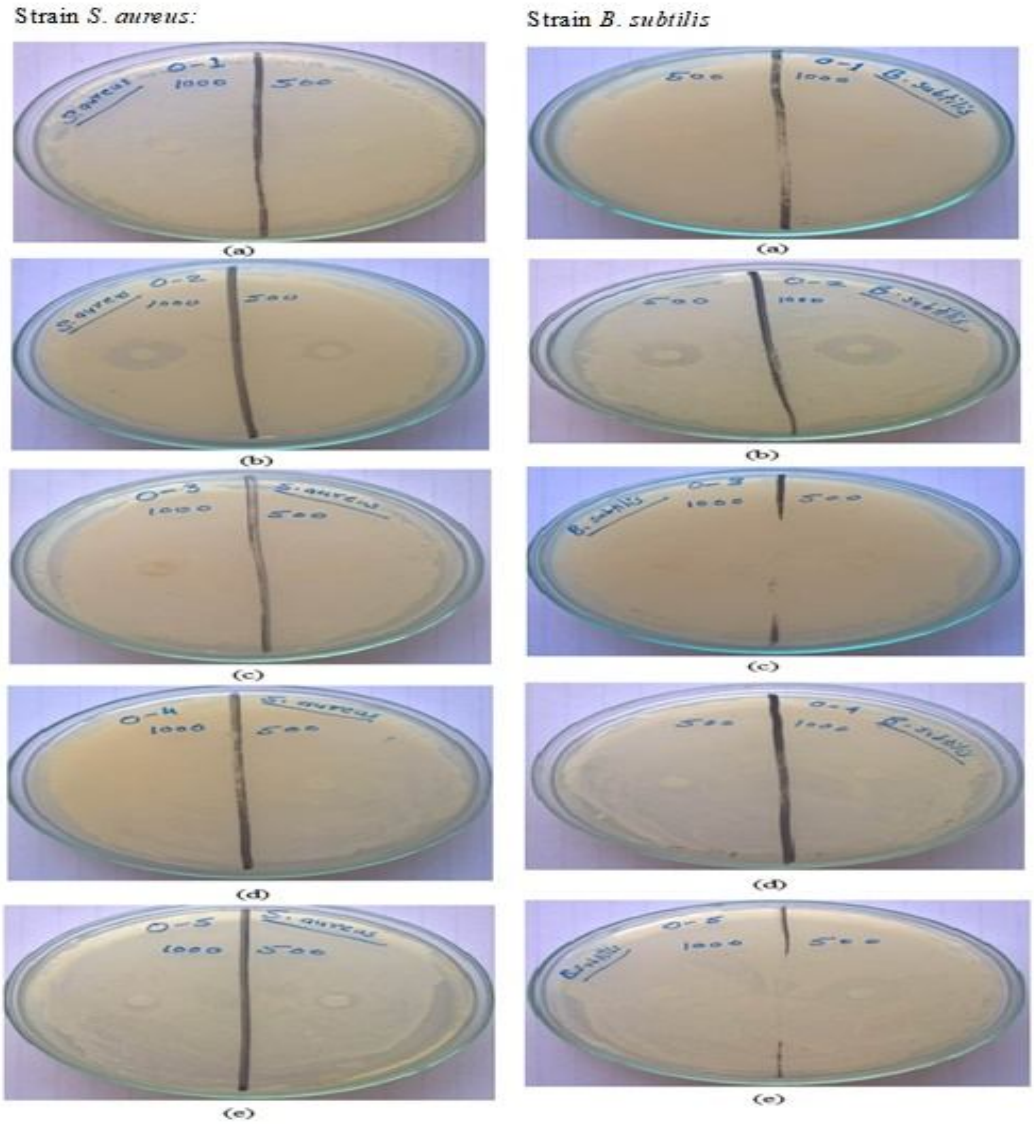


Figure 2: Photographical view for antibacterial action of synthesised dyes I-V on bacterial strain *S. aureus* (a-e) and *B. subtilis* (a-e).

CONCLUSION: No report has been found for the synthesis of Dye II-V whereas the Dye –I has been reported by Ezema, 2014. In present work we reported biological potency of all dyes I-V. The results out-

come has shown that Dye –II is most active toward inhibition of *S.aureus* as well as *B.subtilis* bacterial strain with increasing antibiotic concentration from 500 µg/ml to 1000 µg/ml, this concludes dye-II produces growth inhibiting activity with increasing concentrations while both of the bacterial strains shown resistance against dye-III &V, this might be due to permeability problem of the compounds to reach up to the cell organelles or degradation potency of the bacterial cells.

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