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Synthesis, Characterization and *In vitro* evaluation of antibacterial and antifungal activities of New Schiff Base and Its Metal Complexes

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ABSTRACT

A new potentially pentadentate Schiff base ligand L has been prepared via the condensation of diethylenetriamine and 2'-hydroxyacetophenone in the molar ratio of 1:2 in absolute ethanol and characterized by elemental analyses and UV, IR spectroscopy. Mn(II), Ni(II), Co(II), Cu(II), Fe(III), Mg(II), Cd(II) and Zn(II) complexes were prepared by reaction of L, dissolved in ethanol, with an appropriate metal salt, have been characterized by UV and IR spectroscopy. Furthermore, the synthesized compounds were screened for antibacterial and antifungal activities. Almost all of these compounds showed moderate to excellent antimicrobial activity against four gram negative bacteria (Escherichia coli, Salmonella typhimurium, Acinetobacter baumannii and Citrobacter freundii), two gram positive bacteria (Enterococcus faecalis and Lysteria monocytogenes), pathogenic fungal strains (Candida albicans, Aspergillus niger, Aspergillus flavus and fusarium oxysporum). The activities were confirmed by Activity Index (AI). Activity Index values were found to be higher for Cd(II) followed by Co(II) and the other compounds. The Minimum Inhibitory Concentration (MIC) L and its metal complexes were also determined.

Introduction

Anti-microbial agents are undeniably one of the most important therapeutic discoveries of the 20th century [1]. However, antimicrobial drug resistance is of increasing importance as the phenomenon has considerable impact on human and animal health [2]. The development of new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medical need [3]. In addition, the search and development of more effective antifungal agents are mandatory [4, 5]. However, the lack of new antifungal agents and the long-term use of antifungal drugs in the treatment of chronic fungal infections have caused the emergence of amphotericin-B and azole resistant Candida species [6].
The discovery and development of effective antibacterial and antifungal drugs with novel mechanism of action have become an urgent task for infectious diseases research programs [7]. Many investigations have proved that binding of a drug to a metallo-element enhances its activity and in some cases, the complex possesses even more healing properties than the parent drug [8]. There has been a steady growth of interest in the synthesis, structure, and reactivity of Schiff bases due to their potential applications in biological modeling, catalysis, design of molecular magnets [9, 10].
Schiff bases are important class of ligands and had got wide applications in various fields [11]. The synthesis of new Schiff base complexes becomes widespread due to their potential application in chemistry, biochemistry, medicine and technology [12].
Therefore Schiff bases metal complexes were widely investigated for their antifungal, antibacterial, diuretic and antitumor, antifertility and enzymatic activities [13, 14]. Transition metal complexes of Schiff bases are one of the most adaptable and thoroughly studied systems [15]. In addition, some of the complexes containing N and O donor atoms are effective as stereo-specific catalysts for oxidation [16], reduction [17], hydrolysis [18], biocidal activity [19].
Several research groups have been involved in the synthesis and biological screening of Schiff bases [20, 21]. Lei et al. [22] reported the synthesis of Schiff bases of 5-chlorosalicyldehyde as anti-bacterial and anti-fungal agents. Further, Venkatesh et al. [23] synthesized some novel Schiff base complexes of metal ion and reported anti-microbial activities and anti-fungal activity. Among them the (Cu^{2+} and Zn^{2+} metal complexes of (E)-4-(1-(2, 4-dihydroxyphenyl) ethylidene amino) benzenesulfonamide) showed excellent activity. On the other hand,

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Mishra et al. [24] reported new bidentate or tridentate Schiff bases and their Vo (II) and Co (II) complexes, and some of the complexes have been screened for their antimicrobial activity on different species of pathogenic bacteria/fungi, E. coli, S. aureus, S. fecalis, A. niger, T. polysporum, and reported that all the complexes show higher activity than the free ligand.
The Schiff base derived from 2-furancarboxaldehyde and 2-aminobenzoic acid and its metal complexes with Cu (II), Ni (II), Co (II), and Fe (III) has biological activities against *bacteria staphylococcus pyogenes, E.coli and pseudomonas aeruginosa* [25, 26].

In the present article, we report the synthesis and characterization of Schiff base derived from diethylenetriamine and 2'-hydroxyacetophenone and its metal Mn(II), Ni(II), Co(II), Cu(II), Fe(III), Mg(II), Cd(II) and Zn(II) complexes. We have also investigated antibacterial and antifungal activities of these compounds *F. oxysporum, A. flavus, A. niger , C. albicans,* and against Gram-negative and Gram-positive bacteria.

Experimental

Materials, methods and instrumentation

Each of the following chemicals was purchased and used without further purification:
2'-hydroxyacetophenone from Aldrich; Diethylenetriamine from Sigma–Aldrich.
Each of the following salts, used as received : nickel(II) acetate tetrahydrate, zinc(II) acetate dihydrate and cadmium(II) acetate dihydrate were obtained from Fluka , iron(III) chloride hexahydrate, and copper(II) acetate monohydrate were purchased from Sigma–Aldrich, cobalt(II) sulfate was obtained from Biochem, magnesium(II) acetate tetrahydrate was obtained from Sigma , manganese(II) sulfate was obtained from Panreac. Using commercially available silica plate by Merck, we monitored the progress of reactions by means of thin-layer chromatography (TLC). Melting points were measured on a Stuart melting point /SMP3/.
Infrared spectra (IR) were obtained, as KBr disks, on a Schimadzu FTIR-8400S spectrophotometer. The electronic absorption spectra in the 200–900 nm range were measured on a UV-1800 Schimadzu UV–visible spectrophotometer. Elemental analyses (C, H, and N) were carried out with EuroVector EA3000 instrumentation.

Synthesis of Schiff base L

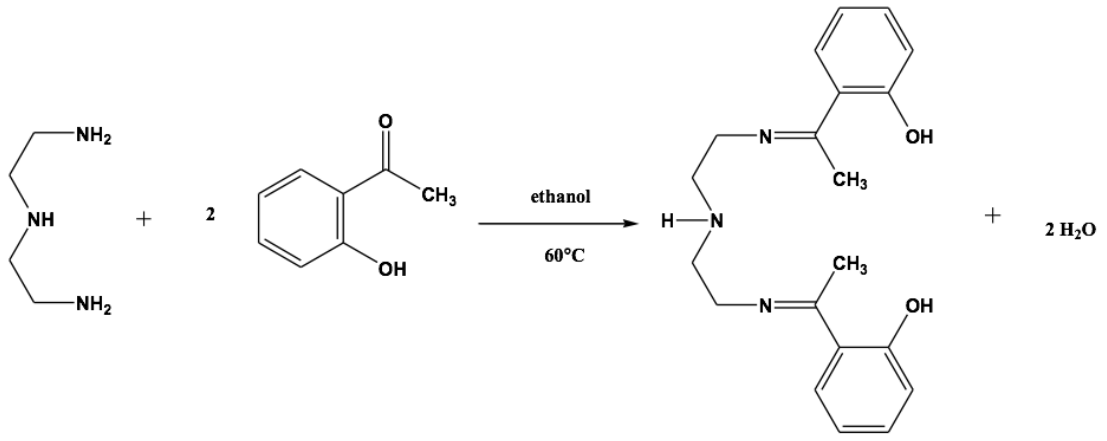
The Schiff base (L, Scheme 1) was been prepared according to a procedure [27]: diethylenetriamine (29 mmol, 9.12g), 2'-hydroxyacetophenone (58mmol, 23.64g) were mixed in 100 ml of absolute ethanol in a round flask. The mixture was refluxed with agitation for 3h at 60°C. After cooling, the volume was reduced, and the yellow precipitate that formed was separated and dried in vacuum.

Synthesis of the Schiff base complexes:

Schiff base–metal complexes were synthesized according to the following modified general procedure outlined by Raman and coworkers [28].
A mixture of L (58 mmol) in ethanol was added to an aqueous solution (ethanol-distilled water 1:2) of nickel(II) acetate tetrahydrate, zinc(II) acetate dihydrate , cadmium(II) acetate dihydrate ,iron(III) chloride hexahydrate, copper(II) acetate monohydrate, cobalt(II) sulfate, magnesium(II) acetate tetrahydrate , manganese(II) sulfate (58 mmol) , refluxed for 3 hours. The solution was left as such for slow evaporation, the precipitated compounds that separated, and dried in vacuum.

Antimicrobial activity (Antibacterial and Antifungal activities)

In vitro antibacterial activity studies were carried out using the standardized disc-agar diffusion method [29] to investigate the inhibitory effect of the synthesized ligand and complexes against Gram-positive bacteria, such as Enterococcus faecalis ATCC 49452 and Lysteria monocytogenes ATCC 15313. Gram-negative bacteria: as Escherichia coli ATCC 25922, Acinetobacter baumanii ATCC 19606, Citrobacter freundii ATCC 8090; and salmonella typhi ATCC 13311 and against of fungi such as F. oxysporum, A. flavus NRRL 391, A. niger 2AC 936 and Candida albicans ATCC 1024. The bacterial and fungal cultures were sub cultured on nutrient agar and potato dextrose agar medium respectively. The antibiotic Gentamicine (10µg/disk) was used as reference antibacterial drug and Econazole was used as reference antifungal drug.
An inhibition zone diameter indicates that the tested compounds are active against the used kinds of the bacteria and fungus. The tested compounds were dissolved in DMSO (which has no inhibition activity). The test was performed on medium potato dextrose agar (PDA) which contains infusion of 200 g potatoes, 6 g dextrose and 15 g agar for antifungal activity [30, 31] and Mueller Hinton Agar for antibacterial



Scheme 1. Synthesis of Schiff base ligand (L).

activity. Uniform size filter paper disks (Whatman, 6 mm-diametre) were cut and sterilized in an autoclave .The paper discs were saturated with 15 µl of the tested compound dissolved in DMSO solution (3 disks per compound) and carefully placed on incubated agar surface. After incubation for 24 h at 37 °C in the case of bacteria and for 48 h at 27°C in the case of fungi [32], inhibition of the organisms, which evidenced by clear zone surround each disk, was measured and used to calculate mean of inhibition zones. The activity of tested compounds was categorized as: (i) low activity = mean of zone diameter is ≤ 1/3 of mean zone diameter of control, (ii) intermediate activity = mean of zone diameter ≤ 2/3 of mean zone diameter of control and (iii) high activity = mean of zone diameter >2/3 of mean zone diameter of control [33].
The Activity Index (AI) and Proportion Index (PI) were calculated using the following formulae:
Activity index (AI) = Inhibition zone of sample /Inhibition zone of standard [34, 35].
Proportion Index (PI) = Number of positive results obtained for individual extract /Total number of tests carried out for each sample [36].

Determination of minimum inhibitory concentration (MIC)

The minimal inhibitory concentrations (MIC) were determined by broth microdilution methods [37-39] with slight modifications. For MIC determination, the inocula of the bacterial strains or Candida were prepared like the following procedure: the broth culture of each bacterial strain or Candida was adjusted to a turbidity equivalent to a 0.5 McFarland standard for 4–6 h and were diluted in Nutrient broth media to give concentration of ≈106 cfu/mL for bacteria.
Twofold serial dilutions of compounds were prepared in Nutrient broth in 96-well plates starting from a stock solution of compounds (30 mg/ml DMSO). An equal volume of bacterial strains or Candida suspension was added to each well on the microtiter plate.
In this manner (the final concentration in each well adjusted to 2.0 x 106 CFU/ml for bacteria and 2.0 x 105 of Candida strains. (Last wells are broth only control well). Then, the inoculated microtiter plates were incubated at 37 °C for 24h. A control well containing the growth medium and the bacteria or Candida was set-up. The growths were spectrophotometrically recorded at 620 nm by using a microplate reader (BioTek). The MIC values were defined as the lowest concentration of compounds whose absorbance were comparable to the negative control wells (broth only, without inoculum).
To determine the fungi-associated MIC, the assay of [40] was used with modifications. A fungal suspension was inoculated with various concentrations of tested compound in sterile 96-well microtiter plates containing PDB (potato dextrose broth) medium. The mixture was incubated at 30°C for 48h. After this time, the absorbance of each well was read at 630 nm using a microplate reader (BioTek).

Statistical analysis

All determinations were conducted in triplicate and results were calculated as mean standard deviation (SD). Statistical analysis was performed with the aid of Student's t test for significance; differences were considered significant at $p \leq 0.05$.

Results and discussions

Synthesis

According to a published procedure [27], condensation of diethylenetriamine with 2'-hydroxyacetophenone in a 2:1 M ratio readily gave rise to the corresponding Schiff base bis-(2-hydroxyacetophene ethyl amine)-amine (L).
The L have been prepared and characterized by FT-IR and elemental analysis.
The IR of each compound confirms the formation of imine band (–C=N-) and absence of the original cetonic band (C=O). A strong band at 1580-1625 cm^{-1} is assigned to the stretching vibration of the imine group ν (C=N), and a broad band at 3450 cm^{-1} due to phenolic (OH) groups was observed. Anal. Calcd.for C20H25N3O2: C, 70.77; H, 7.42; N, 12.38. Found: C, 70.69; H, 7.38; N, 12.32
Mn(II), Ni(II), Co(II), Cu(II), Fe(III), Mg(II), Cd(II) and Zn(II) complexes were prepared by reaction of L, dissolved in ethanol, with an appropriate metal salt, have been characterized by UV and IR spectroscopy.
L, Mn(II), Ni(II), Co(II), Cu(II), Fe(III), Mg(II), Cd(II) and Zn(II) complexes were synthesized in powder form, the physical appearance, percent yield and Rf values are listed on (Table 1).

Table 1. Physical parameters and percent yield of the synthesized compounds

compounds	Color	Yield %	M.P. (°C)	Rf value*
L	Yellow	98	93-95	0.88
Mn(II)-L	Black	95	110-115	0.77
Ni(II)-L	Orange	98.3	126-130	0.76
Cu(II)-L	Bleu	93.2	106-108	0.73
Cd(II)-L	Yellow clear	72.4	241-243	0.61
Co(II)-L	Dark green	96.9	229-230	0.66
Zn(II)-L	Yellow clear	72	135-139	0.74
Mg(II)-L	yellow	83	165-169	0.79
Fe(III)-L	Red	86.5	184-187	0.77

* Ethyl acetate: Ethanol in the ratio of 7:3 was used as mobile phase

Infrared spectra

Main characteristic infrared absorption bands of L and its Mn(II), Ni(II), Co(II), Cu(II), Fe(III), Mg(II), Cd(II) and Zn(II) complexes, along with their assignments, are presented in Table 2.

The infrared spectrum of the L ligand (Scheme 1) exhibits a band at 1616 cm^{-1} assignable to ν (C=N) of the azomethine group, and a wide absorption band of medium intensity in the range of 3260–3450 cm^{-1} observed for the Schiff base is due to the interamolecular linkage (OH…NH). Upon complexation the vibration of azomethine group underwent a shift to lower frequency 1577–1606 cm^{-1}, indicating the bonding of unsaturated nitrogen of the azomethine group of L to the metal ions[41], and the broad band at ~3400 cm^{-1} is assigned to the ν (OH) frequency of the coordinated H_2O [42], and the interamolecular linkage with (NH). The band at 3453 cm^{-1} was absent in the spectra of the complexes; this is indicative of the deprotonation and involvement of the phenolic hydroxyl group of the ligand in bond formation with the metal ions. The bands in the regions 489-660 cm^{-1} and 412–450 cm^{-1} are ascribed to ν (M–O) and ν (M–N) vibrations respectively.

Electronic spectra

The UV-visible spectra of L, Ni(II)-L, Co(II)-L, Cu(II)-L, and Zn(II)-L were recorded in ethanol and Mn(II)-L, Fe(III)-L, Mg(II)-L and Cd(II)-L were recorded in DMSO. Relevant electronic spectra data are presented in Table 3. The UV Vis spectra of L showed two bands at 321 and 389 nm. The first band can be attributed to π–π* transition within the aromatic ring, while the second band would be due to n–π* transition within -C=N group. Upon complexation, n–π* transition of ligand shifts to a longer wavelength; this indicates the coordination of ligand to metal [43].

Biological activity

In this work, the antibacterial and antifungal activity of the synthesized compound was evaluated. From the results presented in Fig.1, it is very clear that some of the synthesized compound inhibited the growth of all the tested strains of bacteria and fungi. The Cd(II) complexes was found to have maximum proportion index (PI) with PI=0.9, followed by Co(II), Cu(II) and Ni(II) complexes, with PI values of 0.7, 0.7 and 0.6 respectively. On the other hand the bis Schiff base ligand L and Mg(II), Mn(II), Zn(II), Fe(III) complexes have less potency against the tested strains, with PI values 0.4, 0.3, 0.3, 0.3 and 0.1 respectively.

Table 3. The UV-Vis. spectral data of Schiff base metal complexes.

Compounds	Electronic transition, λ_{max}(nm, DMSO/EtOH)	Band assignments
Mn(II)-L	258, 314	$\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$
Ni(II)-L	307, 385	$\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$
Cu(II)-L	354, 560	$\pi \rightarrow \pi^*$, d→d
Cd(II)-L	219, 318	$\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$
Co(II)-L	380	$\pi \rightarrow \pi^*$
Zn(II)-L	263, 351	$\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$
Mg(II)-L	319, 392	$\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$
Fe(III)-L	322, 514	$\pi \rightarrow \pi^*$, d→d

Fig. 1 Proportion Index of antimicrobial activity of Schiff base and its metal complexes.

The antibacterial activity of L and its metal complexes were evaluated by the agar diffusion method. Using representative standard strains of Gram (+) and Gram (-) bacteria on nutrient agar media, as listed in Table 4 Dimethylsulfoxide was used as solvent for the test compounds. The data are compared with standard antibiotic, Gentamicine.

The results in Table 4 do reveal that the diameters of inhibition zones for the compounds ranged from 9 to 52.5 mm and the Cd(II) has a high inhibitory activity against Gram-positive and Gram-negative bacteria, except (L. monocytogenes), with inhibition zone of 52.5±0.71, 33.5±2.12, 28.00±1.018, 25.5±0.71, 24.00±1.41 against *C. freundii*, *E. faecalis*, *A. bau-*

Table 2. Infrared spectral data of the ligand and its metal complexes.

	Absorption band ν (cm^{-1})					
Species	ν (C=N)	ν (O-H), ν (H_2O)	ν (C=C)	ν (CH_3/CH_2)	ν (M-O)	ν (M-N)
L	1616	3453	1510	2907,2820	-	-
Mn(II)-L	1606	3393	1530	2994,2926	517	451
Ni(II)-L	1577	3408	1443	2946,2867	556	421
Cu(II)-L	1596	3418	1433	2936,2859	546	441
Cd(II)-L	1587	3260	1413	2936,2877	489	412
Co(II)-L	1606	3408	1500	2916,2897	527	479
Zn(II)-L	1587	3320	1420	2954,2867	508	421
Mg(II)-L	1596	3446	1451	2926,2839	546	430
Fe(III)-L	1596	3389	1530	2887,2829	662	450

manii, *S. typhi* and *E.coli* respectively. However, the Co(II) inhibited the growth of all the tested strains of bacteria. In addition, the results demonstrate that Ni(II) is most active against *E. coli*, *C. freundii* and *A. baumanii*. The antibacterial activity of L and the other complexes follow the order: Cu(II) > L= Mn(II) > Mg(II) > Fe(III) > Zn(II). Similarly, the activity was confirmed by Activity Index (AI), Cd(II) was found to have higher index than corresponding compounds (Fig 2), with AI values of 2.576±0.163, 1.892±0.151, 1.521±0.010, 1.263±0.074, 1.019±0.129 against *E. faecalis*, *S. typhi*, *C. freundii*, *E.coli* and *A. baumanii* respectively.

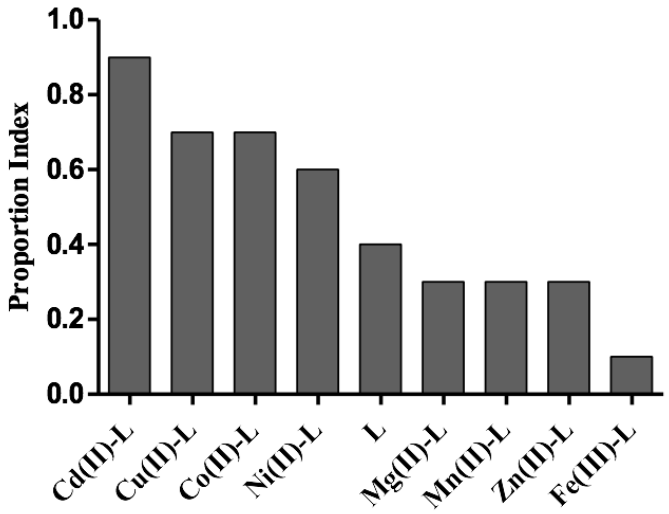


Fig. 1. Proportion Index of antimicrobial activity of Schiff base and its metal complexes.

Based on the assessed MIC values, as can be seen Table 5, the Cd(II) exhibited a substantial activity against C. freundii , A. baumanii, E.coli, E. faecalis and S. typhi with an MIC of 0.0387±0.02, 0.0423±0.001, 0.247±0.001, 0.249±0.091, 0.282±0.014 mg/ml, respectively. Followed by Co(II) with MIC of 0.0337±0.004, 0.243±0.002, 0.317±0.047, 0.458±0.002 0.528±0.002, 3.110±0.087 against *C. freundii, L. monocytogenes, S. typhi, E. faecalis, E.coli* and *A. baumanii* respectively. Whereas, the MIC of L > 5mg/ml against the majority of tested starins. These results are in agreement with previous studies that Schiff bases derived from Diethylene Triamine and its mixed-metal complexes exhibited strong activity against positive and negative bacteria [44].

It is obvious from (Table 4, 5 and Fig. 2) that biological activity of most of metal complexes is increased compared with free bis Schiff base ligand. This can be explained taken into account the principal factors. One of these factors is the chelate effect, i.e., polydentate ligands, such as the Schiff base ligand, show higher antimicrobial efficiency towards complexes with monodentate ligands [45, 46]. According to Tweedy's theory [47], chelation reduces the polarity of the metal atom because of partial sharing of its positive charge with a donor group and the possible ϖ-electron delocalization over the whole chelate ring system, thus formed during coordination. Thus, the process of chelation increases the lipophilic nature of the metal ion, which in turn favors its permeation through the lipid layer of the membrane and this is also increase the hydrophobic character and liposolubility of the molecule in crossing the cell membrane of the microorganism and hence enhances the biological utilization ratio and activity of the testing antibiotic compounds [48].

In the case of antifungal activity, the results were compared with the standard drug (Econazole) we can see that the synthesized compounds were moderately active against C. albicans (Fig. 3, Table 5). Cd(II), Ni(II) and Co(II) exhibited a substantial activity with inhibition zone of 37.00±0.10, 34.00±0.10, 31.00±0.03 mm respectively. The results showed that the effect of Cd(II), Ni(II) and Co(II) is increased compared with the standard Econazole and the ligand L.

In order to evaluate the antifungal activity of L and its metal complexes against *A. niger*, *A. flavus* and *F oxysporum*, the inhibition zone was measured after 48, 72, 96 and 144h. The re-

Table 4. Diameters of inhibition zones of bacterial growth induced by L and its metal complexes, and by the antibiotic: Gentamicine.

Bacterium	Diameter of inhibition zone (mm)					
	E.coli ATCC 25922	*A. baumanii* ATCC 19606	*C. freundii* ATCC 8090	*L. monocytogenes* ATCC 15313	*E. faecalis* ATCC 49452	*S. typhi* ATCC 13311
L	13.5±0.71	9.00±0.00	12.5±0.71	-	12.00±1.41	11.5±0.71
Mn(II)-L	12.00±0.71	9.00±0.00	12.00±00	10.00±0.00	10.5±0.71	-
Ni(II)-L	22.5±0.71	15.5±0.71	19.00±0.35	9.5±0.71	-	-
Cu(II)-L	11.5±0.71	14.00±1.41	14.5±0.71	9.00±0.00	13.5±0.71	10.5±0.71
Cd(II)-L	24.00±1.41	28.00±1.018	52.5±0.71	-	33.5±2.12	25.5±0.71
Co(II)-L	20.5±0.71	24.5±0.71	25.00±0.71	20.00±0.00	21.5±0.71	15.5±0.71
Zn(II)-L	12.5±0.71	-	28.00±0.71	-	-	-
Mg(II)-L	11.5±0.71	11.5±0.71	10.00±0.00	11.00±0.00	-	-
Fe(III)-L	-	10.00±0.00	10.00±0.00	-	12.00±0.00	-
Gentamicine	19.00±0.71	27.00±0.71	35.5±0.71	13.00±0.00	13.00±0.00	13.5±0.71
DMSO	-	-	-	-	-	-

- : compound did not show any activity.

Table 5. Minimum Inhibitory Concentration (MIC/ mg mL^{-1}) values for antibacterial activity of L and its metal complexes.

Bacterium	*E.coli* ATCC 25922	*A. baumanii* ATCC 19606	*C. freundii* ATCC 8090	*L. monocytogenes* ATCC 15313	*E. faecalis* ATCC 49452	*S. typhi* ATCC 13311
L	1.898±0.031	>5	>5	>5	>5	>5
Mn(II)-L	>5	>5	>5	>5	-	-
Ni(II)-L	1.301±0.018	1.854±0.031	1.301±0.018	>5	>5	-
Cu(II)-L	>5	>5	1.98±0.014	>5	>5	>5
Cd(II)-L	0.247±0.001	0.0423±0.001	0.0387±0.02	-	0.249±0.091	0.282±0.014
Co(II)-L	0.528±0.002	3.110±0.087	0.0337±0.004	0.243±0.002	0.458±0.002	0.317±0.047
Zn(II)-L	>5	-	2.58±0.018	-	-	-
Mg(II)-L	>5	>5	>5	>5	-	-
Fe(III)-L	-	>5	>5	-	>5	-
Gentamicine	ND	ND	ND	ND	ND	ND

ND: not determined, (-): compound with low or did not show any activity.

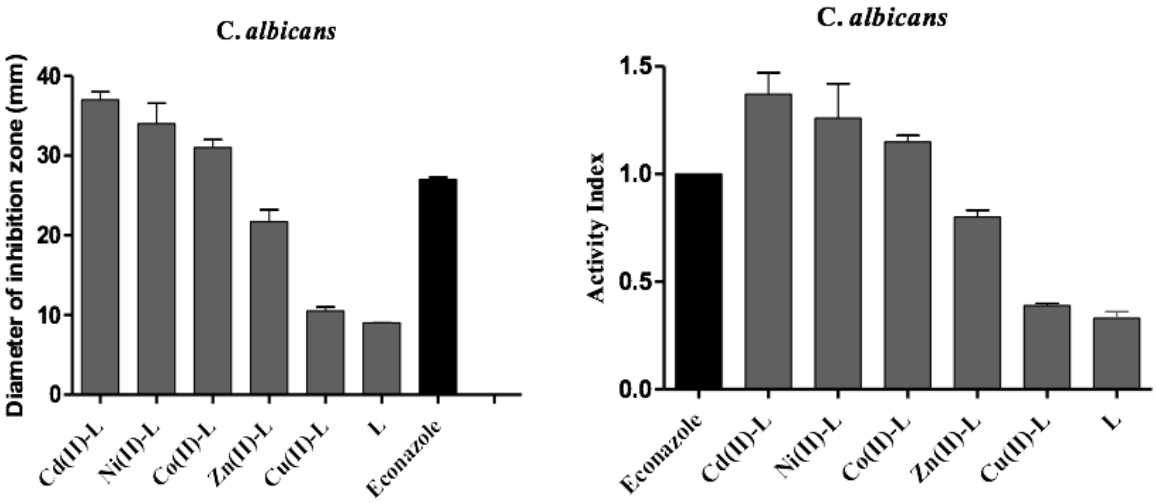


Fig. 3. Diameter of inhibition zone and Activity Index of L and its metal complexes against C. albicans.

Table 6. Diameters of inhibition zones of fungi growth induced by L and its metal complexes, and by the a*ntifungal: Econazol. (C. albicans after 48h*, *A. niger*, *A. flavus* and *F. oxysporum* after 144h).

Fungi	*C. albicans* ATCC 1024	*A. niger* 2AC 936	*A. flavus* NRRL 391	*F. oxysporum*
L	9.00±0.00	-	-	12.00±0.01
Mn(II)-L	-	-	-	-
Ni(II)-L	34.00±0.10	11.50±0.01	9.00±0.00	-
Cu(II)-L	10.50±0.02	16.00±0.00		-
Cd(II)-L	37.00±0.10	40.00±0.00	38.00±0.02	18.50±0.1
Co(II)-L	31.00±0.03	9.00±0.00	-	-
Zn(II)-L	21.70±0.20	-	-	-
Mg(II)-L	-	10.00±0.00	-	11.00 ±0.00
Fe(III)-L	-	-	-	-
Econazole	27.00	34.00	29.00	37.00

- : compound did not show any activity

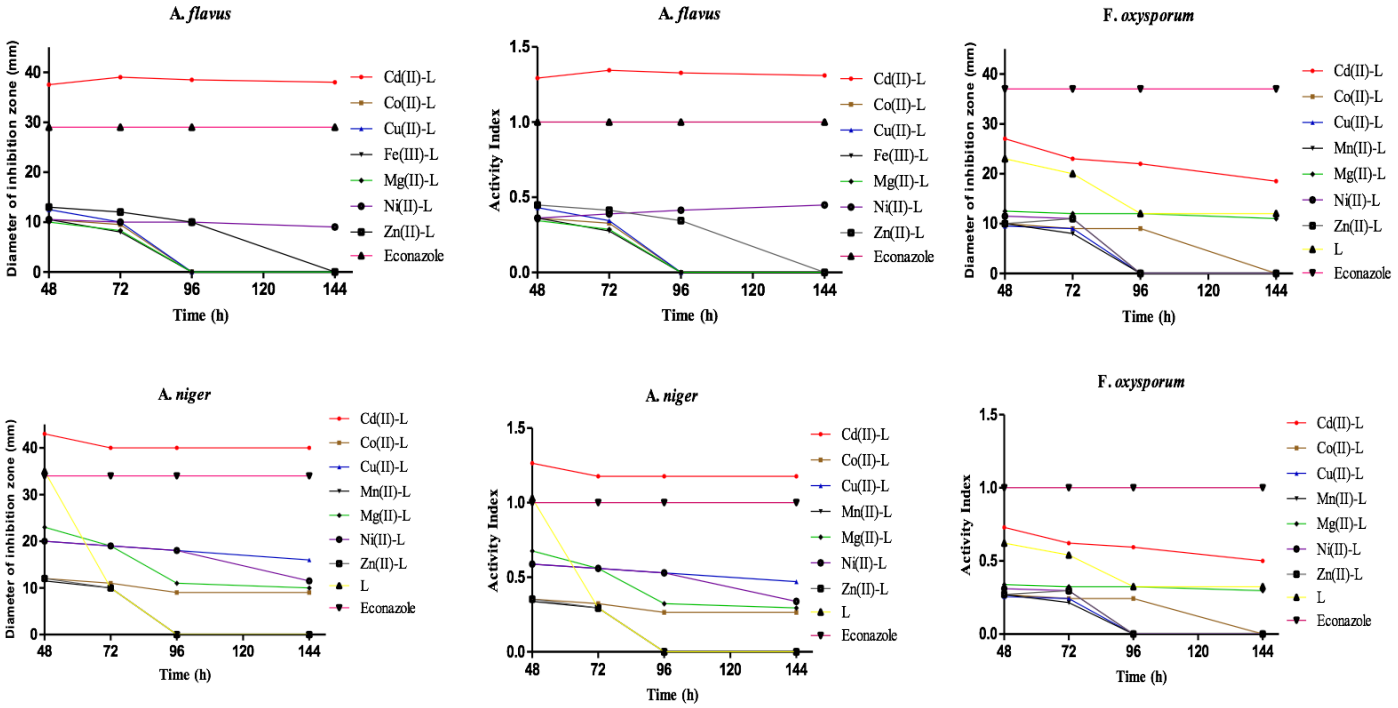


Fig. 4. Evolution of inhibition zone and Activity Index of L and its metal complexes against fungi after 48, 72, 96 and 144 h. Values are expressed as means of triplicate.

sults presented in Fig. 4, showed the increase in the antifungal activity of the majority of the synthesized compound before 72h. But after 72h, the results showed lesser or no activity.

Except, Cd(II) that exhibited the most stability of the inhibition zone with slight decrease against all the tested stains of fungi at 48 to 144h. In addition Ni(II) was also exhibited the stability of the inhibition zone against *A. niger* and *A. flavus*.

The results presented in Table 6 showed the decrease in the diameter of inhibition after 144h.

Minimum inhibitory measurements MIC (Table 7), showed that Cd(II) has lowest concentration (0.427±0.006 for A. niger and 0.218±0.004 for *F. oxysporum*).

The majority of the synthesized complexes showed moderate to excellent antimicrobial activity compared with Schiff base ligand, because metal ions play a very crucial role.

This can be explained as follows:

Protein synthesis is one of the most important step in the microbial growth. The metal ions act as growth-inhibitor of the microbes by adsorbed on the outer surface of the cell wall and obstruct the process of respiration; this in turn stops the synthesis of protein [49] and kills that microorganism [50].

Conclusion

A new potentially pentadentate Schiff base ligand L has been prepared via the condensation of diethylenetriamine and 2'-hydroxyacetophenone in the molar ratio of 1:2 in absolute ethanol. Mn(II), Ni(II), Co(II), Cu(II), Fe(III), Mg(II), Cd(II) and Zn(II) complexes were prepared by reaction of L, dissolved in ethanol, with an appropriate metal salt, have been characterized by UV and IR spectroscopy. Furthermore, the synthesized compounds were screened for antibacterial and

Table 7. Minimal Inhibitory Concentration (MIC/ mg mL^{-1}) values for antifungal activity of L and its metal complexes.

Fungi	C. *albicans* ATCC 1024	A. *niger* 2AC 936	F. *oxysporum*	A. *flavus* NRRL 391
L	>16	-	>16	-
Mn(II)-L	-	-	-	-
Ni(II)-L	3.783±0.054	8.90±0.424	-	ND
Cu(II)-L	3.160±0.02	15.41±1.253	-	-
Cd(II)-L	0.573±0.009	0.427±0.006	0.218±0.004	ND
Co(II)-L	0.226±0.001	>16	-	-
Zn(II)-L	10.91±0.05	-	-	-
Mg(II)-L	-	>16	>16	-
Fe(III)-L	-	-	-	-
Econazole	ND	ND	ND	ND

ND: not determined, (-): compound with low or did not show any activity.

antifungal activities. Almost all of these compounds showed moderate to excellent antimicrobial activity against four gram negative bacteria (*Escherichia coli*, *Salmonella typhimurium*, *Acinetobacter baumannii* and *Citrobacter freundii*). Synthesized complexes showed moderate to excellent antimicrobial activity compared with Schiff base

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Conflicts of interest

Authors declare no conflict of interests.

Notes

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