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Synthesis, Chromatographic Separation and Antimicrobial Evolution of New Azoquinoline-8-ol

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Abstract:-

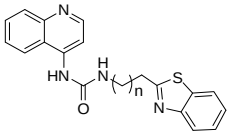
New azo-compounds 4NQ, 3NQ, 4SQ, 3SQ were synthesized through diazo coupling of aminobenzimidazole or aminobenzothiazole with 8-hydroxyquinoline. The azo-compounds structures were confirmed by NMR, IR spectroscopy, and elemental analysis. The new azo-compounds were evaluated for antimicrobial activity by agar diffusion methods and showed a strong to moderate antimicrobial activity. Most of the tested compounds possessed a broad spectrum of activity with *MIC* values ranging from 50 to 250µg/mL

Key words: benzothiazole, benzimidazole, hydroxyquinoline, antimicrobial activity.

INTRODUCTION

The quinoline moieties are the main core of synthetic or natural bioactive compounds, which have been mainly used for treatment of different types of human tumor histotypes [1-4]. Other derivative of quinoline were known to be effective inhibitors of leukemia, colon, and ovarian cancer cells [5-6].

Also, it was reported that the azolylthioalkyl- ureidoquinoline derivatives **(SRA-HX-1)** (Fig. 1) possess higher activity as antitumor agents [7].



SRA-HX-1

Figure 1. Struture of azole quinolines: cytotoxic quinole derivatives with azole group

The heterocyclic compounds, containing benzothiazolyl, moiety have variable biological scientific importance. The benzothiazole derivatives have many biological importance such as antitumor [8—11], antidiabetic, antimicrobial, anti-inflammatory, antipsychotic, anticonvulsants, and schictosomicidal [12].

The imidazole moiety containing compounds have many clinical uses [13-15] and is considered the main core in many drugs such as thiabendazole, flubendazole, candesartan, lansoprazole, telmisartan, mebendazole, omeprazole and pantoprazole [16].

The benzimidazole containing compounds were reported to be antimicrobial [17] anti-inflammatory [18], antioxidant [19] , anti-tuberculosis [20], antihypertensive [21], antiulcer [22]), antiretroviral [23] and anti-parasitic [24]. As a result, from previous interesting work and in continuation of research [25-26], the main aims of work his are the preparation of new azo-quinoline derivatives substituted with benzothiazole or benzimidazole moieties in an attempt to find a new lead compounds. The newly synthesized lead compounds supposed to be highly active against microorganisms, and hopefully comparable in their activity with that of positive standard antifungal and antibacterial.

MATERIALS AND METHODS

Instruments and reagents

Melting points were measured using a Böetius PHMK (VebAnalytik Dresden) instrument. TLC was performed on aluminum plates pre-coated with silica gel 60 or 60 F254 (Merck) and visualized using UV light (254 nm). Nuclear Magnetic Resonance of compounds was carried out on a Varian Gemini 300 and Bruker DRX 400 spectrometer at 25 ºC with TMS as a reference and solvent shift ($(CD_3)_2SO$ δ H 2.50 and δ C 39.5). Coupling constants are expressed in Hz. Mass spectrometry was performed using a Varian FINNIGAN MAT 212 machine. The Infra-red spectra were investigated (KBr) by means of a Jasco FT/IR-410 apparatus. Elemental analysis was measured using the Perkin Elmer 240 instrument. The starting anilinocompounds **Ia-d** were prepared according to the reported methods [10-11].

Synthesis

General procedure for synthesis of compounds 4NQ, 3NQ, 4SQ,3SQ

To a stirring solution of compounds **1a-d** (0.01mol) in HCl (10%, 10 ml), sodium nitrite solution (0.011 mol in 10 ml water) was added dropwise with stirring on ice bath for 1hr. The reaction mixture was added with stirring to a solution of 8-hydroxyquinoline (0.01mol in 10 ml NaOH 10%). The stirring was continued for 4 hr in the ice bath. The precipitated was collected, dried, filtered and purified using the adapted method of chromatography.

5-[4-(1*H*-Benzimidazol-2-yl) phenylazo]-quinolin-8-ol (4NQ).

After the reaction completion, the final product was monitored by TLC plate using solvent mixture of *n*-hexane: ethyl acetate (1: 1), the chromatogram showed **5** minor spots and a major one which supposed to be the target molecule **(4NQ)** according to the co-TLC plate and comparison to authentic standards. The mixture was successively chromatographed on a silica gel column and eluted with *n*-hexane: ethyl acetate (100% to 40:60) mixture to give five different fractions **(1-5)**, fraction **2** was repeatedly chromatographed on a series of column chromatography to yield four sub-fractions **($4NQ_{2;\ 1-4}$)** from which the sub-fraction **2** afforded the target molecule **4NQ (140 mg)** after purification on sephadex LH-20 with methanol as eluent.

Compound 4NQ was obtained as red powder, Yield 83%; mp, 219-221 °C; IR (KBr): 3600 (OH), 3186 cm^{-1} (NH), 3040 cm^{-1} (CH aromatic) ^{1}H NMR (DMSO-d_6) δ/ppm: 6.50-6.52 (m, 1H, quinoline H-7), 7.21-7.88 (m, 3H, benzimidazole H -5,6, NH), 8.0-8.19 (m, 4H, benzimidazole H-4,8, phenyl H-2,6,), 8.21-8.26 (m, 3H, phenyl H-3, 5 , quinoline H-6), 8.32-8.62 (m, 2H, quinoline H -3,4), 9.20 (m, 1H, quinoline H-2), 9.22 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ/ppm: 111.25 ,112.35, 118.24, 120.23, 121.61, 122.45, 123.18, 124.34, 125.24, 125.64, 130.40, 130.60, 131.21, 132.25, 132.60, 151.86, 155.53, 162.74, 160.25, 162.20, 165.35; Elemental Analysis Calcd. for $C_{22}H_{15}N_5O$; C, 72.32; H, 4.14; N, 19.17; Found: C, 72.20; H, 4.20; N, 19.00

5-[3-(1*H*-Benzimidazol-2-yl)-phenylazo]-quinolin-8-ol (3NQ)

Compound 3NQ was purified from its reaction mixture by different chromatographic techniques after monitoring with TLC plate, a mixture of DCM and MeOH in a ratio of (88: 12) was used as mobile phase, two major spots were detected together

with another four ones, one major spot was selected as the target molecule and the others were excluded depending upon co-TLC plate and authentic standards. The mixture was subjected to vacuum liquid chromatography (VLC) using silica gel 60, 0.04 - 0.063 mm mesh size (Merck). Gradient fractionation with 100% DCM up to 15 % MeOH in DCM yielded six fractions ($3NQ_{1-6}$). Fraction 4 (the major and the most pure one) was furtherly purified on normal silica gel column chromatography using a mobile phase mixture of DCM and MeOH (90: 10), three sub-fractions **($3NQ_{4; 1-3}$)** were obtained from which the sub-fraction **2** (120 mg) was selected to be chromatographed on Sephadex LH-20 to yield a pure compound **(3NQ)** with MeOH as mobile phase.
The powder 3NQ had dark red with a yield 80%; mp., 279-281 °C; IR (KBr): 3406 cm^{-1} (OH) 3190 cm^{-1} , (NH), 3055 cm^{-1} (CH aromatic); ^{1}H NMR (DMSO-d_6) δ/ppm: 6.79-6.81 (m , 1H, quinolone H-7 **)**, 7.20-7.22 (m, 2H,benzimidazole H-5, H-6), 7.95-7.97 (m, 4H, benzimidazole H-4,7, phenyl H-5,6) , 8.10-8.12 (m, 2H, phenyl H-4, quinoline H-6), 8.35-8.76 (m,3H, quinoline H-3,4 , phenyl H-2), 8.75 (m, 1H, quinoline H-2), 9..25 (s, 1H, OH); ^{13}C NMR (DMSO-d_6) δ/ppm: 111.64, 114.40, 115.53, 116.30, 119.28, 122.31, 122.67, 123.42, 127.00, 127.94, 129.20, 129.29, 129.99, 130.53, 132.05, 133.26, 136.58, 142.09, 147.78, 151.53, 153.58, 154.74. Elemental Analysis Calcd. for $C_{22}H_{15}N_5O$: C, 72.32; H, 4.14; N, 19.17. Found: C, 72.30; H, 4.10; N, 19.10.

5-(4-Benzothiazol-2-yl-phenylazo)-quinolin-8-ol (4SQ):
Compound **4SQ** appeared semi-pure form upon monitoring on TLC plate, it has been chromatographed three times on gel chromatography with MeOH to obtain pure red powder of compound **4SQ (100 mg)** with yield 84%; mp., 272-274 °C; IR (KBr): 3450 cm^{-1} (OH), 3040 cm^{-1} (CH aromatic), 1600 cm^{-1} (C=N), 1520 cm^{-1} (C=C), cm^{-1}; ^{1}H NMR (DMSO-d_6) δ/ppm: 7.27-7.28 (m, 1H, quinoline H-7), 7.51-7.61 (m, 2H, benzothiazole H-5, H-6), 7.80-7.83 (m, H, quinoline H-6), 8.08-8.34 (m, 8H, phenyl H-2,3,5,6, benzothiazole, H-4,7, pyridine H -3,4), 9.36 (m, 1H, o-pyridine proton), 9. 38 (s, 1H, OH); Elemental Analysis Calcd for $C_{22}H_{14}N_4OS$: C, 69.09; H, 3.69; N, 14.65. Found: C, 69.10; H, 3.60; N, 14.70.

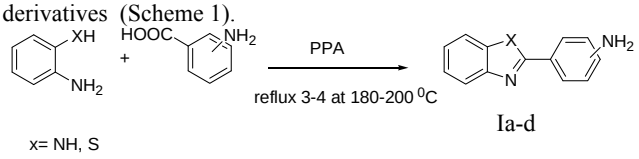
5-(3-Benzothiazol-2-yl-phenylazo)quinolin-8-ol (3SQ)
The reaction mixture of **3SQ** was examined by TLC plate using a solvent mixture of DCM: MeOH (90: 10), it showed couples of spots with a major one expected to be the molecule of interest compared with authentic standards. The product was chromatographed on silica gel column and eluted with DCM: MeOH (100% to 9:1) to give five different fractions (1-5), fraction 3 was successively subjected to silica gel column chromatography to obtain the purest spot of the target molecule **(3SQ)**. urther purification was performed by size exclusion chromatography followed by crystallization from Methanol.
The dark red powder obtained with yield 83%; m.p. 263-265 °C; IR (KBr): 3450 cm^{-1} (OH), 3040 cm^{-1} (CH aromatic), 1600 cm^{-1} (C=N), 1520 cm^{-1} (C=C); ^{1}H NMR (DMSO-d_6) δ/ppm: 7.18-7.20 (m, 1H, quinoline H-7), 7.50-7.61 (m, 2H, benzothiazole H-5, 6), 7.71-7.81 (m, 1H, phenyl H-5), 8.08-8.32 (m, 8H, benzothiazole H-4, 7, phenyl H-2,4,6., quinoline H-3,4,6), 9.33(m, 1H, pyridine H-2) 9..35 (s, 1H, OH);. Elemental Analysis Calcd for $C_{22}H_{14}N_4OS$: C, 69.09; H, 3.69; N, 14.65. Found: C, 69.20; H, 3.70; N, 14.60.

Antibacterial and antifungal activity (*Agar diffusion assay*)
Samples Preparation
All samples were dissolved in dimethyl sulfoxide (RFCL Limited, New Delhi, India) DMSO at 10 mg/ml concentration as shown in the Table (1) in comparing with different standard antibiotics.

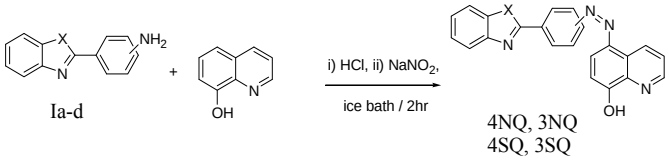
Test organisms:
A-Bacteria: e.g. *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (NRRL-B-4219), B-Test fungi such as *Aspergillus niger* (ATCC 16888) and *Candida albicans* (ATCC 10231). The antimicrobial activity of newly synthesized compounds was evaluated using an agar disc diffusion assay [27-28]). The samples were dissolved in DMSO. Briefly, a 24 hours old culture of bacteria and 48 hours old culture of fungi was mixed with sterile physiological saline (0.9%) and the turbidity was adjusted to the standard inoculum of MacFarland scale 0.5 (106 colony forming units (CFU) per ml). Petri plates containing 20 ml of Mueller Hinton Agar (Lab M., Bury, Lancashire, UK) and Sabouraud-dextrose agar (Lab M., Bury, Lancashire, UK) was used for antibacterial and antifungal activity. The inoculums were spread on the surface of the solidified media and Whatman No. 1 filter paper discs (6 mm in diameter) impregnated with the test compound (40 µl/disc) were placed on the solidified media. Standard Bacterial antibiotics (Streptomycin (S-10 µg), Tetracycline (TE- 30 µg) and fungal antibiotic (Neomycin (N- 30 µg); Nystatin (NY-100 µg) were used as positive control for bacteria and fungi respectively along with DMSO ( ) as a negative control. A zone of inhibition was recorded in millimeters after incubating bacterial strains at 37 °C (24 h) and fungal strains at 25 °C (72 h). Tests were performed in triplicate and the values were expressed as mean ± SD [29-30]). Determination of minimum inhibitory concentration (MIC): The in vitro antibacterial activity of the extract and minimum inhibitory concentration was determined by the macrobroth dilution methods (NCCLS, 1993). The nutrient broth medium was used to prepare different concentrations ranging from 0.0195 to 10 mg/ml by serial dilutions. Each prepared concentration in tubes was inoculated with 100 *µl* of each of the 106 cfu/ml bacterial strain. The blank nutrient broth was used as negative control. The tubes were incubated aerobically at 37°C for 18 to 24 h. The first tube in the series with no sign of visible growth was taken as the MIC.

RESULTS AND DISCUSSION

Chemistry
The starting compounds, namely 4-(1*H*-benzo[d]imidazol-2-yl)-aniline, 3-(*1H*-benzo[d]imidazol-2-yl)-aniline, 4-(benzo[d]thiazol-2-yl)-aniline and/or 3-(benzo[d]thiazol-2-yl)-aniline **Ia-d** were prepared as previously reported methods [10-11] and used as a key compound for the preparation of novel 5-(quinoline -8-ol) derivatives (Scheme 1).

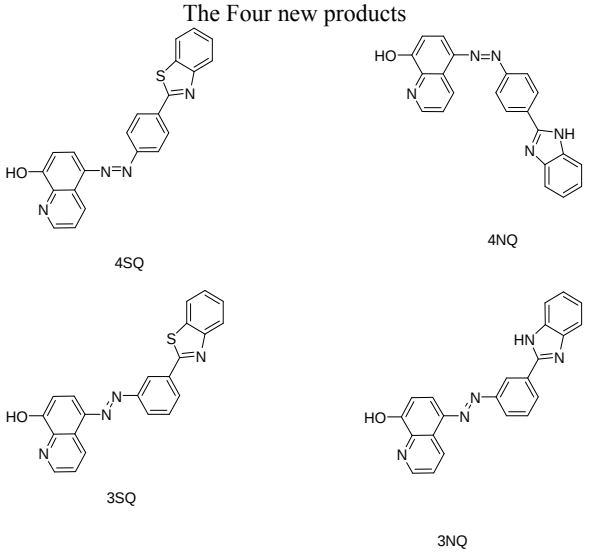


Scheme 1; reaction of *o*-phenylene diamine or *o*-aminothiophenol with *p*-aminobenzoic acid and/or *m*-aminobenzoic acid
Thus, Coupling of aniline derivatives **Ia-d** with 8-hydroxyquinoline in the presence of sodium nitrite under strong acidic condition using HCl resulted in the formation of target compounds 5-[4-/3-(*1H*-benzo[d] imidazole/thiazol-2-yl)] quinoline derivatives **3SQ.4SQ, 3NQ and 4NQ** in good yields (Scheme 2).



Scheme 2: Coupling of aniline derivatives Ia-d with 8-Hydroxy quinoline

The Four new products



The IR spectrum of the azo derivatives **3NQ, 4NQ, 3SQ and 4SQ** showed the characteristic absorption band corresponding to the phenolic OH at 3600 cm^{-1}. Also the ^{1}H NMR spectrum showed the corresponding broad singlet signal of phenolic OH at δ/ppm 9.22. Also The ^{1}H NMR spectra of the new azo compounds are in agreement with the assigned structure revealing the absence of the signal for the NH_2 group of their precursor aniline derivatives **Ia-d.**

Table1 : Antimicrobial activity of compounds *at concentration of 10 mg/mL*

Sample No	Bacteria		Fungi	
	G+ve	G+ve	unicellular	filamentous
	B.S	***E.coli***	***C. albicans***	***Aspergillus niger***
Inhibition zone (mm)				
3NQ	23	25	25	20
4NQ	30	29	30	20
35Q	15	18	20	15
45Q	00	00	20	00
Standard Antibiotics				
NA= 30 µg	20.18±0.2475	16.68±0.4596	00.00±0.0000	00.00±0.0000
S =10µg	14.00±0.0000	00.00±0.0000	12.00±0.0000	00.00±0.0000
N =30 µg	00.00±0.0000	00.00±0.0000	00.00±0.0000	16.00± 0.4142
Ny=100µg	00.00±0.0000	00.00±0.0000	00.00±0.0000	00.00±0.0000
NV=30µg	29.99±0.0141	30.13±0.1768	00.00±0.0000	00.00±0.0000
T =30µg	30.63±0.8838	27.98±0.0354	00.00±0.0000	00.00±0.0000
SDZ=30µg	00.00±0.0000	20.18±0.2475	00.00±0.0000	00.00±0.0000
VA=30µg	21.99±0.0141	23.18±0.2475	00.00±0.0000	00.00±0.0000

Standard Bacterial antibiotics NA = Negram (nalidixic acid), S= Streptomycin; N = Neomycin,Ny = Nystatin; VA= T= Oxytetracycline, Vancomycin, CDZ =Cefodizime, NV= Novobiocin

Antimicrobial activity

The results in **Table 1, Figures 1** showed a variation antimicrobial effect against each test pathogenic microorganisms. Compounds **3NQ** and **4NQ** showed a strong inhibitory effect and recorded 23 to 30 mm, but showed a moderate antifungal inhibition effect of 20 mm against *A. niger*, while the compound **35Q** recorded a moderate antibacterial and antifungal inhibition effect against all test pathogenic microorganisms in compared to the standard antibacterial and antifungal antibiotic that used in this study. On the other hand, the compound **45Q** showed a moderate inhibition effect against *C.albicans* and characterized by negative inhibitory effect against the other tested microorganisms in comparing to the standard antibacterial antibiotic that used in this study. *Aspergillus niger*, is one of the fungal pathogens that can affect the respiratory tract. *A. niger* is a causative agent of pulmonary diseases including asperagillosis, bronchial asthma and acute allergic alveolitis. The fungus colonizes old tuberculosis or bronchi static cavities, in which it forms a large colony (aspergilloma); or it may actually invade the lung tissue to produce hemorrhagic and necrotizing pneumonia [31]. Finally, the in vitro antimicrobial activity of the synthesized compounds was screened against gram-positive *(Bacillus subtilits*) and gram-negative *(Escherichia coli*) bacteria, yeast (*Candida albicans*) and filamentous fungi (*Aspergillus niger*). Most of the samples have the strongest antibacterial activities. The demonstration of the activity against both gram-positive, gram-negative bacteria and fungi is an indication that the compounds can be used in the treatment of gram-negative, gram-positive and fungus pathogens or these compounds have a broad spectrum effect.

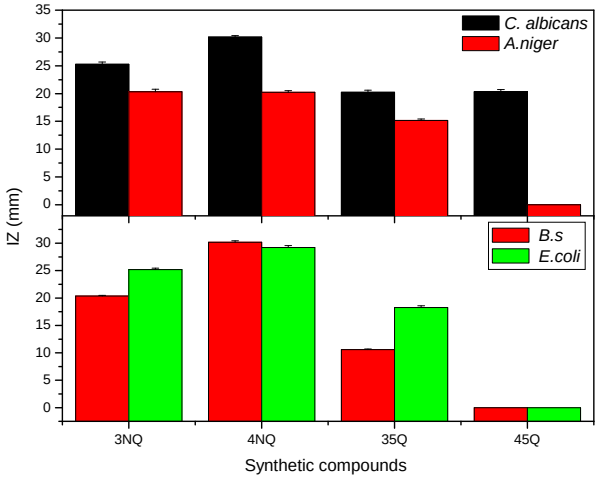


Figure: 2 Antimicrobial activity of different compound ***sat concentration of 10 mg/mL***

MIC of 3NQ and 4 NQ compounds against different pathogenic microorganisms

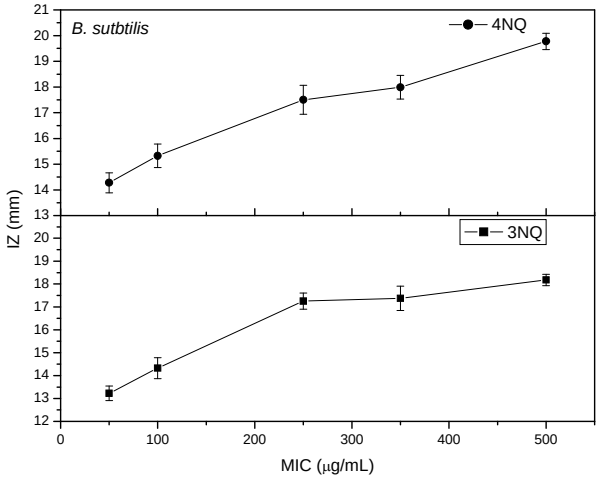


Fig.3 (A) MIC of 3NQ and 4NQ compounds against *B.subtilis*

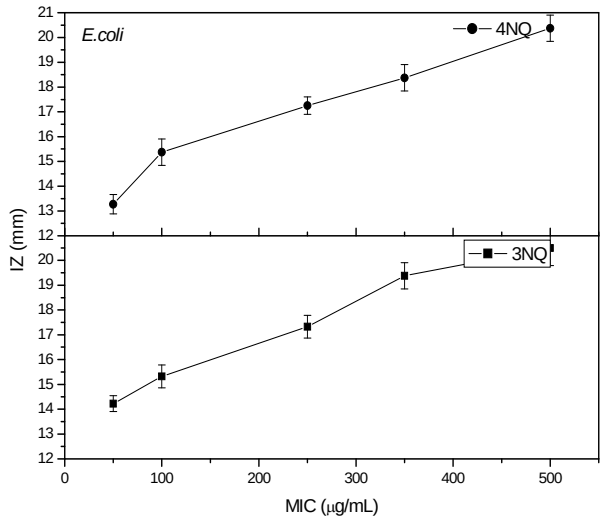


Fig.4 (B)MIC of 3NQ and 4 NQ compounds against *E.co*

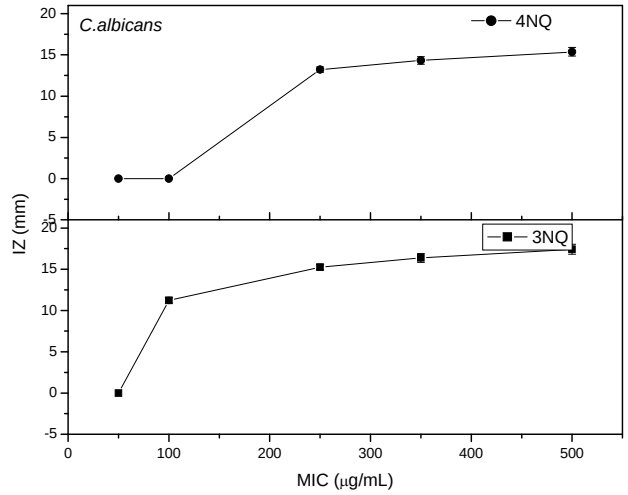


Fig.5 (C)MIC of 3NQ and 4 NQ compounds against *C.albicans*

Table 2: MIC of 3NQ and 4 NQ compounds against different pathogenic microorganisms

G3 Compound No.	Inhibition Zone diameters (mm)	MIC(µg/mL)
Bacillus subtilits		
3NQ	13	50
4NQ	14	50
	E.coli	
3NQ	14	50
4NQ	13	50
	C. albicans	
3NQ	11	100
4NQ	13	250

The MIC of the synthesized compounds are presented in the **Table 2**, **Figure 2**(**A**, **B** and **C**). The MIC recorded 50 µg/ml based on the compounds tested, but was differed in inhibition zone obtained and ranged from 12 to 14 mm. The MIC of the compound 3NQ and 4NQ were 50 µg/ml against *Bacillus sutbtilis* and *E. coli* (Fig.2: A and B), but it is 100 µg/ml and 250µg/mL against *C.albicans* for 3NQ and 4NQ respectively (Fig.2: C).

CONCLUSION

In conclusion, four new compounds 5-(4- benzimidazole-2-yl-phenylazo) –quinoline-8-ol (4NQ), 5-(3-benzimidazole-2-yl-phenylazo)-quinoline-8-ol (3NQ), 5-(4-benzothiazol-2-yl-phenylazo)-quinolin-8-ol (4SQ) and 5-(3-benzothiazol-2-yl-phenylazo)-quinolin-8-ol (3SQ) were synthesized and evaluated for their antimicrobial. Furthermore, the attachment of benzothiazole and/or benzimidazole moiety to 8-hydroxyquinoline via azo scaffold enhance the activity whether antimicrobial or antifungal. 5-(4- Benzimidazole-2-yl-phenylazo) –quinoline-8-ol (4NQ), showed the highest activity against gram positive and gram negative microorganism.

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