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DNA Binding three Azo Dyes as new Antibiotics

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Abstract. The antimicrobial activities of (E)-4-((2-nitrophenyl) diazenyl) benzene-1,3-diol (1), (E)-4-((3-nitrophenyl) diazenyl) benzene-1,3-diol (2) and (E)-4-((4-nitrophenyl) diazenyl) benzene-1,3-diol (3), were screened in vitro toward *Staphylococcus aureus* strain NCTC 6571 and *Candida Krusei*. The results were revealed that the azo dye (2), which has NO₂ group in the meta- position, was more active toward *Staphylococcus aureus* using 0.3 mg/ mL and 0.4 mg/ mL concentrations. This activity was seemed to be higher than the activity of (1) and (3), which have substituted NO₂ group in ortho- and para- positions respectively. However, the azo dye (1) was gave well activity against *Candida Krusei* than (2) and (3) using same concentrations. These results were then compared with that obtained by antibiotics (erythromycin capsules, amoxicillin capsules and metheprim tablet). The results were displayed that the activities of different concentrations of each azo dye were better than the antibiotics in the treatment of the disseminated infection. Further, the three azo dyes were showed non-toxic effects toward of the hemolytic red blood cells and didn't show any hemolysis effect in the cells. Further the three azo dyes were variable in their bonded to the infected human DNA. The azo dyes (1) and (3) can affect the human DNA resulting DNA damage, which inhibits DNA transcription and replication. However, the azo dye (2) was right bind the human DNA without damaged it in contrast with the control. Due to recommend the three synthetic azo dyes as new antibiotics for disseminated infection. The first section in your paper

Key words: Antimicrobial activity, Azo dyes, *Staphylococcus aureus*, *Candida Krusei*, Human DNA

1. Introduction

Azo dyes were received high attention in scientific research [1], and they have great importance in the chemical analysis. Azo dyes contain one or more azo groups (-N=N-) which are linked to SP₂ hybridized carbon atoms, based on the number of such groups [2]. A strongly colored compounds extremely importance as dyes and also as pigments for a long time. [3] Further, the azo dye is reactive compound that was reported for its pharmaceutical importance as antidiabetic, [4] antineoplastic, [5] antibacterial, [6] and anticancer agent. [8] The presence of -N=N- in the molecular structure of azo is responsible for the interaction with the active site of the protein. [7].

The synthetic azo dyes that named, (E)-4-((2-nitrophenyl) diazenyl) benzene-1,3-diol (1), [8] (E)-4-((3-nitrophenyl) diazenyl) benzene-1,3-diol (2) [8] and (E)-4-((4-nitrophenyl) diazenyl) benzene-1,3-diol (3) [8],[9] were characterized using melting point, infrared spectrum and ultraviolet-visible spectrum. Add to which, the antimicrobial activity of each synthetic azo dye was studied against two bacterial strains: *Staphylococcus aureus* strain NCTC 6571, and *Escherichia coli* strain ATCC 25922, and fungal strain of *Candida albicans* using Agar-well diffusion method. [10] The results of this study were showed that the three azo dyes were biologically active and the best reactivity was observed in (2). However, the biological activity of (1) with NO₂ group in ortho- position remained reasonable against *Candida albicans*. But, this effect was resisted by *Staphylococcus aureus* and *Escherichia coli* using low concentration. However, the azo dye (2) and (3) with substituted NO₂ group in meta- and



para- positions respectively were showed better activities than (1) towered *Candida albicans* and *Staphylococcus aureus* using same concentration. [8]

2. Methods

2.1 Antimicrobial investigations in vitro

- The biological activity of the synthetic azo dyes (1), (2) and (3) were studied towered fungi and gram positive bacteria: *Candida Krusei* and *Staphylococcus aureus* strain NCTC 6571 as following:
- Sabouraud dextrose agar (SDA) and general nutrient agar cultures media were prepared and added to the petri dish followed by reactivate the isolates and then by developed of agricultural sector which then incubated at 30°C for 1-3 days for *Candida Krusei* sector, but at 37°C for 24 hours for *Staphylococcus aureus*.
- When the time of incubation was finished, A fungal suspension of each isolate was obtained by taking a small fraction of the fungal colony and bacteria and adding it to a test tube containing 2 mL sterile sterilized water with shaking a solution using an electrode (Vortex) until a fungal suspension become rude, the density was measured by using Malgeland device No. 0.5 to obtain 1×10^6 cell mL concentration.
- The antimicrobial activity in vitro using agar solid diffusion assay by adding (0.2 mL) of each of the fungal suspension on the surface of the agricultural medium SDA and the bacterial suspension on the surface of the agricultural medium (glucose nutrient agar GNA) followed by diffusing each equally on media. Finally, by using a glass rod in a letter L, which was then the dishes were left for 10 minutes. The suspension was absorbed by the medium and all the names of the isolates and the chemical compounds tested were then labelled. Each hole was applied using a cork hole to create a circular hole of 0.9 mm diameter. Add 1 mL/ 100 μ L per chemical compound in the agricultural middle pit of each of the isolates and in a double repeat of each isolation.

2.2. Cellular toxicity

The Xian-guo and Ursola method,[11] was applied to measure the toxicity of azo dyes under study using hemolytic red blood cells as following: A stock solution of 200 mg / mL was prepared and followed by preparing a series of diluted (0.2, 0.3 and 0.4 mg/ mL) solutions. 0.8 mL of each diluted solution was added to Eppendorf tubes. 0.2 mL of red blood cells was also added to each tube. In addition, two Eppendorf tubes were equipped. In the first tube, 0.8 mL of Ringer solution was added as a negative control, but the tap water as a positive control was added to second tube. Then 0.2 mL of red blood cells was added to each tube. The results were recorded after the incubation of these tubes for 37 minutes in a special incubator and the changes in the solutions were followed checked.

2.3. Human Genomic DNA extractions

Nucleic acids from each 200 μ L of EDTA-whole blood sample were extracted. After cell lysis and protein denaturation, according to the procedure of Sambrook *et al.*[12] and stored frozen until use.

2.3.1. Effect of azo dyes (1), (2) and (3) on human DNA

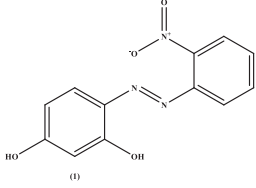
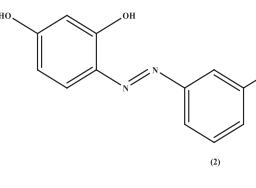
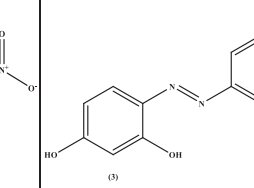
To study the effect of azo dyes (1), (2) and (3) on infected genomic human DNA, 8 μ L of each was mixed with 8 μ L of human genomic DNA, the mixture incubated at 37 °C, and then subjected to 0.8% agarose gel electrophoresis at 60V.

3. Result and dissection

Antimicrobial activities of (*E*)-4-((2-nitrophenyl)diazenyl)benzene-1,3-diol (1), [8] (*E*)-4-((3-nitrophenyl) diazenyl)benzene-1,3-diol (2) [8] and (*E*)-4-((4-nitrophenyl)diazenyl) benzene-1,3-diol (3) [8] were screened in vitro. These activities were achieved towered *Staphylococcus aureus* strain

NCTC 6571 and *Candida Krusei* infections by using different concentrations (0.2, 0.3 and 0.4 mg/mL) from each in DMSO, (Table 1).

Table (1): The diameter of inhibition zones of (1), (2) and (3) against bacterial and fungal infections

Id	Inhibition zones (mm)								
									
Conc. mg/ mL	0.2	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.4
<i>Staphylococcus aureus</i>	20	25	27	30	37	39	28	33	35
<i>Candida Krusei</i>	35	38	42	30	36	41	29	35	41

The results from table (1) above were showed that the azo dye (2), which has NO₂ group in meta-position more reactive than (1) and (3), which have substituted NO₂ group in ortho - and para-positions respectively, towered *Staphylococcus aureus* using 0.3 mg/ mL and 0.4 mg/ mL concentrations. But, the azo dye (1) was gave well activity against *Candida Krusei* than azo dyes (2) and (3) using same concentrations, (Figure 1).

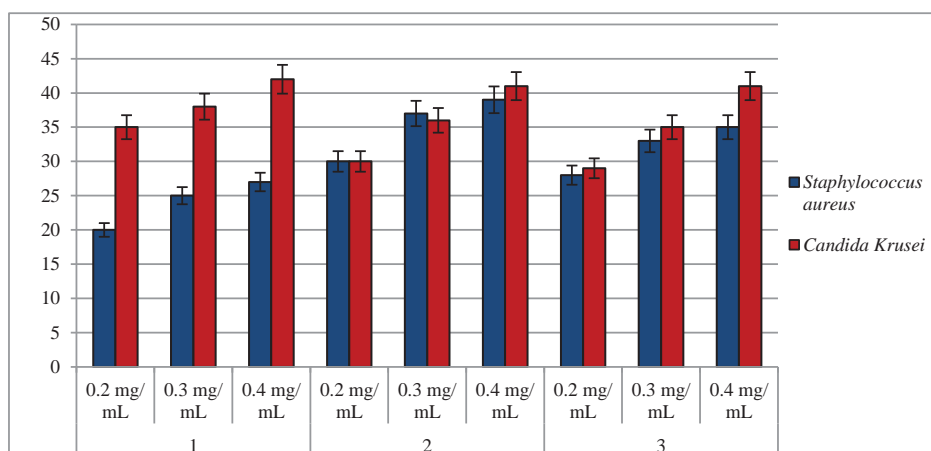


Figure (1): Antimicrobial activity of different concentrations from (1), (2) and (3).

Azo dye (1) as realized in figure (1) above is given good antimicrobial activity against fungal infection and reasonable activity against gram positive bacteria, using 0.2 mg/ mL, 0.3 mg/ mL and 0.4 mg/ mL concentrations, but, the azo dye (2) was showed high reactivity against the two microorganisms, and better than (3) using same concentrations. These activities were made the synthetic azo dyes as new candidates against the dual infections with the two microorganisms. Also, these activities were increased using 0.4 mg/ mL concentration.

Then, the results of the synthetic azo dyes (1), (2) and (3) were compared with that attended by using different antibiotics (erythromycin capsules, amoxicillin capsules and metheprim tablet) [13] using same concentrations.

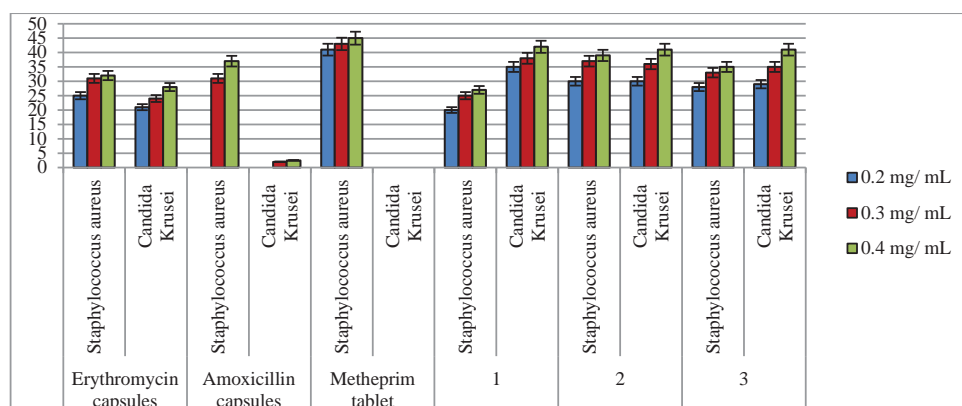


Figure (2): Antimicrobial activity of (1), (2) and (3) in contrast with antibiotics in different concentrations.

The figure displays high activity of methheprim tablet against *Staphylococcus aureus*, but no reactivity toward *Candida Krusei*. [13] However, the diameters of inhibition zones of (1), (2) and (3) against *Candida Krusei* in the three (0.2, 0.3 and 0.4 mg/mL) concentrations were seemed to be higher than that achieved by antibiotics. Further, the activities of the three azo dyes were seemed to be better than erythromycin and amoxicillin drugs in the dual treatment of the *Staphylococcus aureus* and *Candida Krusei* disseminated infections. Thus, the biological activity of each synthetic azo dye against the two microorganisms was realized in Figure (3) below.

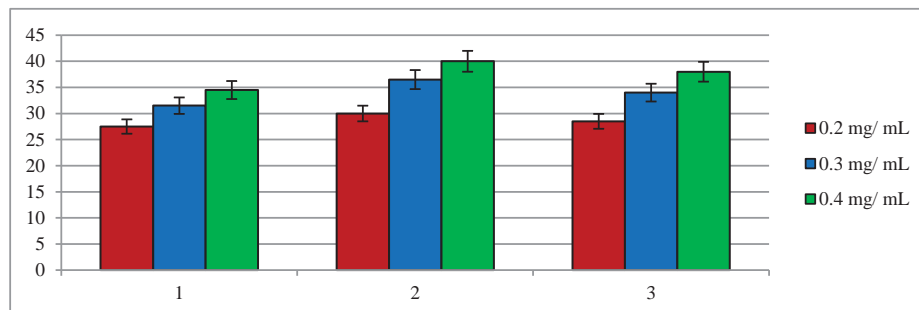


Figure (3): The effect of (1), (2) and (3) in the dual treatment of the disseminated infections.

The results were showed that the best reactivity was obtained by (2) in contrast with other synthetic azo dyes in treated of the disseminated infection.

Further, the method of Xian-guo and Ursola was then applied to measure the toxicity of (1), (2) and (3) using hemolytic red blood cells in vitro. The results were showed that the three azo dyes were provided non-toxic effects using different concentrations of each, and didn't show any hemolysis effect in the cells. Due to recommend these azo dyes as new drugs can treat the dull infection with the two microorganisms. Numerous antimicrobial agents' activity they can be toxic to human beings. Antimicrobials have to be non-toxic, non-allergenic, effective and selective, chemically stable, active against possibly more than one bacterium and inexpensive. [14]

There are certain drugs that are shown to effect the very vital functions of living organisms, such as protein biosynthesis, nucleic acid replication and gene expression, collectively called antibiotics. [15] These non-protein molecules that are known to bind DNA molecules includes natural products such as antitumor, antibiotics and other secondary metabolites from bacteria and fungi and plants, synthetic compounds, and also heterocyclic and multi ring heterocyclic

aromatic compounds and homo pyrimidine oligonucleotides. The interaction of relatively small molecules of this type with DNA may lead to a "useful" results such as antibacterial and anticancer activity, but may also involve undesirable biological responses, such as carcinogenesis or mutagenesis.[15] Therefore, the DNA binding of each dye was studied as seen in Figure (4) below.

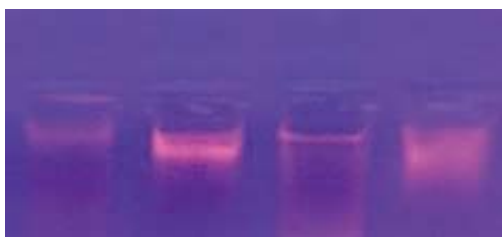


Figure (4): The DNA binding of (1), (2), (3) and the control (C).

The figure displays that the three azo dyes were variable in their bonded to the infected human DNA. The azo dyes (1) and (3) can affect the human DNA resulting DNA damage as seen in figure (4) above, which inhibits DNA transcription and replication. The azo molecules are known to be involved in the inhibition of DNA, RNA, carcinogenesis, and protein synthesis.[7] However, the azo dye (2) was right bind the human DNA without damaged it in contrast with the control. The DNA in which two helical chains of nucleotides are held together by the hydrogen bonds that occur in a selective fashion between a purine and a pyrimidine nucleic base giving rise to the Watson-Crick pairs adenine-thymine (AT) and guanine-cytosine (GC).[16] The hydrogen bonds were conceived as predominantly electrostatic phenomena that in the case of DNA base pairs are reinforced by polarization of the π -electron system (Resonance Assisted Hydrogen Bonding, RAHB).[16] the analyses of the bonding mechanism that donor-acceptor orbital interactions between the DNA bases in the Watson-Crick pairs are of comparable strength as electrostatic interactions. The donor acceptor or charge-transfer term is provided by the interactions of lone-pair orbitals on O or N of one base with N-H σ^* acceptor orbitals of the other base. Our observation, that the synthetic azo dyes can bind DNA in same manner. But, with variable strength in their interactions depend on the NO_2 group positions.

Conclusion

The Azo dyes (1), (2) and (3), can be synthesized inexpensively because the starting materials are readily available, and most of the chemistry was done below the room temperature. Also, the synthetic dyes were gained a good color, delivered non-toxic effects using different concentrations from each and didn't show any hemolysis effect in the cells. Further, the synthetic azo dyes were well bonded the infected human DNA and displayed well activity towered *Staphylococcus aureus* and *Candida Krusei* than antibiotics, spicily in the treatment of the disseminated infection. Due to recommend these azo dyes as novel antibiotics for *Staphylococcus aureus* and *Candida Krusei* disseminated infections.

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