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Synthesis, Characterization, Corrosion inhibition and Biological Evaluation of Schiff Bases

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Abstract: Four schiff bases, namely 4-((thiophene-2-yl) methyleneamino) phenyl sulphonamide (**1**), (2E)-2-((5-((E)-(2-hydroxyphenylimino) methyl) thiophene-2-yl) methyleneamino) phenol (**2**), 2-(5-methoxy-2-hydroxybenzylideneamino)phenol(**3**) and N,N'-bis((thiophene-2 yl)methylene)benzene-1,2-diamine (**4**) were synthesized. The Chemical structures of the products were confirmed by IR, ^{1}H NMR, Uv-Vis, mass spectral and elemental analyses techniques. Corrosion inhibition of the schiff bases were evaluated using weight loss method in a 0.1MHCl solution for mild steel. The Schiff bases had exhibited a good inhibitory action against corrosion of mild steel in the medium investigated. The schiff bases were screened for their in vitro antimicrobial activities against S. aureus, K.pneumoniae, C. albicans and C. krusei. The result revealed that, except 4 all the synthesized Schiff bases showed significant antimicrobial activity against all microbial under the study.
Keywords: Schiff bases, Corrosion inhibition, antimicrobial activities.

Introduction

Schiff bases are usually synthesized from the condensation of primary amines and active carbonyl groups. They are one of the important compounds owing to their wide range of biological activities and industrial applications. They have been found to posses pharmacological activities such as antimalarial [1], Anticonvulsant[2], anti-inflammatory[3], anticancer [4], anti-tumour [5], anti-HIV [6] and antibacterial and antifungal [7-9]. They also serve as a back bone for the synthesis of various heterocyclic compounds [10]. The azomethine linkage and the donor atoms in the back bone of the schiff bases are responsible for their biological activity and industrial application, which can be altered depending upon the type of substituent present on the aromatic rings. In addition, Schiff bases, since they contain an azomethine-N, are well-known organic inhibitors of metal corrosion[11, 12]. Research work revealed that the inhibition efficiency of Schiff bases is much greater than that of the corresponding amines and aldehydes due to the presence of an –HC=N– group in the molecules[13].

In view of the above importance of Schiff bases, we report herein the synthesis of new Schiff bases by condensing sulphanilimide, o-aminopehenol and o-pheneylediamne with 2-Hydroxy-5-Methoxybenzaldehyde, 2-thiophenecarboxaldehyde and 2,5-thiophenedicarboxaldehyde in the presence of glacial acetic acid (Scheme1). All the synthesized Schiff bases have been characterized on the basis of their elemental analysis, FT-IR, ^{1}HNMR, MS-EI and UV-Vis spectral data. The effect of the schiff bases on the corrosion behavior of mild steel in a 0.1MHCl solution was studied by weight loss method. Furthermore, the in vitro antimicrobial activities of the synthesized schiff bases were also evaluated against bacterial: Klebsiella pneumonia and Staphylococcus aureus and fungal: Candida albicans and Candida krusei by the agar well diffusion assay. The results were compared with standards.

2. Experimental Part

2.1. Materials and Analytical Methods

All chemicals used in this investigation were of analytical reagent grade (AR) and of highest purity

available and hence used as received. The Schiff base components were purchased from Sigma Aldrich and Alfa. Melting points were recorded in open capillaries in Stuart Melting point, SMP10, apparatus. IR spectra of the compounds were recorded using KBr pellets on FT-IR spectrometer Perkin-Elmer Infrared model 337. Electronic absorption spectra were obtained on a Perkin-Elmer Lambda 35 UV-Vis spectrophotometer. ^{1}H NMR spectra of the Schiff bases in $CDCl_3$/ DMSO-d_6 were recorded on a BRUKER AVANCE III 500 MHz FT NMR Spectrometer. The EI mass spectra of the Schiff bases were recorded on GC-MASS spectrometer. Elemental analyses were performed on a CHN-Analyser: PERKIN-ELMER CHN-2400 analytical instrument.

2.2. Synthesis of 4-((thiophene-2-yl) methyleneamino) phenyl sulphonamide (1)

Sulfanilamide (1.84 g, 0.0107 mole) dissolved in a 40ml of mixture of THF/EtOH in a ratio of 1:4,v/v was mixed with 2-thiophenecarboxaldehyde (1 ml,0.0107 mole) dissolved in 30ml of a mixture of same solvent. To this solution 2.5 ml of glacial acetic acid was added. The reaction mixture was refluxed on oil bath at 75^{o}C for 6hrs with continuous stirring. The progress of the reaction was monitored by TLC**.** The mixture was cooled and poured into ice-cooled water to precipitate Schiff base. The resulting colored precipitate was filtered and dried in vacuum oven at 80^{o}C. The dried solid product was recrystallized twice from hot ethanol and then dried under reduced pressure over anhydrous $CaCl_2$ in a desiccator. The product isolated as Shiny yellow powder in 92% yield. The melting point of the product was found to be 217^{o}C.

IR (KBr cm^{-1}) **:** 3290 (υ(N-H);-NH_2), 1607 (υ(C=N)), 1335 (υ_{asym}(S=O)), 1314(υ_{sym}(S=O)), 1153(υ(C-N); aromatic),846 (υ(C-S-C)*asym),* 821(,υ(C-S-C)*sym*); **Uv-Vis** (Acetonitrile, nm): 269, 328; **^{1}HNMR (DMSO-D_6)**: δ=10.3 (s, 2H, NH_2), 8.82 (s, 1H,CH=N), 7.255-7.895 (m,7H, aromatic); MS [EI] m/z 267 [M+1]; **Elemental analysis (CHN):** ($C_{11}H_{10}O_2N_2S_2$) Found (Calc.) (%), C 49.52(49.56), H 3.77(3.75), N 10.48 (10.51).

2.3. Synthesis of (2E)-2-((5-((E)-(2-hydroxyphenylimino) methyl) thiophene-2-yl) methyleneamino) phenol (2)

Similar procedure was used to synthesize the title schiff base from o-Aminophenol (0.1454 g, 0.0014 mole) and 2,5-Thiophenedicarboxaldehyde (0.0993gm,0.0007 mole), except that the solid product was precipitated out on cooling of the resulting reddish clear solution at room temperature. The product isolated as Golden yellow powder in 97% yield. The melting point of the product was found to be 189^{o}C.

IR (KBr cm^{-1}) **:** 3357 (υ(O-H)), 1612 (ν(C=N)), 1456 (ν(C–O) phenolic), 2698(ν(OH)), 820,807 (ν(C-S-C) (asym, sym)), 744 (ν(C-S)), 1152 (ν(C-N) aromatic); **Uv-Vis**(Acetonitrile, nm): 228, 268, 329 ; **^{1}HNMR** (DMSO-D_6) : δ= 13.08 (s, 1H,OH), 8.593(s, 1H,CH=N), 6.87-7.367 (m,10H, Aromatic); MS [EI] m/z 323 [M+1]: **Elemental analysis (CHN):** ($C_{18}H_{14}O_2N_2S$) Found (Calc.) (%), C 66.89 (66.99), H4.33 (4.34), N8.71(8.68).

2.4. Synthesis of 2-(5-methoxy-2-hydroxybenzylideneamino) phenol (3)

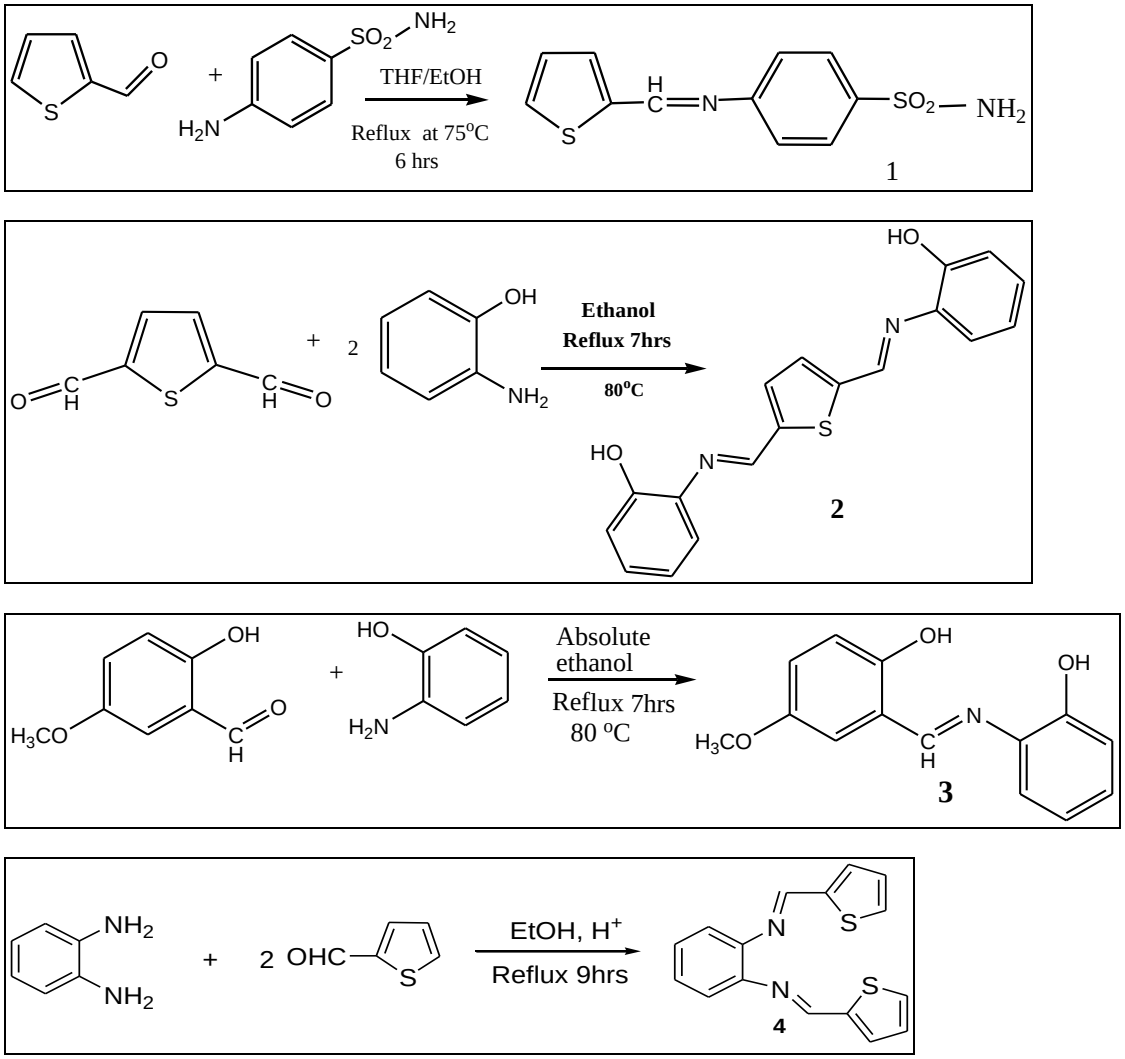
The title schiff base was synthesized from o-Aminophenol (0.874 g, 0.0080 mole) dissolved in a 30ml of absolute ethanol and 2-Hydroxy-5-Methoxybenzaldehyde (1.22gm,0.0080 mole) dissolved in 30ml of same solvent by the similar procedure employed for synthesis of **1**. The product isolated as reddish powder in 97% yield. The melting point of the product was found to be 157^{o}C.

IR (KBr cm^{-1}) **:** 3444 (υ(O-H)), 1627 (υ(C=N)), 1517 (υ(C–O) phenolic), 2700 (υ(OH)), 1140,1040 (υ (C-O-C) (asym, sym)), 1222 (υ(C-N)), 2985-2830 (υ (C-H) -CH_3) ,737 (δ (C-H) aromatic); **Uv-Vis (**DMSO, nm**):** 238, 270, 370; **^{1}HNMR (DMF-D_6): δ =**12.678ppm (s,1H,OH), 8.689 (s,1H, CH=N), 6.945-7.447(m, 7H, 7ArH). MS [EI] m/z 244 [M+1]: **Elemental analysis (CHN): (**$C_{14}H_{13}O_3N$) Found (Calc.) (%) C 69.08 (69.06), H 5.35(5.34), N 5.75(5.75).

2.5. Synthesis of N,N'-bis((thiophene-2-yl)methylene)benzene-1,2-diamine(4)

The title schiff base was synthesized from o-phenylenediamine (4g, 0.037 mole) and 2-thiophenecarboxyaldehyde (8.3g, 0.07398 mole) by the Similar procedure employed for synthesis of **1**, except that the reaction mixture was refluxed at 85 ^{o}C for two extended hours. The product isolated as yellow powder in 73% yield. The melting point of the product was found to be 150 ^{o}C.

IR(KBr cm^{-1}) **:** 1600 (υ (C=N)), 851 (υ(C-S-C)*asym),* 834(υ(C-S-C)*sym*), 733 (υ(C-S)); **Uv-Vis**(DMF, nm): 242, 291,349; **^{1}HNMR**($CDCl_3$): δ= 8.7 (s, 1H,CH=N), 6.8-7.5 (m,10H, aromatic); **MS [EI]** m/z 297[M+1]. **Elemental analysis (CHN):** ($C_{16}H_{12}N_2S_2$) Found (Calc.) (%), C 64.78(64.83), H 3.96(4.05), N 9.37(9.45).



Scheme 1 Synthetic route for the new Schiff bases (1-4)

2.6. **Gravimetric measurements**

Aggressive solution (0.1M HCl) was prepared by dilution of reagent grade 37% HCl with double distilled water. Inhibitor solutions with concentrations of 400 and 800ppm were employed for inhibition studies and were prepared by dissolving the required amount of the schiff bases in 80 ml of 0.1 M HCl by stirring at room temperature. 80 ml of 0.1 M HCl without inhibitor was used as blank test solution.

In the weight loss experiment, beakers of 100ml capacity were labeled 1 to 9, each containing 0.1M of HCl solution. The first beaker was reserved as blank while each of the remaining beakers contained the schiff bases at concentrations of 400 and 800 ppm. All placed at room temperature. Mild steel coupons having 1 × 1 × 0.1 cm size were abraded with emery paper and washed with ethanol, acetone, distilled water then dried and weighed. The area of the mild steel coupons was measured. The coupons were immersed in hanging position in the experimental solutions with the help of glass hooks for two days. The weights of the specimens were noted before immersion. After immersion time of 48 hours, the specimens were removed, polished with emery papers, washed in distilled water, degreased with acetone, dried in oven, and reweighed. Duplicate experiments were conducted at same time and average values were taken. From the initial and final weights of the specimens, the loss of weights was calculated, ΔW, as follows:

$$\Delta W = \frac{m1 - m2}{A} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where $m1$ is the mass of the specimen before corrosion, $m2$ the mass of the specimen after corrosion, and A the exposed area of the specimen.

The corrosion rate (in mmy^{-1}) was computed from the following equation [14]

$$Corrosionrate, CR = \frac{87.6 \times W}{DAt} \quad \text{.....(2)}$$

where W is the weight loss in mg, D is the density of the specimen (7.85 g/cm^3), A is the surface area of specimen (cm^2) and t is the time of exposure of the sample in hours .
The efficiency of the inhibitor was computed using the following equation [15]:

$$Inhibitionefficiency, \%IE = \frac{\Delta W1 - \Delta W2}{\Delta W1} \times 100 \quad \text{.....} \quad (3)$$

Where $\Delta W1$ is the weight loss without inhibitor and $\Delta W2$ is the weight loss with inhibitor.

2.7. Antimicrobial studies

The in vitro antimicrobial activity of the synthesized Schiff bases was determined by the agar well diffusion assay [16]. The bacteria strains: Klebsiella pneumonia and Staphylococcus aureus and the fungal strains: Candida albicans and Candida krusei were selected because of their clinical relevance. The bacterial and fungal strains used for the assay were maintained on Nutrient agar and Sabouard dextrose agar, respectively. In the agar diffusion method, Muller-Hinton agar and Sabouard dextrose agar were sterilized in flasks and poured into sterile petriplates and allowed to solidify. The inoculum was prepared by suspending the colonies of the organisms to be tested in 0.9% sterile saline and turbidity adjusted to 0.5 McFarland standard (10^8UFC/mL). Sterile swabs were used for inoculating the surface of the petriplates and wells of 7 mm diameter were aseptically bored into the culture medium and 50µl of compounds dissolved in DMSO were added to these wells. DMSO alone was used as negative control along with the antimicrobials, Voriconazole and Ampicillin as positive controls for fungi and bacteria, respectively. The plates were incubated aerobically at 37°C for 24 hours and the antimicrobial activity was assessed by measuring the inhibition halo of microbial growth around the well.

3. Result and Discusion

3.1. Characterization of the Schiff Bases (1-4)

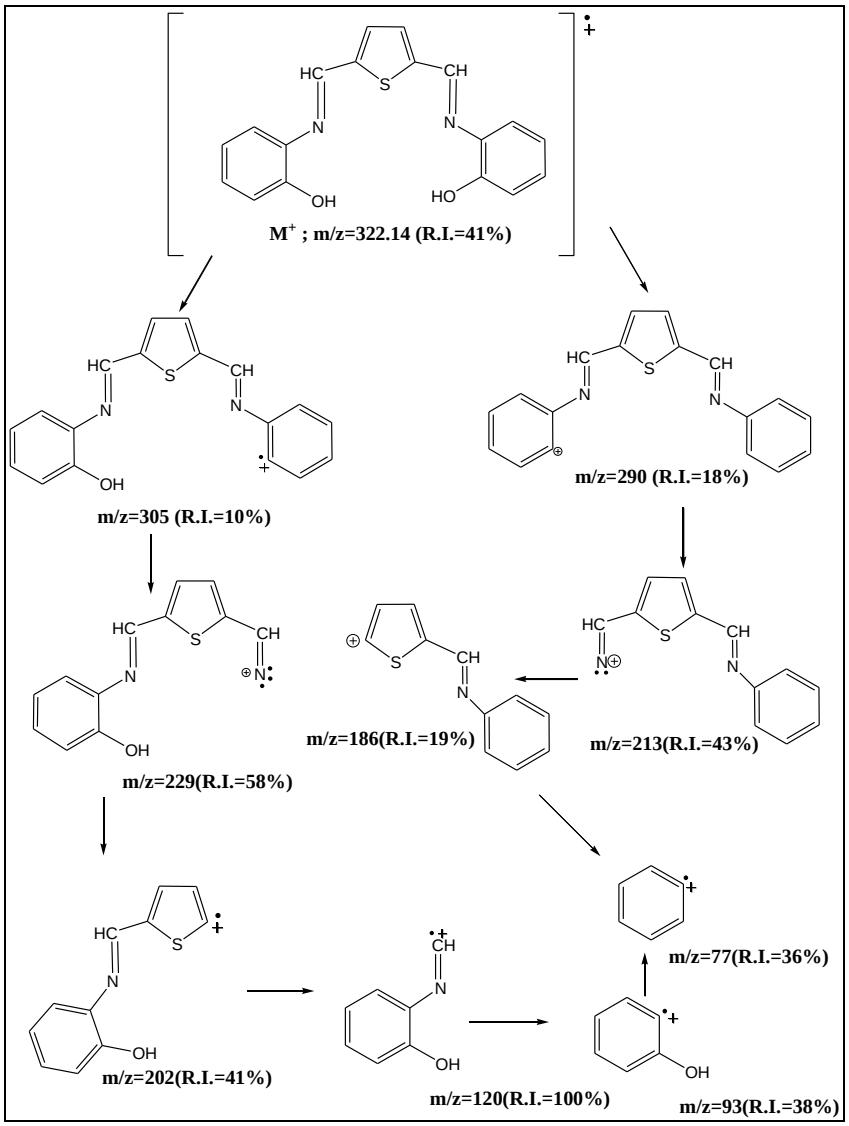
The route for the synthesis of Schiff bases (1-4) and their structures is illustrated in Scheme 1. The synthesized Schiff bases were checked by comparing the TLC with the starting materials, which resulted in a single spot different from the starting materials. The Synthesis achieved in high yields**.** The structures of all synthesized compounds in Scheme 1 were confirmed on the basis of elemental analyses, IR, Uv-Vis, ^{1}H NMR and mass spectral data.

IR studies of each compound confirms the formation of -C=N- bonds as well as lack of -C=O- from original aldehydic compounds. The IR spectra of compounds 1-4 showed absorption bands in the range of 1600-1627cm^{-1} due to -C=N- stretching. The bands in the range 1456-1517 and 3357-3480 cm^{-1} were due to C-O and O-H stretch, respectively, for compounds of 2 and 3. The spectra of the Schiff bases of **2** and **3** exhibited a band in the range 3290-2560 cm^{-1} due to intramolecularly hydrogen bonded vibration (O-H---N) [17, 18]. In the IR spectrum of **1**, N–H stretching and N-H bending vibrations were observed at 3290 and 1579 cm^{-1}, respectively. These values are in agreement with those observed for similar compounds [19, 20]. In addition, **1** exhibited two bands at 1335 and 1314 cm^{-1} due to υ_{asym}(S=O) and υ_{sym}(S=O) stretching vibration of -SO_2- moiety, respectively. Similarly Schiff bases **1, 2** and **4** exhibited two bands at 846,820 and 851, and 821,807 and 834 cm^{-1} due to υ(C-S-C) asymmetric and υ(C-S-C) symmetric stretching vibration of thiophene moiety, respectively.

The ^{1}H NMR spectra of compounds 1-4 showed singlet in the range of 8.59-899 ppm which was due to the presence of azomethine,–CH=N-, proton[21]. The signal at13.08 and 12.678 ppm were due to the resonance hydroxyl groups of 2 and 3, respectively. The down field signal of OH group could be attribute to the contribution of the OH group to the intramolecular and intermolecular hydrogen bonding[22, 23]. In addition to this, multiple signals lying in range 7.757-7.895, 6.87-7.367, 6.945-7.447 and 6.8-7.5 ppm were attributed to resonance of aromatic protons of schiff bases of 1, 2, 3 and 4, respectively [24,25].

The mass spectra of the schiff bases confirmed the structure of the Schiff bases as indicated by the peak corresponding to their molecular masses. The spectra showed a signal with m/z ratio of 266.35 (R.I.100%, base peak), 322.41, 243.27 (R.I.100%, base peak) and 296.4379 (R.I.51%) which are same as the calculated formula m/z= 266.35,322.14, 243.12 and 296.44 for Schiff bases of 1,2,3 and 4, respectively. The m/z ratio of 266.35,

322.41, 243.27 and 296.43 confirm their molecular formulas are $C_{11}H_{10}N_2O_2S_2$, $C_{18}H_{14}O_2N_2S$, $C_{14}H_{13}O_3N$ and $C_{16}H_{12}N_2S_2$, respectively. It is observed that, the molecular ion peaks are in good agreement with their suggested empirical formula as indicated from elemental analyses. As representative example, the fragmentation of **2** obtained from the rupture of different bonds inside the molecule is shown in scheme **2**. The fragment was in good agreement with the proposed formula of the Schiff base. Similarly the spectral values for all the compounds and C, H, N analyses are given in the experimental part. The elemental analysis of the prepared Schiff bases is consistent with the calculated result from the empirical formula of the compounds. The melting points are sharp, which indicate the purity of the prepared Schiff bases.



Scheme 2 Suggested mass fragmentation of Schiff base 2

3.2. The effect of Schiff Bases (1-4) on acid Corrosion of Mild Steel

In order to study the effect of the synthesized Schiff bases on corrosion of mild steel in 0.1 M HCl, gravimetric measurements of mild steel was carried out in absence and presence of 400 and 800 ppm of the compounds in the same solution at room temperature. The percentage inhibition efficiency and corrosion rate calculated from the weight loss results for 48 h are given in Table 1.

Table 1 The weight loss, percentage inhibition efficiency and corrosion rate obtained for a mild steel coupons immersed in 400 and 800ppm of 0.1MHCl solutions of Schiff bases (1-4) at RT for 48 h duration

Inhibitor	Inhibitor Concentration (ppm)	Weight loss ($mgcm^{-2}$)	IE(%)	CR(mmy^{-1})
Blank	-	470	-	109.27
1	800	183.91	60.87	42.76
	400	229.22	51.23	53.29

2	800 400	111.39 148.52	76.3 68.4	25.90 34.53
3	800 400	100.11 141.0	78.7 70.0	23.27 32.78
4	800 400	126.57 159.80	73.07 66.00	29.43 37.15

It can be seen from the data that the Schiff bases synthesized in this study exhibited good corrosion inhibition efficiency against corrosion of mild steel in a 0.1MHCl solution. This might be due to coordination by the donor-acceptor interactions between the unshared electron pairs of donor atoms of the schifff base and metals in the steel [26,27]. The order of inhibition efficiency is **3>2>4>1**.The higher in inhibition efficiency of **3** compared to **2** was due to the effect of additional $–OCH_3$ substituent group on the aromatic ring. The methoxy and hydroxyl groups exhibit an inductive effect that results in changing the electron density and activate the aromatic ring, which may impart better absorptivity to the inhibitor. The effect would result in improved adsorption of the chemisorptions type through the aromatic ring and confer better protection than the others. The poor inhibition effect of **1** was rather due to the sulfonamide substituent that deactivates the aromatic ring. Further in the case of **2** and **3**, the presence of –OH groups in the ortho position would facilitate the formation of chelates that stabilize the interaction between the Schiff bases and the metals. The inhibition efficiency increased with increased concentration of the compounds. This suggests corrosion inhibition is a result of adsorption of inhibitor on the metal surface and the compounds acts as adsorption inhibitors. The inhibition efficiency of the Schiff bases was better at higher concentration. This might be due to larger coverage of the steel with inhibitor molecules [28].

Thus, among all the compounds synthesized in this study, 2 and 3 could be best used as corrosion inhibitors to protect and control metals deployed whenever aggressive acid solutions are used in industry for cleaning, descaling and pickling of steel structures, processes which are normally accompanied by considerable dissolution as well as consumption of the metal.

3.3. Antimicrobial Activity

The Schiff bases were tested for their in vitro antimicrobial activity against S.aureus, K.pneumoniae, C. albicans and C.krusei. The results of the in vitro growth inhibitory activities of the schiff bases (1-4) are listed in Table 2. The antifungal behavior of the Schiff bases against C. albicans was also shown in Figure 1. The result indicates that, all the synthesized compounds exhibited varying degree of inhibitory effect on the growth of all the organisms tested. The difference in the magnitude of the antimicrobial activity of the Schiff bases might be due to the differences in cell wall structure of the microbial and solubility and stability of the Schiff bases.

Except 4 all the Schiff bases showed antimicrobial activity against all microbial under the study. This is due to additional substituent in these compounds that enhances their toxicity. Compounds 1, 2 and 3 showed the highest activity against C.krusei, S.aureus and C. albicans, respectively. Among these Schiff bases, compounds bearing methoxy and sulfonamide substituent groups have shown promising activity against all the tested fungi.

Table 2 Antimicrobial activity of Schiff bases (1-4) (Concentration 50µg/ml)

Compounds	Diameter of inhibition zone (in mm)			
	S.aureus	K.pneumoniae	C. albicans	C.krusei
1	14	18	20	44
2	34	25	26	27
3	16	R	40	15
4	R	R	R	R
DMSO	-	-	-	-
Ampicillin	40	38	-	-
Voriconazole	-	-	45	36

R: Resist

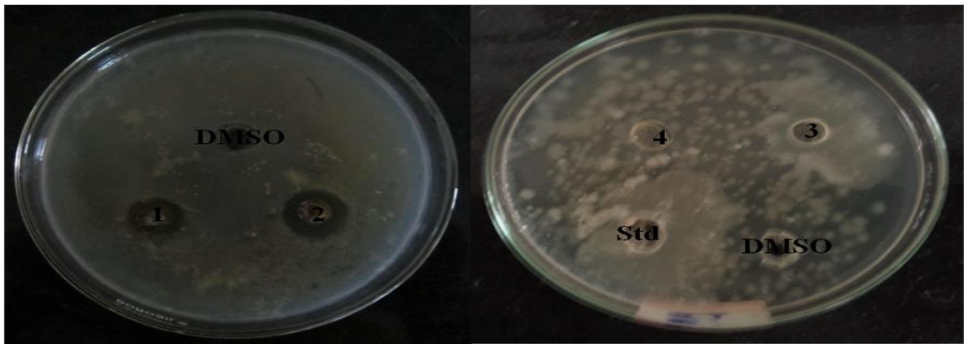


Figure 1: Photograph showing antifungal studies of the schiff bases against C. albicans

Conclusion

In the present work, four new Schiff bases were synthesized and characterized by analytical and spectral studies. The compounds were evaluated for their corrosion inhibition behavior against corrosion of mild steel and in vitro antimicrobial activities against, S.aureus, K.pneumoniae, C. albicans and C.krusei. In conclusion, from the corrosion inhibition study and *in-vitro* antimicrobial activity results, it was observed that both electron donating and electron withdrawing groups of the compounds influenced the efficiency and activity of the compounds. Among all the compounds tested **3** showed the best corrosion inhibition behavior against the corrosion of mild steel in acid. Except 4, the other Schiff bases showed good *in-vitro* antimicrobial activity. The antifungal activities of **1** and the antibacterial activities of **2** could further be studied for the treatment of infections caused by any of the test bacteria and fungi strains under the study and for some other similar diseasing causing bacteria and fungi strains.

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