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Original Article

Synthesis and Evaluation of Novel Azo-Schiff's Bases with Antimicrobial Potentials

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ABSTRACT

Background: Antimicrobial agents occupy a significant importance today due to the increasing challenge posed by microbial resistance to existing drugs. Azo dyes, time tested compounds, known for their antimicrobial efficacy, have demonstrated potent activity against both bacteria and fungi. However, the emergence of resistance among microorganisms to these compounds necessities the development of new azo dye derivatives with better activity profile. **Materials and Methods:** In the present study, a series of azo dye compounds were synthesized from coupling diazotized Schiff bases with β-naphthol. The synthesized compounds were characterized using various spectroscopic techniques The *in silico* studies were conducted to know the potency of these compounds on selected bacterial proteins 5 XDT and 1Q8I from pdb belongs to selected gram+ve and gram-ve bacteria, and the results have shown significant potency towards their ability to inhibit the bacteria. **Results:** Based on the *in silico* studies, the compounds were subjected for *in vitro* antimicrobial analysis and the results revealed that the synthesized compounds exhibited prominent antimicrobial activity against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. **Discussion:** These results highlighted the antimicrobial potential of the compounds as promising lead molecules for the development of antimicrobial agents. Furthermore, these findings suggest the possible application in both healthcare and industrial settings as antimicrobial agents. **Conclusion:** These findings indicate that the synthesized Schiff's Bases-Azo dyes are promising antimicrobial compounds for antimicrobial and industrial applications.

Keywords: Azo dye, Antimicrobial agents, Schiff's bases derivatives, Diazo compounds.

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INTRODUCTION

Azo compounds, characterized by the -N=N- (azo) linkage, are classified in IUPAC nomenclature as diazenes and have played a key role in organic chemistry since P. Griess's discovery in 1858. These compounds commonly serve as bridges between two or more molecular groups and may even contain multiple azo linkages. Today, synthetic azo compounds are widely used across many industries, including pharmaceuticals, cosmetics, food, dyes, paints, plastics, and more. Aromatic azo compounds are especially significant due to their diverse practical applications: they are utilized as dyes, pigments, indicators, and food additives, and are important in textiles, pharmaceuticals, and cosmetics for therapeutic as well as diagnostic purposes. Additionally, modern research explores their roles as anticancer, antibacterial, antifungal, antioxidant, anti-inflammatory, and antiviral agents, as well as their use as corrosion inhibitors and drug carriers. One notable feature is their good thermal stability and the tunability of their optical properties by changing substituents, which further broadens their utility in science and industry (Razali *et al.,* 2023; Kyei *et al.*, 2020).

The rise of resistant pathogens is a major global health threat, reducing the effectiveness of existing antimicrobial drugs. This has sparked urgent research to develop new treatments, as bacterial resistance is inevitable through evolution. Without new drugs, there's a risk of returning to a pre-antibiotic era. Additionally, helminth-related neglected tropical diseases cause significant illness and death worldwide. Limited effective antimicrobials and anthelminthics push the need for ongoing drug discovery. Nitrogen-containing heterocyclic compounds, particularly

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pyrazole derivatives, are vital in drug development due to their broad biological activity and potential for creating new medicines to combat resistance and neglected diseases .It has been noticed that significant development has taken in the research of Schiff 's bases that have shown variety of pharmacological activities, still a threat from various factors including microbes to these organic compounds led to the development of new coordinate compounds containing schiff's bases with different metals (Joseyphus *et al.*, 2022; Dinnimath *et al.*, 2024). This chelation of Schiff bases with metals has resulted in the development of enhanced potency in their pharmacological activities, mainly anticancer activity and hence this has gained momentum now (Ibrahim *et al.*, 2024; Mishra *et al.*, 2024).

Azo-Schiff bases combine azo groups (-N=N-) with Schiff bases (-CH=N-) to form versatile compounds. These photochromic azo dyes can undergo reversible trans-cis isomerization under light or heat, making them useful in catalysis, antimicrobial applications, photo pharmacology, food, and textiles. Typically, azo groups attach to benzene or naphthalene rings but may also bind to aromatic heterocycles or enolizable aliphatic groups. Schiff bases form through amines reacting with carbonyl compounds, creating imine linkages. Recent syntheses of azo-Schiff base ligands via coupling diazonium salts with imidazole derivatives have expanded their chemical and biological applications, especially enhancing photostability and antimicrobial activity, making them promising for medicinal and industrial uses (Di Martino *et al.*, 2022; Tahir *et al.*,2021; Abd El Salam *et al.*, 2023).

MATERIALS AND METHODS

Materials

All the chemicals used were of LR grade purchased from Himedia. Initially the compounds were identified preliminarily by TLC by conventional method.

FTIR has been used to identify the functional groups in final products, using Shimadzu IR Affinity using DRS 8000 spectrometer. To predict the hypothetical binding interactions of ligand with the receptor. Molecular docking studies were performed by using glide and Qikprop module of schrodinger software.

Methods

The proposed Azo-Schiff's base derivatives were synthesized in three steps (Moaeaa *et al.*, 2022: Naeimi *et al.*, 2015; Razali *et al.*, 2023).

Step 1: Synthesis of diazotised Schiff's bases.

Step 2: Synthesis of Diazotised Schiff base derivatives.

Step 3: Coupling of Diazotised Schiff's bases with beta naphthol.

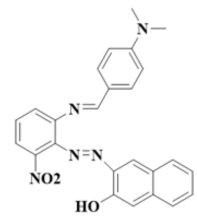
Step 1: Synthesis of Schiff's base

Equimolar concentration of substituted aromatic aldehyde and Orthophenelene diamine dissolved in 20 mL abs. Ethanol and refluxed for 6-7 hours using $AlCl_3$ as a catalyst. This has yielded a coloured product, which was filtered, washed and recrystallized from ethanol and water (1:1).

Step 2: Diazotisation of Schiff's bases

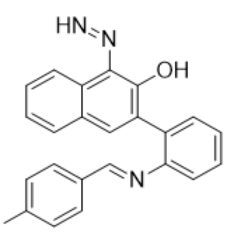
In an RBF weighed quantities of (1 g) of Schiff's base was beaker 2 mL of distilled water with 4 mL of Conc.Hcl is taken and added to the RBF and is kept on a magnetic stirrer with temperature between (0-5°C). In a separate beaker 3.2 g of sodium Nitrite is dissolved in 15 mL of distilled water and the temperature of the solution is maintained (0-5°C). Add solution 2 in 1 slowly with vigorous stirring (0-5°C).

Details of the synthesized compounds (C1 to C7)



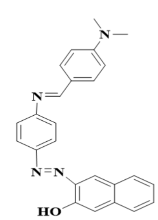
3-((*E*)-(2-(((*E*)-4-(dimethylamino)benzylidene)amino)phenyl)diazenyl)naphthalen-2-ol **(C1)**

(Mol. Weight-418)



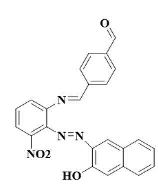
(4-chlorobenzylideneamino)phenyl diazenyl naphthalen-2-ol

Molecular Weight: 385.85



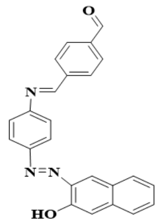
3-((*E*)-(4-(((*E*)-4-(dimethylamino)benzylidene)amino)phenyl)diazenyl)naphthalen-2-ol **(C3)**

(Mol. Weight-418)



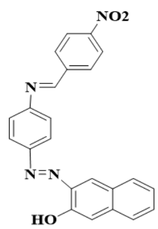
4-((*E*)-((2-((*E*)-(3-hydroxynaphthalen-2-yl)diazenyl)-3-nitrophenyl)imino)methyl)benzaldehyde **(C4)**

(Mol. Weight-465)



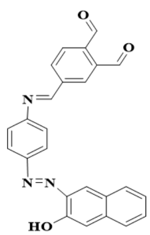
4-((*E*)-((4-((*E*)-(3-hydroxynaphthalen-2-yl)diazenyl)phenyl)imino)methyl)benzaldehyde **(C5)**

(Mol. Weight-403)



3-((*E*)-(4-(((*E*)-4-nitrobenzylidene)amino)phenyl)diazenyl)naphthalen-2-ol **(C6)**

(Mol. Weight-624)



4-((*E*)-((4-((*E*)-(3-hydroxynaphthalen-2-yl)diazenyl)phenyl)imino)methyl)phthalaldehyde **(C7)**

(Mol. Weight-450)

Step 3: Coupling of beta naphthol with Diazotised Schiff's base

In a separate beaker dissolved weighed quantity (6.24 g) of β-naphthol in 40 mL NaOH solution with temperature (0-5°C). Now slowly added solution of Diazotised Schiff's base to it and refluxed the whole mixture for 30-40 min on the magnetic stirrer and then filtered the product by vacuum filtration and dried. The reaction is monitored by TLC in (3:7) ethyl acetate: Hexane and melting point.

RESULTS

Molecular docking (*in silico* study) studies

Molecular docking is a computational technique used in drug discovery to predict the preferred orientation of one molecule (the ligand) when bound to another molecule (the receptor) to form a stable complex. It's essential for understanding how potential drug interact with their target proteins at the molecular level. Molecular docking study was performed using the Schrodinger's glide software. Compounds were subjected to docking with enzymes of the bacteria downloaded from pdb (Kakkassery *et al*., 2021; Hissam *et al*., 2024).

Table 1: Spectral data (IR) for compounds (C1 to C7).

Name of Compound	Functional Group	Vibration in cm^{-1}
C1	OH C=N C=C N=N	3201.01 - 3322.53 1514.19 1602 1631
C2	OH C-H C=C C=N N=N	3221-3357 cm^{-1} 2902 cm^{-1} 1602 cm^{-1} 1631 cm^{-1} 1514 cm^{-1}
C3	C=N C=C C-H	1515 cm^{-1} 1631 cm^{-1} & 1602 cm^{-1} 2897 cm^{-1}
C4	C=O C=C N=N C=N	1707 cm^{-1} 1487 cm^{-1} 1600 cm^{-1} 1500 cm^{-1}
C5	OH C=N C=C N=N C-H	3221-3303 cm^{-1} 1514 cm^{-1} 1602 cm^{-1} 1631 cm^{-1} 2922 cm^{-1}
C6	OH C=N C=C N=N	3209-3246 cm^{-1} 1550 cm^{-1} 1602 cm^{-1} & 1631 cm^{-1}
C7	C=C -OH C=N N=N	1602 cm^{-1} 3217-3444 cm^{-1} 1514 cm^{-1} 1631 cm^{-1}

Antimicrobial study

Antibacterial analysis

Gram +ve bacterium (S. aureus)

The MBCs were recorded Instead of MIC for the samples against *S. aureus* at a concentration of 10 mg/mL where as the standard taken was amoxicillin with 1 mg/mL of working solution.

Gram $^{-ve}$ bacteria (*E. coli*)

Initially, MIC and MBC were recorded for samples C2, C3, C4, and C6 at a concentration of 1 mg/mL. Due to these negative results, a second round of testing was performed using a higher concentration of 10 mg/mL. The standard drug was amoxicillin with 1 mg/mL of working solution.

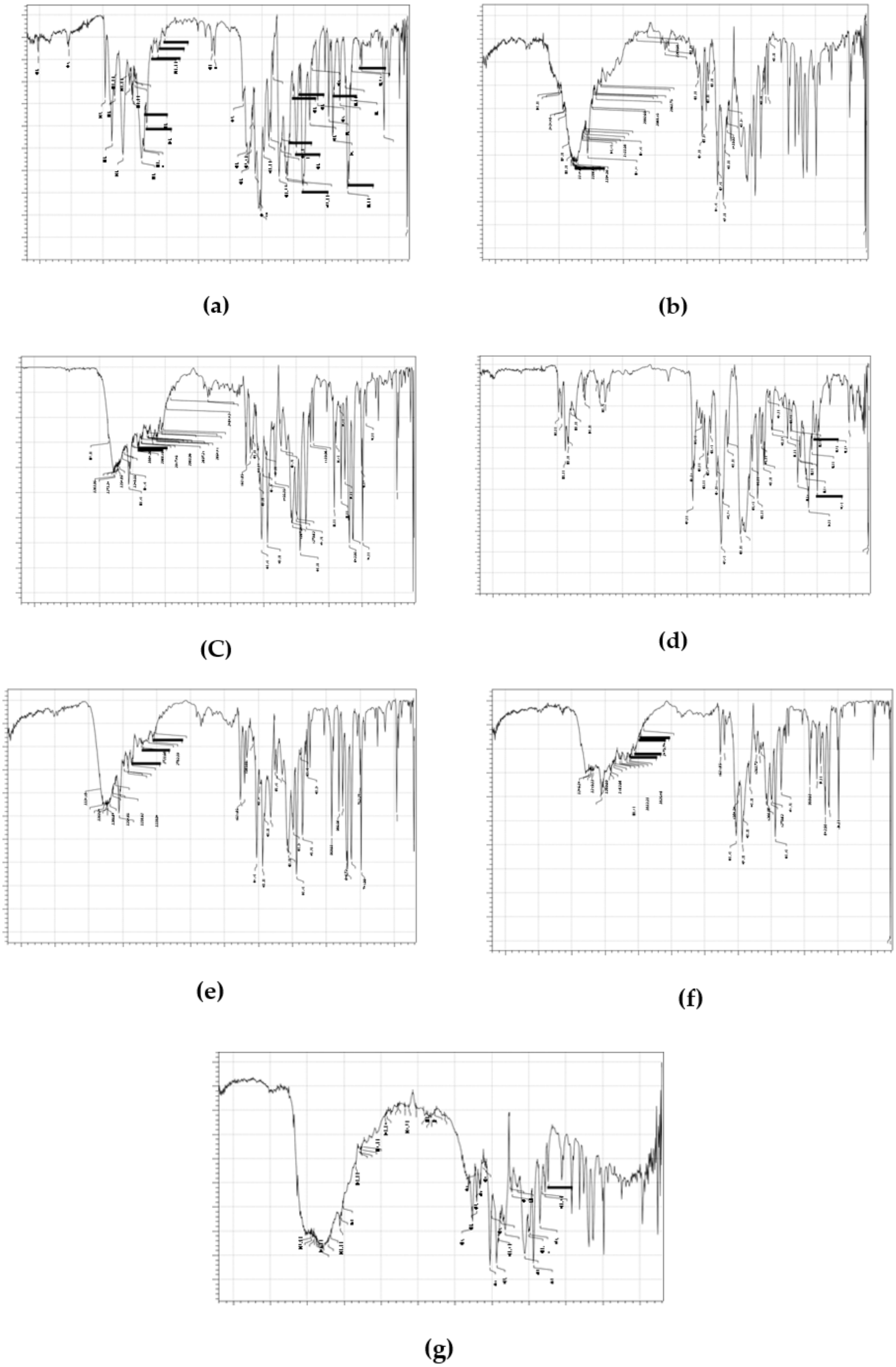


Figure 1: IR Spectrum compounds; a-C1, b-C2, c-C3, d-C4, e-C5, f-C6, g-C7.

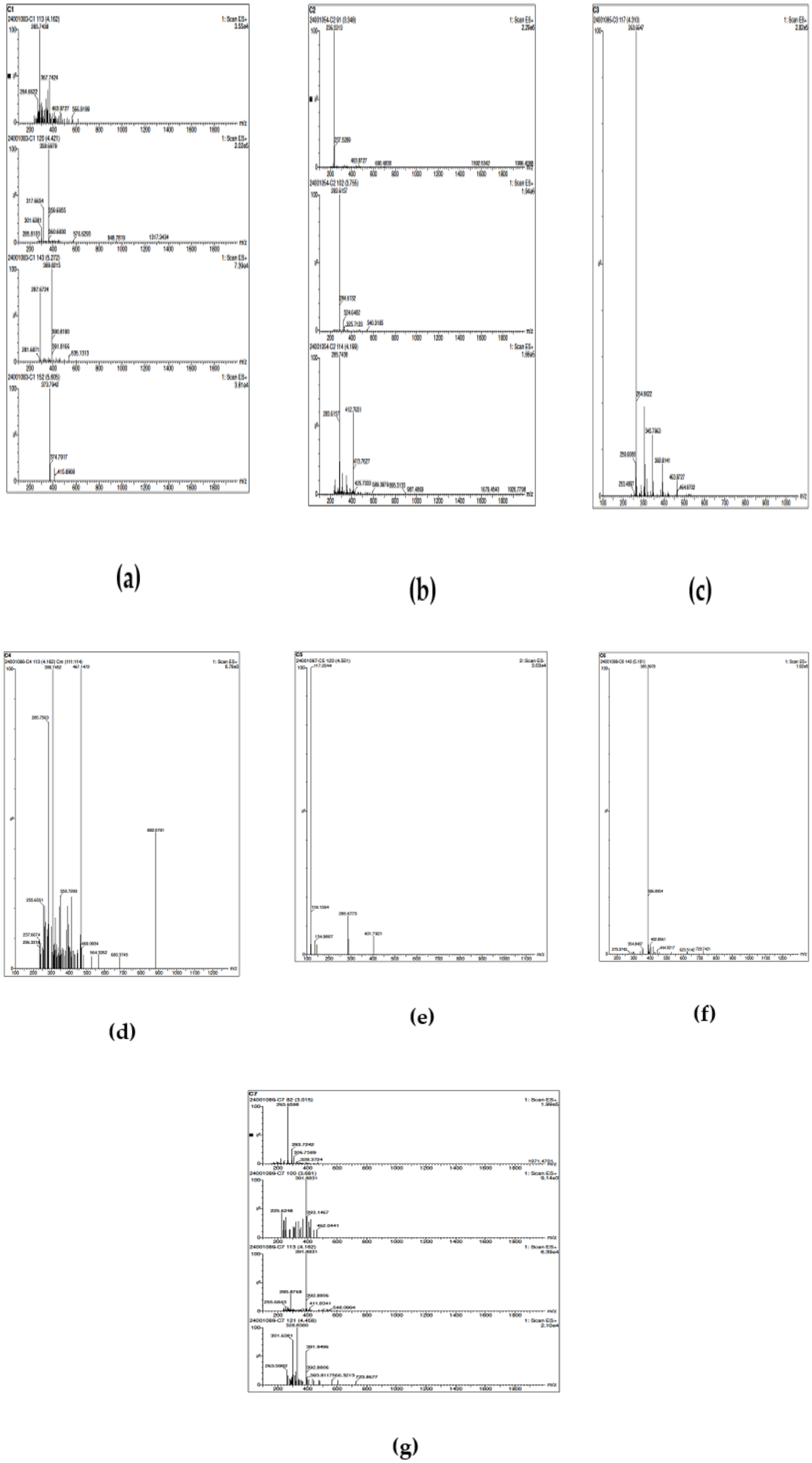


Figure 2: LC-MS Spectra compounds; a-C1, b-C2, c-C3, d-C4, e-C5, f-C6, g-C7.

Table 2: LCMS data of the compounds (C1 to C7).

Name (code) of the compound	Molecular weight	Observed peak value
C1	418	415
C2	463	463
C3	418	392.81
C4	465	460
C5	403	401.79
C6	624	623.51
C7	450	442

Table 3: Details of docking scores and their energy of interactions.

Drug	Scores	Glide energy (K cal/mol)
C4	-4.779	-37.096
C6	-4.97	-40.077
C5	-4.213	-37.194
C7	-05.111	-42.507
Amoxicillin (std drug)	-4.363	-38.057
Microorganism & Protein	E. coli (1 Q81)	---
C4	-8.589	-60.335
C6	-5.649	-40.036
C5	-5.718	-44.25
C7	-5.743	-45.805
Amoxicillin (std)	-4.396	-44.589
Microorganism & Protein	S. aereus (5 XDT)	----
C2	-5.066	-42.76
C4	-8.084	-60.137
C6	-5.116	-42.583
C5	-5.368	-42.436
C7	-6.334	-48.58
Fluconazole (std)	-6.302	-38.039
Microorganism & Protein	C. albicancs (4 HOE)	----

Table 4: Antimicrobial activity results.

Compound name	Concentration of the compounds (mg/mL) 10 mg/mL to 0.019 mg/mL	Microorganism	MBC(MIC) value (mg/mL)
C2		S. aureus	2.5
C3		S. aureus	1.25
C4		S. aureus	0.625
C6		S. aureus	0.625
Standard drug (Amoxycillin)	1 mg/mL	S. aureus	0.0625
C2		E. coli	1.25
C3		E. coli	0.0195
C4		E. coli	0.625
C6		E. coli	0.625
C7		E. coli	2.5
Standard drug (Amoxycillin)	1 mg/mL	E. coli	0.125
C2		C. albicans	0.625
C3		C. albicans	0.625
C4		C. albicans	2.5
C6		C. albicans	0.3125
C7		C. albicans	1.25
Standard drug (Fluconazole)	1 mg/mL	C. albicans	0.0625

While the MIC results cannot be presented due to the compounds' distinct colors affecting the visual analysis, hence the MBC results are as follows:

Antifungal analysis (*C. albicans*)

Since the compounds were coloured, MFCs were recorded instead of MFC against *Candida albicans* for the study and the standard Fluconazole with 1 mg/mL as working solution was used.

DISCUSSION

Privileged Schiff bases were synthesized in one step, which then diazotized and coupled with beta-naphthol to yield new azo dye compounds. These new compounds were preliminarily identified using IR analysis, the confirmation of Schiff base formation by characteristic functional group vibrations such as phenolic-OH, imine (-CH=N), C=C, aromatic CH, and aldehyde (-CHO). As mentioned in Table 1, the prominent group of Schiff's bases imine group (-CH=N-) was clearly notable in the range of 1500 cm^{-1} to 1650 cm^{-1}. Another important functional group which is characteristic of diazotisation was N=N noticed in the range of 1030-1630 cm^{-1} for all the compounds. Figure 1 illustrates that the IR spectra of diazotized and coupled schiff's base derivatives confirmed the attachment between the diazotized Schiff base and beta-naphthol. Table 2 reveals the Molecular ion values of peaks in LCMS spectra were in the range of 400 to 625 for all the compounds almost close to their molecular ion values which indicates the formation of the compounds and hence confirmed the compounds' structures as observed in Figure 2.

The synthesized compounds were evaluated for their antibacterial potentials using Schrödinger Glide software, in which docking with bacterial and fungal proteins was carried out and compared with amoxicillin as a standard. As evident in Table 3, among all, compound C7 showed superior docking scores and binding energies while interacting with key amino acids such as THR, VAL, PHE, and LEU, suggesting probable good antibacterial potential and scope for structural optimization. As illustrated in Figures 3 and 4, the docking images clearly reveal the potential interactions of the synthesized compounds with amino acids, wherein the molecules clearly inserted into the pockets of the targeted proteins. The compounds C4 and C5 have shown glide energy -37.096 and -37.194 respectively which is lower than the glide energy of std, drug -38.057, which indicates the significance

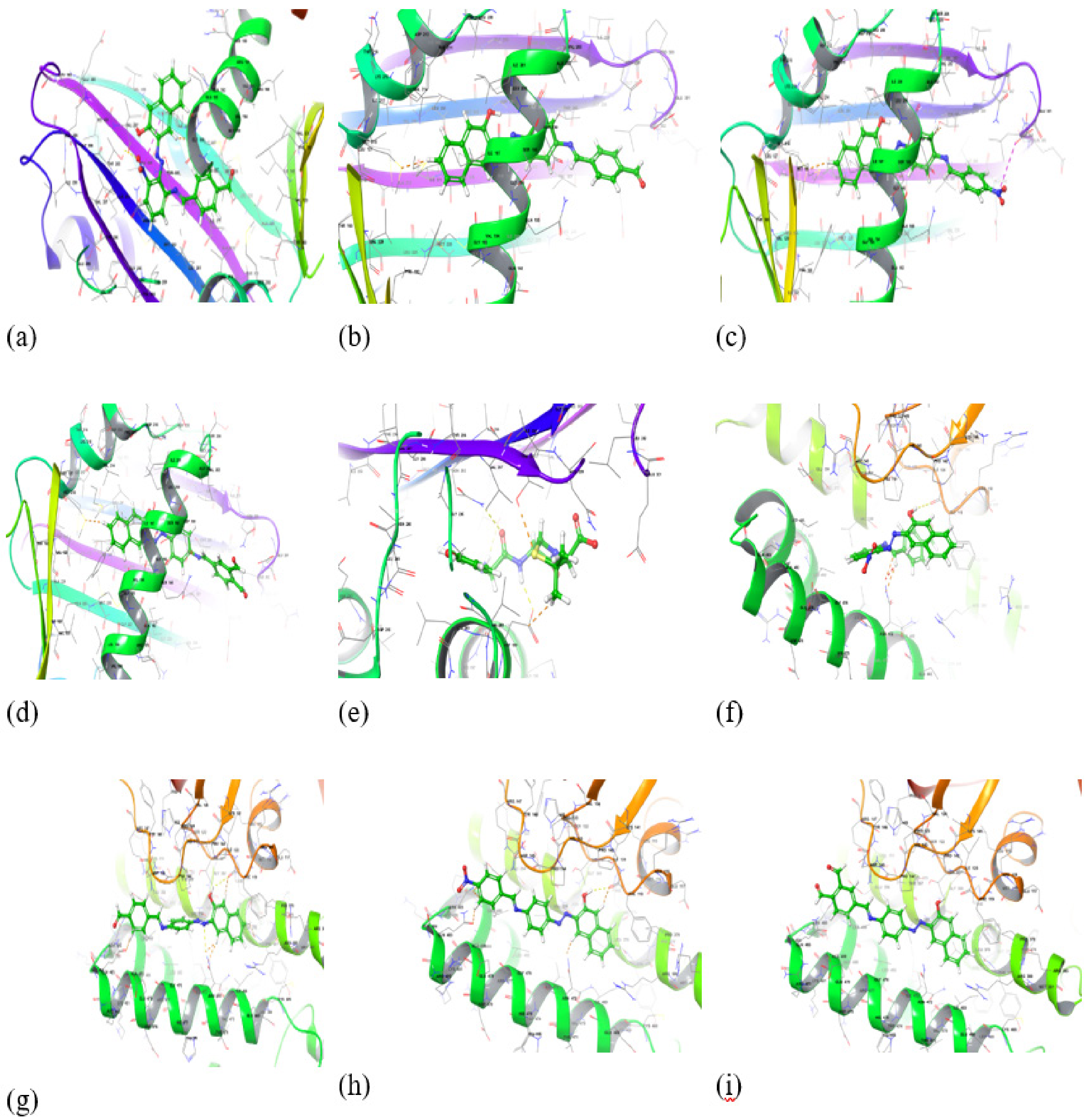


Figure 3: Docking 3-D of Gram +ve bacterial protein [5XDT]; a-C4, b-C5, c-C6, d-C7, e-std.drug(Amoxicillin). Docking 3-D of Gram -ve bacterial protein [1Q81]; f-C4, g-C5, h-C6, i-C7.

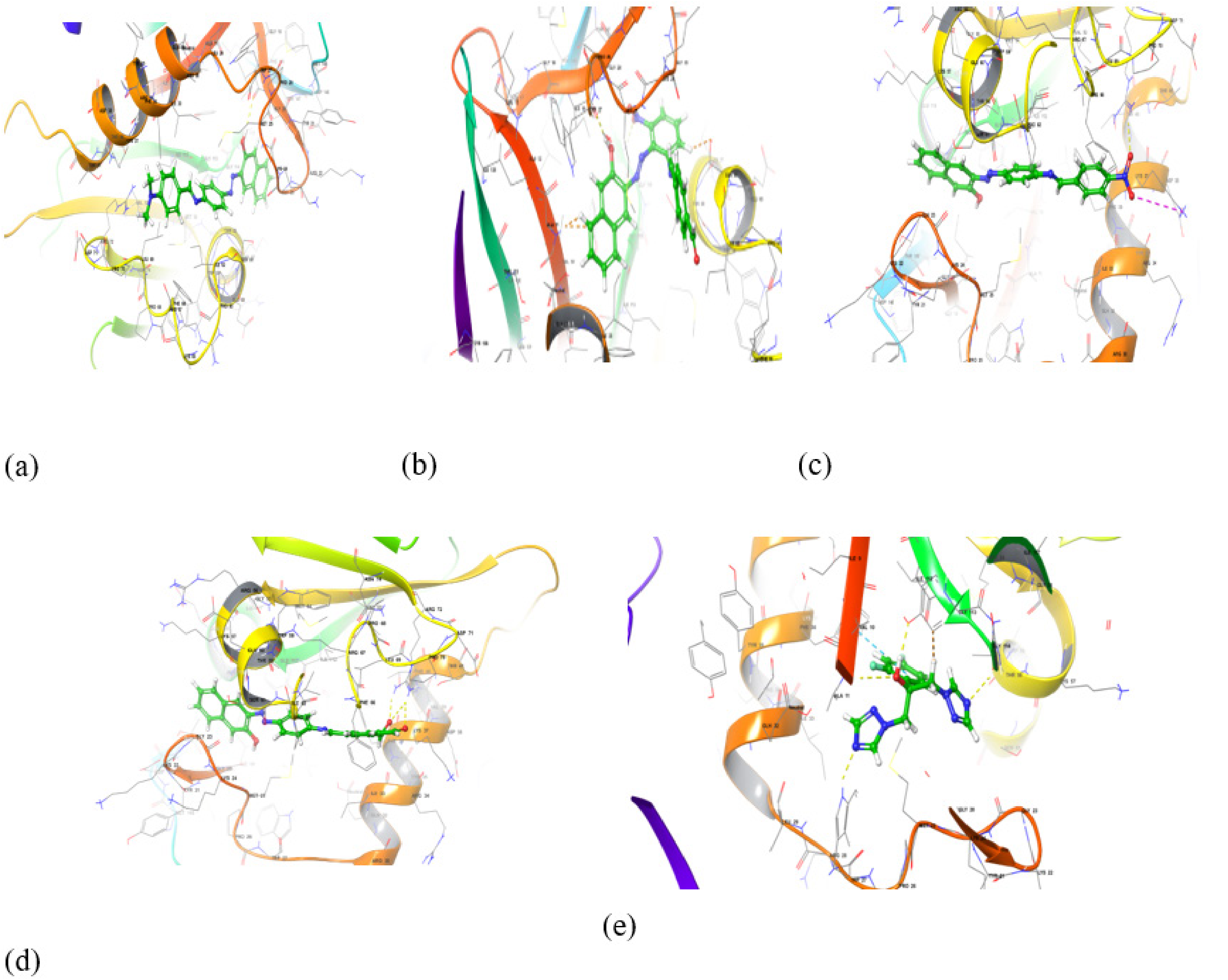


Figure 4: Docking 3-D of Antifungal bacterial protein [4HOE]; a-C2, b-C4, c-C6, d-C7, e-std.drug(Fluconazol).

of the interactions of these compounds with the target proteins. The glide energy given by C5 and C6 were -44.25 and -40.036 respectively which is lesser that that of the std. drug against proteins. However, the glide energy of the compounds against the proteins of fungus *C. albicans*, is more than the energy generated by the std. drug which was38.039 (Fernandez *et al*., 2021; Joseyphus *et al*., 2022; Hissam *et al*., 2024).

In vitro tests assessed antibacterial activity against *S. aureus* and *E. coli*, and antifungal activity against *C. albicans* over range of concentrations from 10 to 0.019 mg/mL. Since compounds were coloured, MBC and MFC were measured instead of MIC, using amoxicillin and fluconazole as standards, ensuring consistent, comparable results.

As mentioned in Table 4, among all the compounds tested against Gram-positive bacterium *S. aureus*, C2 showed the highest MBC of 2.5 mg/dL, compared to C4 and C6 had the lowest MBCs of 0.625 mg/dL, indicating strong antibacterial effects likely due to functional groups such as OH, NO_2, and alkyl amine. C_2 contained OH, NO_2, and alkyl amine; C4 possess -CHO, -NO_2, and -OH; and C_6 contains -NO_2 and -OH groups as seen in Figure 5. Similarly, against Gram-negative *E. coli*, Figure 6 illustrates that C7 exhibited the highest MBC of 2.5 mg/mL, while C3 showed the lowest at 0.0195 mg/mL. These functional groups probable contributions to antibacterial activity which may be due to their electronegative property sufficient to damage the cell wall and it require further study in understanding their mode of action; however, these compounds showed promising, dose-dependent activity compared to the standard drug (Kaur *et al*., 2020; Kasare *et al*., 2022; Kyei *et al*., 2020).

Table 4 also reveals regarding the antifungal activity study of the compounds in which compound C2 exhibited the highest MBC of 2.5 mg/mL, while C4 and C6 exhibited lower MBC values of 0.625

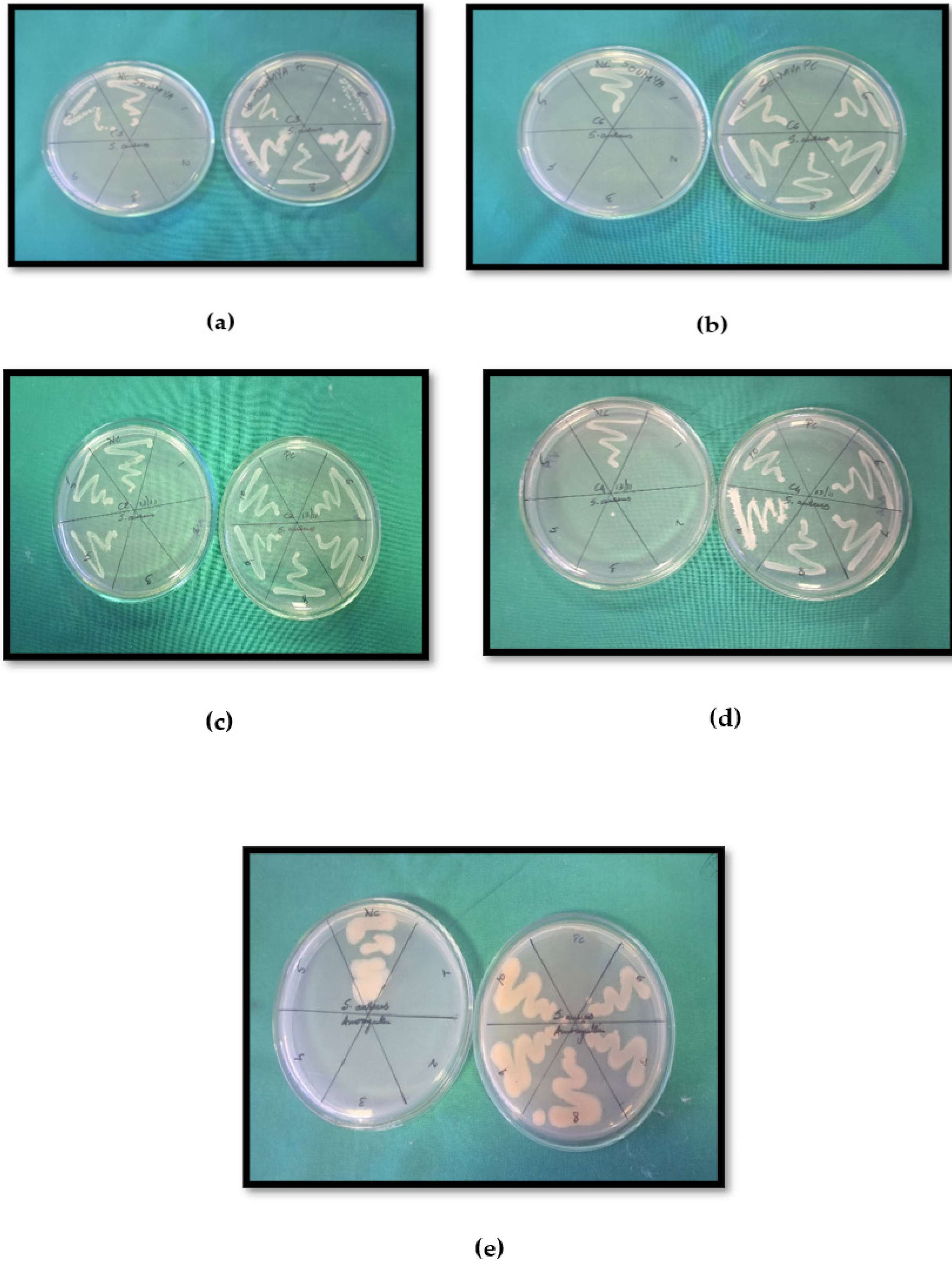


Figure 5: Gram +ve bacteria (S.aureus); a-C3, b-C6, c-C2, d-C4, e-std.drug (Amoxicillin).

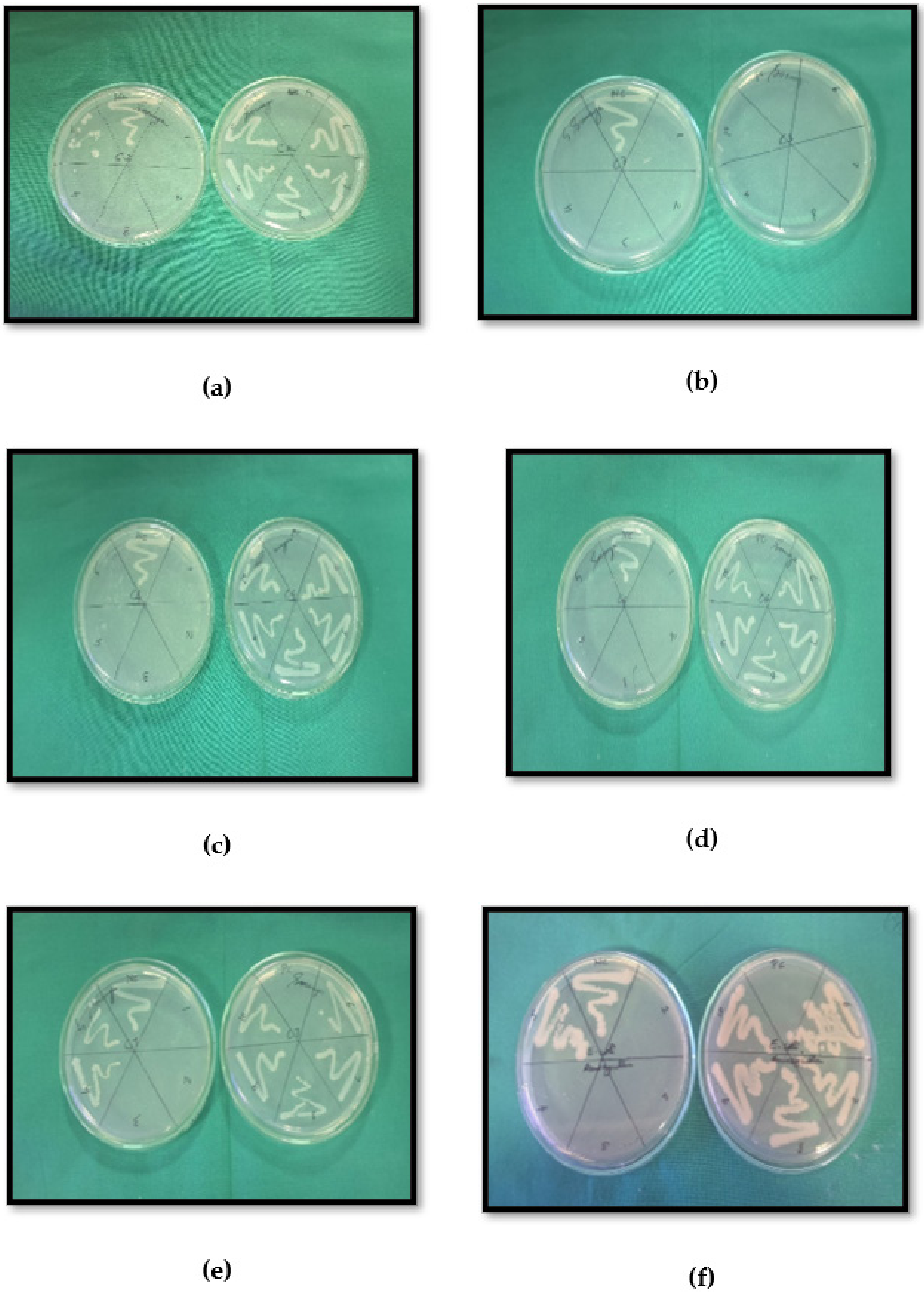


Figure 6: Gram -ve bacteria (E.coli); a-C2, b-C3, c-C4, d-C6, e-C7, f-std.drug (Amoxicillin).

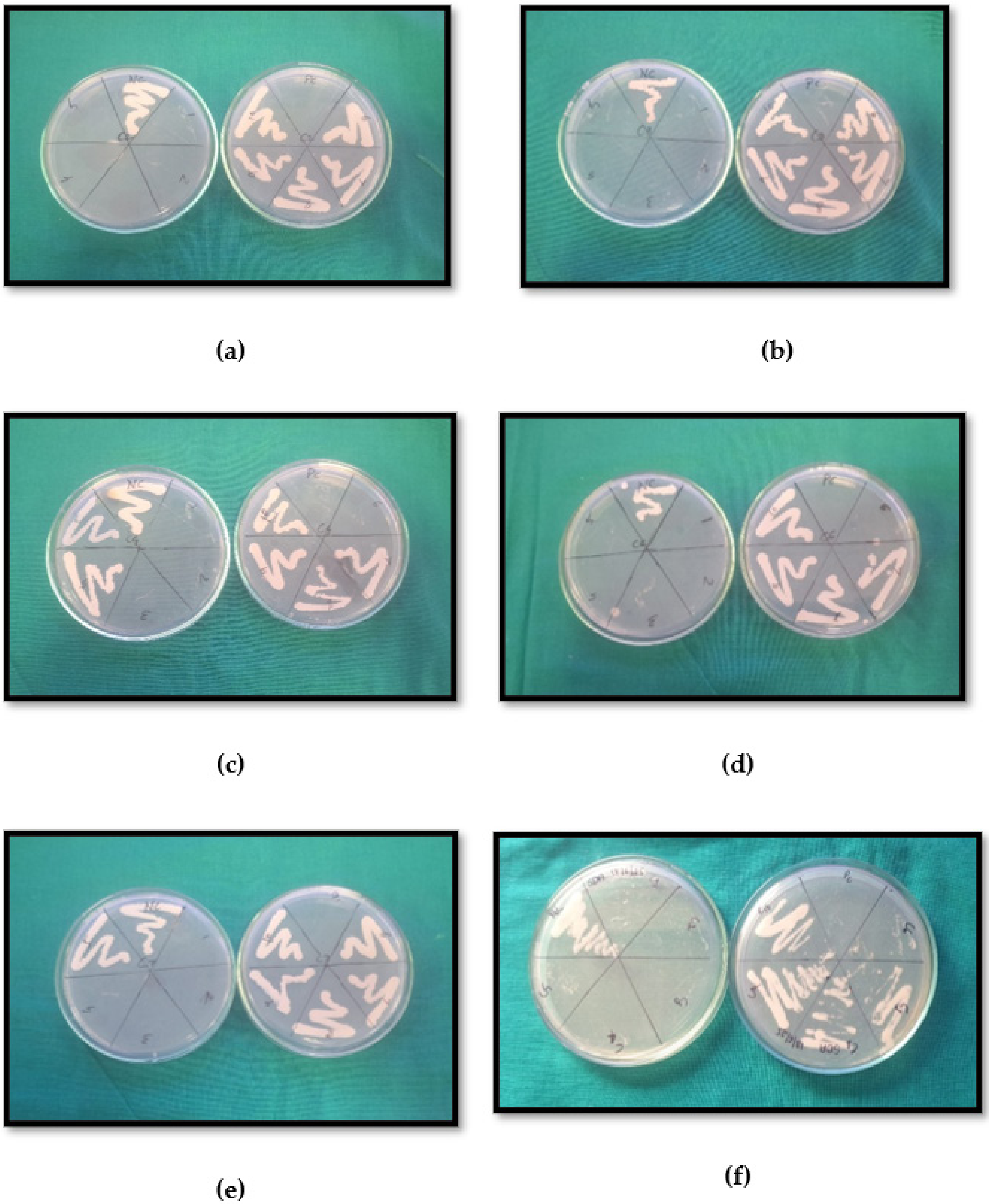


Figure 7: Antifungal activity (C. albicans); a-C2, b-C3, c-C4, d-C6, e-C7, f-std.drug (Fluconazol).

mg/mL. This indicates that basic structure with electronegative groups like -NO2 and -OH likely to contribute to their activity. It is observed from Figure 6 and Figure 7, that compound C7 demonstrated superior activity against both Gram-negative bacteria and fungi, possibly may be due to dimethoxy and hydroxyl groups because of their electronegative property which may damage the cell or inhibit the growth of the cell. Hydrophilic groups, especially hydroxyls, enhance the penetration of Schiff bases through microbial cell walls, improving their efficacy. Among tested compounds, it is evident from Figure 7 that C6 demonstrated the strongest antifungal effect against *C. albicans*, followed by C2 and C3. It is also evident that these effects were dose-dependent compared to the standard drug. However, further study is needed to justify this claim (Morsy *et al*., 2023; Ibrahim *et al*., 2024).

In nutshell, the synthesized compounds were proven efficacious as antimicrobial agents in this study and open new opportunities for their utility. These compounds deserve an opportunity to be explored commercially, as antimicrobial and industrial dyes on various products which elicit new opportunities for the Azo dyes due to their antimicrobial applications.

CONCLUSION

A series of Schiff base derivatives coupled with beta naphthol via diazo link were synthesized and characterised by spectral analysis. Later, these compounds were screened for their antibacterial and antifungal activity by *in silico* followed by *in vitro* methods which have justified their antimicrobial potentials. As these compounds are coloured in nature, and possess antimicrobial potentials, these new Azo dyes would possibly find commercial application as dyes for pharmaceutical and industrial utility along with healthcare benefits in future.

ACKNOWLEDGEMENT

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ABBREVIATIONS

C1 to C7: Compounds 1 to 7; ***S. aureus*:** *Staphylococcus aureus*; ***E. coli*:** *Escherichia coli*; ***C. albicans*:** *Candida albicans*; **MBC:** Minimum Bactericidal Concentration; **MFC:** Minimum Fungicidal Concentration; **MIC:** Minimum Inhibitory Concentration; **IR/FTIR:** Infrared/Fourier-Transform Infrared Spectroscopy; **TLC:** Thin-Layer Chromatography; **LCMS:** Liquid Chromatography-Mass Spectrometry; **pdb:** Protein Data Bank.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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