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Research Article

Antimicrobial Activities Along With Spectrophotometric Assessment of Stability Constants of Copper (II) and Cobalt (II) With 1,2-Bis(2,5-dimethoxybenzylidene) Hydrazine

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The stability constants of 1,2-bis(2,5-dimethoxybenzylidene) hydrazine (DMBH) were determined using the modified Job's method with the addition of the UV-Vis approach using salts of copper (II) (chloride and acetate) and salts of cobalt (II) (chloride and nitrate). Copper (II) chloride with a ligand at 1L:2M (stoichiometric ratio) was discovered to have a higher stability constant log value (6.995) than other metal salts. The stability constant log value (5.811) for cobalt (II) nitrate at 1L:2M (stoichiometric ratio) was found to be less stable than the other stability constants evaluated. Antimicrobial properties of the ligand were tested against *Entamoeba coli*, *Candida albicans*, and *Staphylococcus aureus*. The ligand was found to be effective against these strains, with positive results.

1. Introduction

Azines were found to possess antimicrobial and anticancer activities. Complexes of asymmetric aldazines were synthesized and characterized and their antimicrobial studies were reported [1–3]. In vitro antimicrobial activity of azaphenothiazine derivatives was evaluated [4–6]. N,N-carbazolyl hydrazine and N,N-biscarbazolyl azine derivatives were synthesized and characterized, and their antimicrobial were screened [7–9]. Facile heterocyclic synthesis of polysubstituted and condensed pyrazolopyranopyrimidine and pyrazolopyranotriazine derivatives and their antimicrobic activity were studied [10, 11]. N (1)-arylidene-N(2)-cis-2,6-diphenyltetrahydrothiopyran-4-one azine products were constructed and their antiseptic performance against *Escherichia coli*, *Bacillus subtilis*, and *Streptococcus faecalis* were reported [12]. The construction and microbial performance of n(1)-arilidene-n(2)-t(3)-methyl-r(2), c(6)-diaryl-piperidin-4-one azine products were evaluated [13]. Screening of antimicrobic activity using asymmetrical azines obtained from naphtho[2,1-b] furan was investigated [14].

In recent years, the synthesis of (1)-arylidene-n(2)-cis-2,6-diphenyltetrahydrothiopyran-4-one azine products and cis-2,6-diarylpiperidin-4-one products was reported with their antimicrobial assessment [13, 15–17]. Synthesis and antimicrobial evaluation of 2,6-diarylpiperidin-4-one derivatives, and t(3)-methyl-r(2),c(6)-diarylpiperidin-4-one azine byproducts and their antimicrobic activities were explored [18]. The cobalt (II)-piroxicam complex ratio was determined (1 : 1) by the constant modification method using the spectrophotometric technique at 570 nm $\lambda_{\max}$. Investigation of the stability constant of the associated complex was estimated as 2.174 × 104 [7]. The previous studies revealed that the stability constants of chelating ligands utilizing various methods such as spectrophotometric and potentiometric techniques are more stable [19–23].

The stability factor of a complexion is affected by parameters such as metal ion type, ligand type, solvent, and counterions [24–28]. In the same way, a thermodynamic examination of complex growth between various ligands and metal ions has been stated [25, 29–31]. Nevertheless, in spite of the several studies conducted in the area of thermodynamic constraints and stability constants, the fact that stability constant reliance on temperature through complexation interaction has been found beneficial in finding thermodynamic restrictions as described [26, 31–33]. We noticed that it is still appropriate to perform more of such estimations due to the numerous roles performed by chelating ligands and the several utilizes of the subsequent chelate complexes in genetic approaches [4].

1.1. Preparation of DMBH. 2,5-dimethoxybenzaldehyde (2.1 g) in 25 mL absolute ethanol was refluxed for 12 minutes at 79°C with a little quantity of acetic acid, and then, 0.11 mL hydrazine hydrate was added and refluxed for another 50 minutes. The reaction suspension was cooled to 25°C and filtered to get excellent yellow crystals that were then cleaned with 100% ethanol and recrystallized (yield = 92.44%; color = pure yellow; melting point = 173°C).

1.2. Antimicrobial Activities. Antibacterial activity was performed against three pathogens such as *Entamoeba coli*, *Candida albicans*, and *Staphylococcus aureus* by the disc diffusion method [31, 32, 34] on the nutrient agar plate. DMBH was dissolved in DMSO with a concentration of 0.1%. Two (20 μL and 40 μL) were loaded on filter paper discs. The azine inhibited the growth of pathogen *Staphylococcus aureus*. The inhibition zones measured were from 9 mm to 13 mm with 20 μL, 40 μL, and impregnated disc. Against *Entamoeba coli*, the range of inhibition zone was from 6 mm to 7 mm when 20 μL and 40 μL of azine were loaded on the disc. The azine showed no antibacterial activity with *Candida albicans* even at high concentration, inhibition zone was from 4 mm to 8 mm when 20 μL and 40 μL of azine was loaded on the disc. The highest zone (13 mm) was recorded with azine.

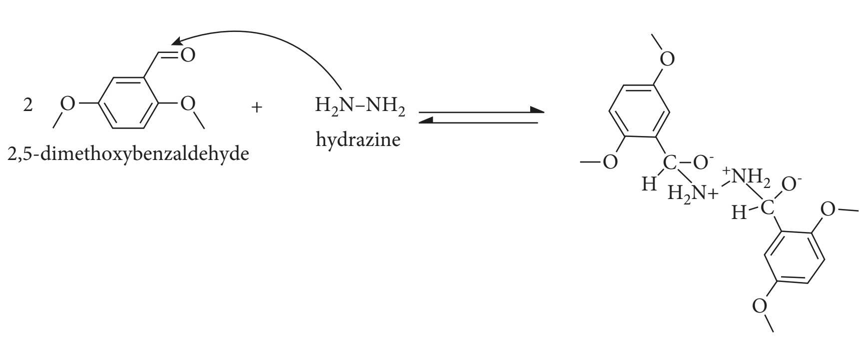
1.3. Assessment of Stability Constants. The stability constants of DMBH with copper (II) and cobalt (II) precursors such as $Cu(CH_3COO)_2.H_2O$, $CoCl_2.6H_2O$, $CuCl_2.2H_2O$, and $Co(NO_3)_2.6H_2O$ were evaluated by modified Job's method [31, 33, 35, 36] with a UV-Vis at $\lambda_{\max}$570 nm. The stability constants were estimated by employing the following formula:

$$K_s = \frac{A/A_{ex}C'}{\left[\left(C_m - (A/A_{ex})C''\right)\left(C_x - A/A_{ex})C'\right]}, \quad (1)$$

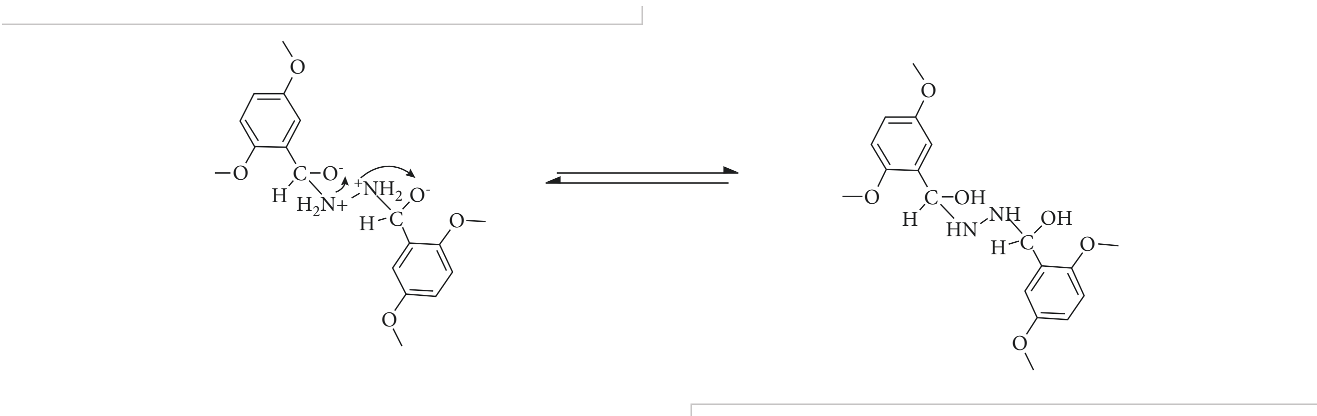
where A is the absorbance peak, A_{ex} is the extraploted absorbance, C_m is the concentration of metal, C′ is the concentration of complex, and C_x is the concentration of ligand.

2. Results and Discussion

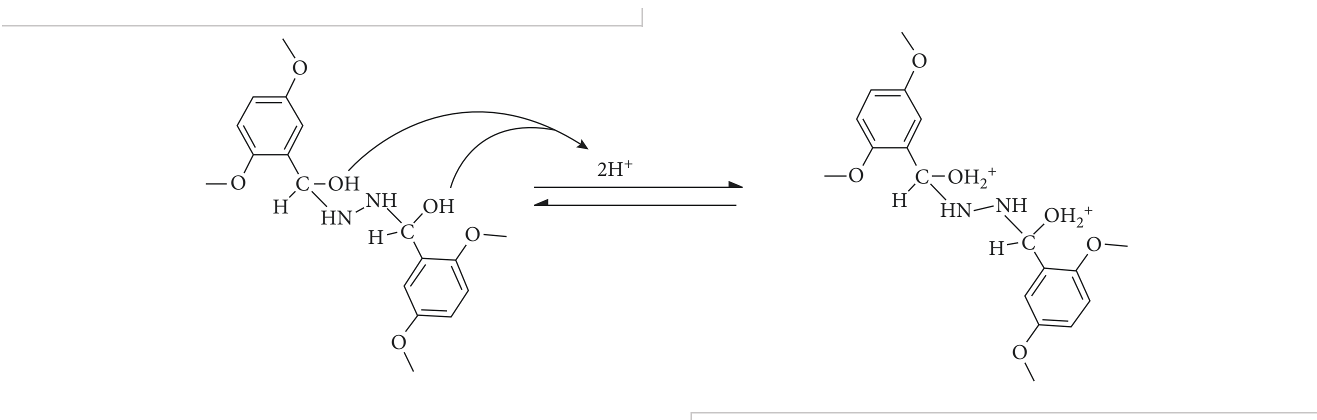
We noticed that the pure yellow crystals of DMBH were solvable in methanol and ethanol but insoluble in water [30] (Scheme 1). The condensation mechanism of DMBH is proposed and mentioned in the scheme with the following steps:



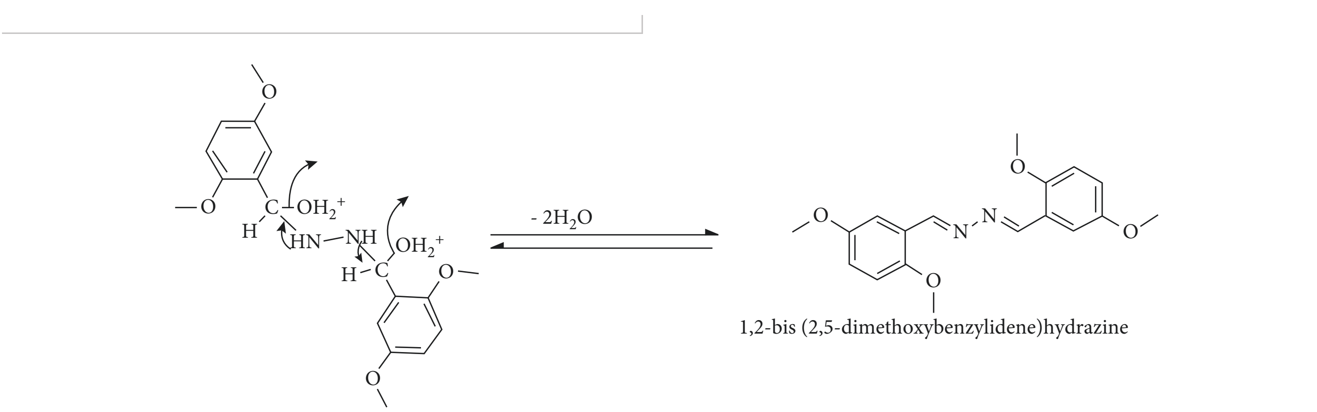
Step 1: attack of nucleophilic nitrogen to a carbonyl carbon atom.



Step 2: protonation by nitrogen group.

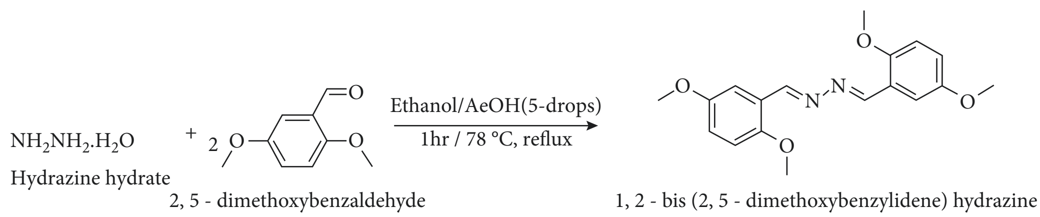


Step 3: protonation of the hydroxyl group.



Step 4: elimination of H_2O.

In this study, DMBH was evaluated by using a visible spectrophotometer, EIMS (electron impact mass spectrometer), and FTIR. We observed that the pure yellow crystals of DMBH were designed by the condensation reaction of 2,5-dimethoxybenzaldehyde with hydrazine hydrate. The obtained percentage yield was 92.44%, and the



Scheme 1: Preparation of DMBH.

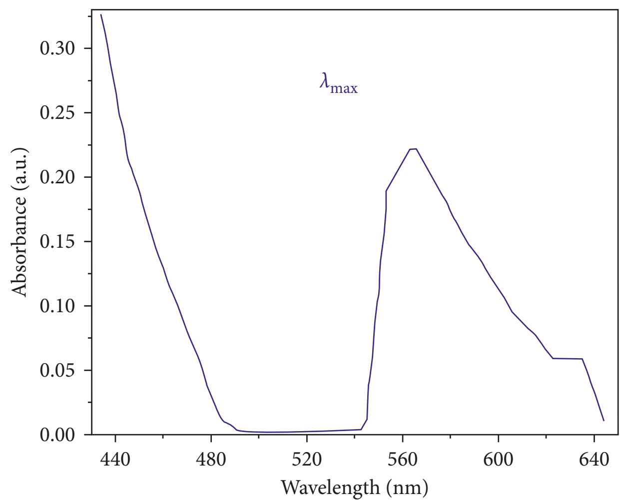
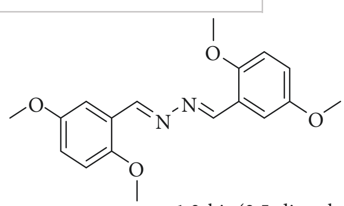


Figure 1: Maximum absorbance (λ_{max}) of DMBH.

melting point was 173°C. The ligand was soluble in methanol and ethanol but insoluble in water.

Our findings revealed that DMBH was insoluble in deionized water, but soluble in ethanol and methanol. The melting point of the crystalline yellow ligand was 173°C, and the yield was 0.6 g (92.44%). λ_{max} of the ligand was found as 570 nm in methanol (Figure 1). The molar mass of DMBH possessing molecular formula $C_{18}H_{20}N_2O_4$ was 328 g/mol which was established by EIMS and was noticed approximately equivalent to the intended molecular mass.



1,2-bis (2,5-dimethoxybenzylidene)hydrazine

The FTIR measurements of DMBH were conducted as KBr-disk and foremost absorption bands are stated in Table 1. FTIR measurements predicted 5 functional group edges such as OCH_3, aromatic C = C, C = N, aromatic C-H, and N-N. The furthermost bulging and intense absorption edge wad categorized by the absorption result from the aromatic (C = C) that seemed at 1492.55 cm^{-1}. On the other hand, the distinct representative intense band of OCH_3 has seemed at 1042.39 cm^{-1}. Two average bands of N-N and C = N were found at 1168.99 cm^{-1} and 1617.52 cm^{-1}. λ_{max} of DMBH was found at 570 nm in absolute methanol.

The molecular formula of DMBH was $C_{18}H_{20}N_2O_4$ and its experimental molecular mass (328 g/mol) confirmed (Figure 2) by EIMS was equal to the calculated molecular mass. The modified Job's method of incessant variation procedure was performed for the assessment of stability constants (Figures 3, 4, and Table 2) of constructed DMBH with transition metal precursors such as copper (II) chloride, copper (II) acetate, cobalt (II) nitrate, and cobalt (II) chloride by using a visible spectrophotometer at λ_{max} 570 nm. The current study mainly focused on the stoichiometry and stability determination in ethanol. The antimicrobial activities of

Table 1: FTIR measurements of DMBH.

S. no.	Functional group	Frequency (cm^{-1})	
		Spectrum value	Theoretical value
1	Ar C = C	1492.55 (s)	1493
2	OCH_3	1042.39 (s)	1021
3	C = N	1617.52 (m)	1611
4	N-N	1168.99 (m)	1076
5	Ar C-H	2831.30 (w)	2998

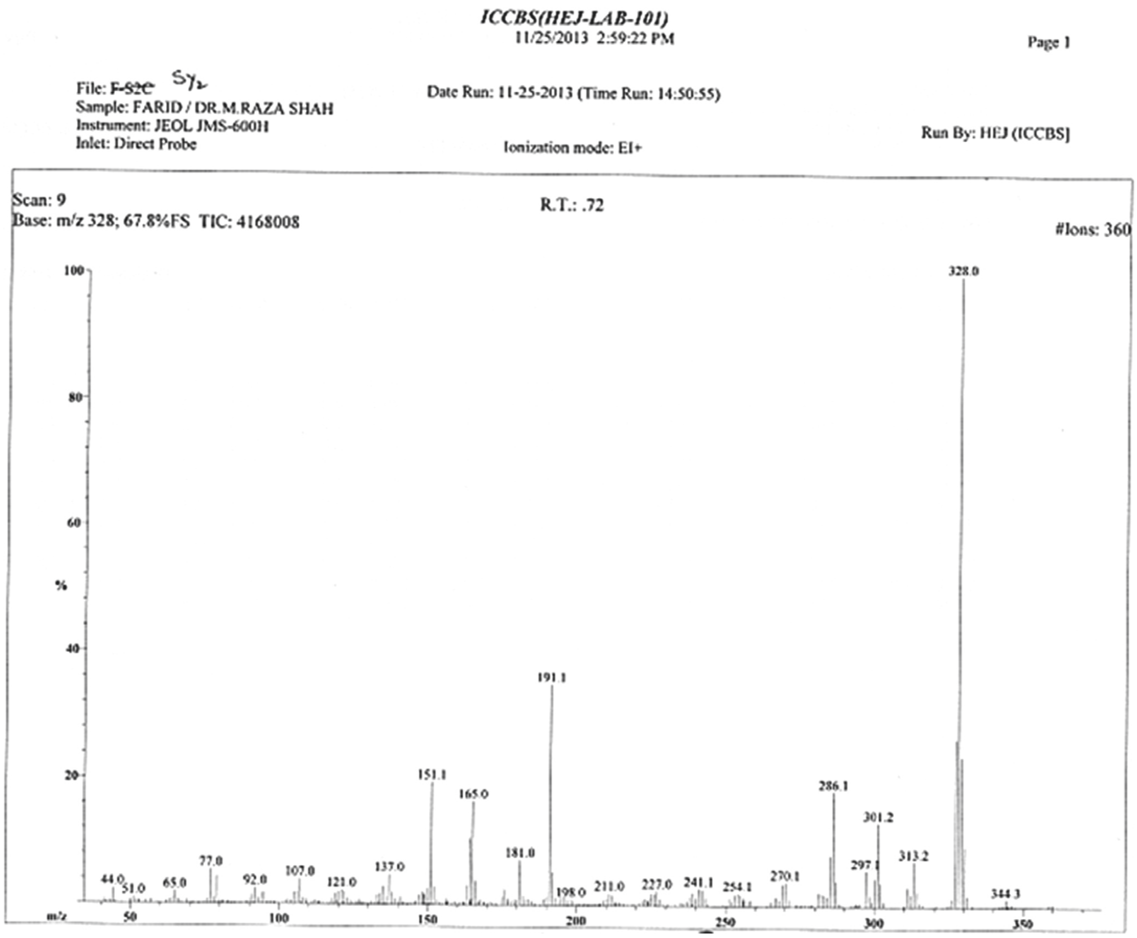
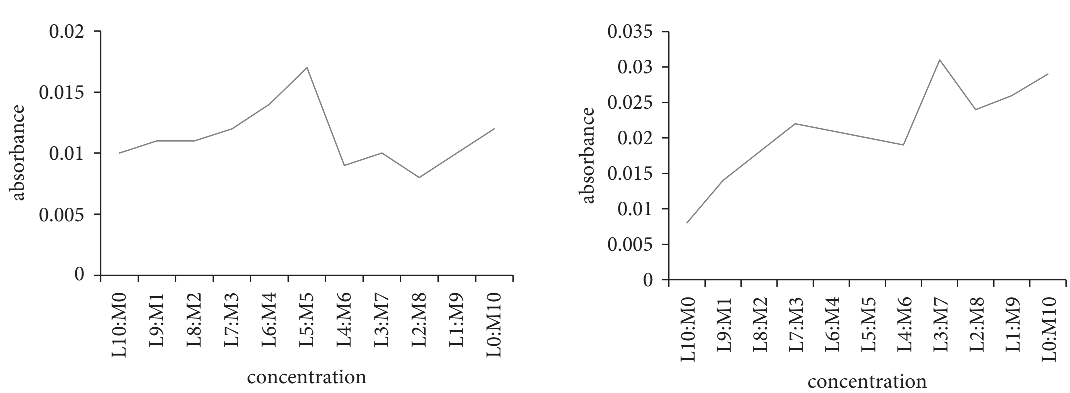


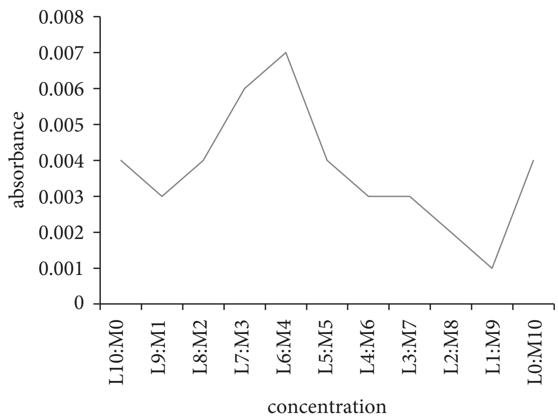
Figure 2: EIMS measurements of DMBH.



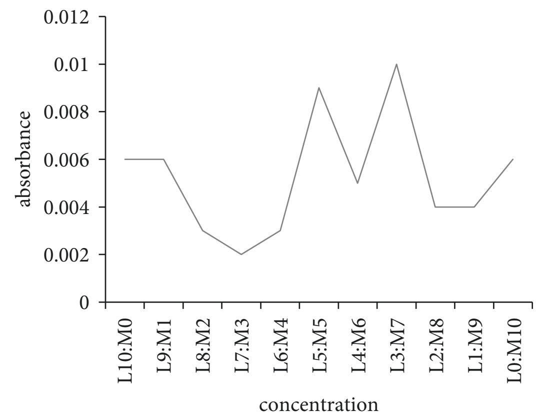
(a)

(b)

Figure 3: Continued.



(c)



(d)

FIGURE 3: Stability constant of DMBH with (a) $CuCl_2.2H_2O$, (b) $Cu(CH_3COO)_2$, (c) $CoCl_2.6H_2O$, and (d) $Co(NO_3)_2.6H_2O$.

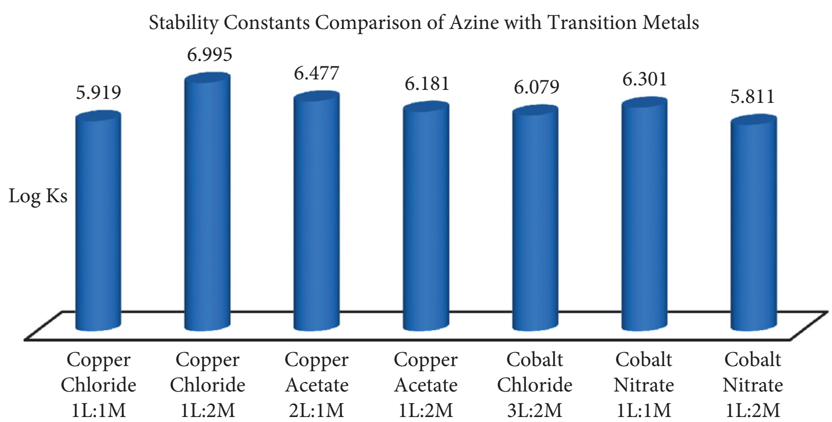


FIGURE 4: Stability constants of metal precursors with DMBH.

TABLE 2: Stability constants of metal precursors with DMBH.

S. no.	Metal salts	Stability constants (K_s)	L:M	Log K_s
1	Copper chloride	8.3×10^5	1 : 1	5.919
		9.9×10^6	1 : 2	6.995
2	Copper acetate	3.0×10^6	2 : 1	6.477
		1.5×10^6	1 : 2	6.181
3	Cobalt chloride	1.2×10^6	3 : 2	6.079
4	Cobalt nitrate	2.0×10^6	1 : 1	6.301
		6.48×10^5	1 : 2	5.811

TABLE 3: Antimicrobial performance of DMBH.

S. No.	Organism	Zone of inhibition A (mm)		Zone of inhibition B (mm)	
		Mean	Std.	Mean	Std.
1	*E. coli*	6.10	0.10	7.10	0.11
2	*S. aureus*	9.08	0.12	13.06	0.12
3	*C. albicans*	4.07	0.13	8.11	0.11

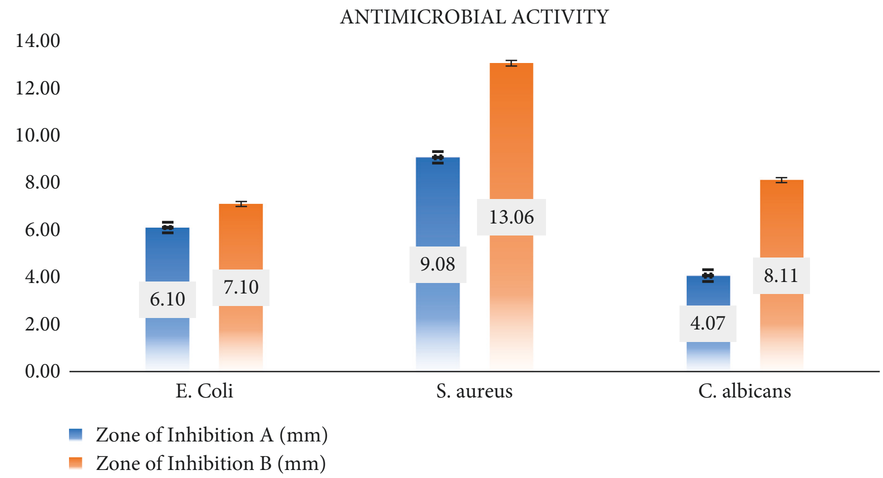


Figure 5: Antimicrobial activity of ligand.

DMBH against bacterial strains were checked quantitatively, and the zone of inhibition (ZOI) is shown in Table 3 and Figure 5.

The stability constants of 1,2-bis(2,5-dimethoxybenzylidene) hydrazine with transition metal salts are in the following order:

Copper chloride 9.9 × 106 (1L : 2M) > copper acetate 3.0 × 106 (2L : 1M) > cobalt nitrate 2.0 × 106 (1L : 1M) > copper acetate 1.5 × 106 (1L : 2M) > cobalt chloride 1.2 × 106 (3L : 2M) > copper chloride 8.3 × 105 (1L : 1M) > cobalt nitrate 6.48 × 105 (1L : 2M).

In the antimicrobial assay (Table 3 and Figure 5), it is evident that *Staphylococcus aureus* has been more affected by the ligand as compared to the *Entamoeba coli* and *Candida albicans*. This may be attributed to the structure-activity relationship of the ligand with these microbes. A moderate level of the zone of inhibitions has been observed for *Entamoeba coli* and *Candida albicans*.

3. Conclusions

It may be concluded that the stability constant of copper chloride with a ligand at 1L:2M (stoichiometric ratio) was found greater than the other determined values. On the other hand, the stability constant value for cobalt nitrate was found least stable than the other values the modified Job's method found to be useful for the determination of stability constants. Antibacterial activity was performed against three pathogens such as *Entamoeba coli*, *Candida albicans*, and *Staphylococcus aureus* by the disc diffusion method on the nutrient agar plate. It was noticed that increased chemical concentration has a positive relationship with the growth of inhibition. Against *Staphylococcus aureus* pathogen, moderate antibacterial activity was evaluated with DMBH except for the pathogens *Candida albicans and Entamoeba coli*.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare no conflicts of interest.

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