Synthesis, Characterization, Industrial And Biological Application Of Co(II),Ni(II),Cu(II) And Zn(II) Complexes With Azo Ligand Derived From Metoclopramide Hydrochloride And 3,5-Dimethylphenol

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Abstract

Four complexes of Co(II),Ni(II),Cu(II) and Zn(II) with the azo ligand (4-chloro-N-(2-(dimethylamino)ethyl)-5-((2-hydroxy-4,6-dimethylphenol)diazenyl)-2-methoxybenzamide) L. The structure of ligand and complexes were confirmed on the basis of their analytical and spectral data, these dyes were tested as dyeing in cotton fabric, and also testing in light and cleaner firmness. Also, antimicrobial and antifungal activities of ligand and their complexes were evaluated and the results showed that the ZnL compound showed the higher antibacterial activity with inhibition zone of 13mm against Staphyloco-ccus epidermidis, Steptococcus sp. and Escherichia coli compared with ligand and other metal complexes. In case of ZnL compound the antifungal activity of Candida albicans showed higher inhibition area of 15mm is higher activity from ligand and rest metal complexes. , there is no doubt, that the effort exerted by the scholar was good enough. But I found a few point must corrected.

Keywords: metoclopramide, azo dyes, dyeing, 3,5-dimethylphenol.

1. Introduction

Azo molecules are widely used in different fields, such as printing systems, dyes of textile and photoelecrionic application(1). Because of exhibit biological and medicinal importance, azo compounds are application for biocidal treatment of textile materials and their application, such as antiseptics, antineoplastics, antidiabetics and antitumor(2,3). Azo compounds have been formed five or six membered loop while other coordination site on ortho position due into stable metal chelates(4). Many application of azo dyes such as, chemistry, pharmaceutical and radiochemical industry, these azo dyes are used complexing and pharmaceutical agent for many of metal ions(5,6). Because of widely used in application, such as optical data storage, non-linear optics, dyes and pharmaceuticals, transition metal chelates appeared good thermal and chemical properties(7). Metal chelates for azo ligands are widely important type for dyeing the wool, nylon and silks into higher fastness for washing and light(8). Recently, metal chelates of azo dyes have been increased attention for electronic interesting and relationship with their using for non-linear optical elements, printing system, molecular memory storages and industrial fields(9,10). At that work, synthesis, identification and chelating of some metal complexes containing metoclopramide azo dye as a ligand.

2. Experimental

Instrumentation

Microelemental analysis (C.H.N.O) have been done in Dimashq University, Syria, utilizing Euro vector EA 3000, single V.3.Osingle. Conductivity with compounds solved at dimethylsulfoxide (10⁻³ M/L)
has been registered on 25°C utilizing Philips PW-Digital Conductimeter. Magnetic properties were completed during employing Auto Magnetic Susceptibility Balance Sherwood Scientific instrument on 25°C. Atomic absorption was registered through utilizing a Shimadzu A.A-160A Atomic Absorption/Flame Emission Spectrophotometer. 1H-NMR spectrum was observed at a Brucker-400 MHz Spectrometer in Mashhad University utilizing dimethylsulfoxide like the solvent also trimethylsaline like the reference. UV-Vis spectrum have been registered at a Shimadzu UV-160A Ultra Violet-Visible Spectrophotometer. IR-spectrum have been taken at Shimadzu, FTIR-8400S Fourier Transform Infrared Spectrophotometer at 4000- 400 cm⁻¹ spectrum areas for models produced like KBr discs. Mass spectra were measured on LC-MS QP50A: Shimadzu(E170 EV) Spectrometer, at Dimashq University, Syria. Other than, melting points have been performed utilizing Stuart Melting Point Apparatus.

Materials and reagents

Chemicals were used to obey also collected of supplier: CoCl₂.6H₂O, NiCl₂.6H₂O, CuCl₂.2H₂O and ZnCl₂ (Merck), metoclopramide, 3,5-dimethylphenol (fluka).

Preparation of the ligand

A solution was produced,(11) of metoclopramide hydrochloride (0.84 gm, 1mmole) in mixture (10ml ethanol, 2ml conc. HCl), and diazotized at 50°C for 10% solution from NaNO₂. Diazotized solution was added collyrium wise for stir to a cool ethanolic solution at (0.305 gm, 1mmole) for 3,5-dimethylphenol. Thereafter 25 ml at 1M NaOH solution was followed to the deep colored mix as well precipitation with azo ligand has been noticed. This deposit have been filtrated, washed number ounces with (1:1) C₂H₅OH: H₂O, leave the mixture later in a dry place. Reaction is appear at scheme 1

![Scheme 1. Synthesis for azo ligand (L).](image)

Buffer solution

Ammonium acetate (0.01M, 0.771 gm) was solved in one liter for doubly deionized water. For only pH rate (4-9) has been the use of CH₃COOH or NH₃ solution.

Standard solution

Standard solutions for metal salts were maded in changing condensation (10-5-10-3 M/L) in pH average (4-9). On the same time a bulk of ethanolic solutions for ligand during the extent for condensations (10-5-10-3 M/L) has been and produced.

Preparation for metal chelats

(0.404gm, 2mmole) EtOH solution for ligand was added drop wise for stirring into 0.118, 0.118, 0.085 as well 0.064 gm of CoCl₂.6H₂O, NiCl₂.6H₂O, CuCl₂.2H₂O and ZnCl₂ dissolved in the pH solution with the wanted pH. The mixture was cooled until dark color precipitate was contained, filtered, and washed number ounces with 1:1 H₂O: C₂H₅OH mixture. The preparation method is offered at scheme-2, other than the physical estates and (C.H.N.O) analysis are listed in Table 1.

2.7. Antimicrobial activity

The ligand and all synthesized compounds have been evaluated to examine their in vitro antibacterial efficacies versus (Staphylococcus aureus, Staphylococcus epidermidis and Pseudomonas Aeruginaso) as gram positive bacteria and (Streptococcus sp., Escherichia coli as well Klebsiella sp.) as gram negative bacteria as well as antifungal activity such as Candida albicans (Yeast) by employing disk diffusion method(12). The solvent utilized for working exam samples and level was DMSO and sample of (1- 200 μg/ml) were utilized. Antibacterial and antifungal activity of any compound were estimated by means of the well-diffusion method. 1cm² at a 24h broth the culture including 10⁶CFU/cm³ was placed in sterilized Petri-dishes. Molten nutritio us agar (15cm³) was kept in ca.45 °C and teeming in the Petri-dishes and pliable into stiffen. Other holes of 6mm diameter were punctured accurately employing a sterilized cork borer and these were perfectly stuffed for the exam solutions. The dishes were brood into 24h. within 37 °C.

![Scheme 2. Expected geometry of the metal (II) complexes for azo ligand](image)
Dyeing manner

Dyeing estates from generated compounds have been tasted as well applied during cotton fabric such as (1% shade). Fabric dye was formative in (15-20) Co) on (1 hr), as well at pH (10).

Table 1: Physical properties with azo ligand and their metal chelates.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Color</th>
<th>M.P °C</th>
<th>Yield%</th>
<th>AnalysisCalc(Found)</th>
<th>M%</th>
<th>C%</th>
<th>H%</th>
<th>N%</th>
<th>O%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand(L)</td>
<td>Orange</td>
<td>300 &gt;</td>
<td>81</td>
<td></td>
<td>59.25</td>
<td>6.17</td>
<td>13.82</td>
<td>11.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(58.93)</td>
<td>(6.03)</td>
<td>(12.96)</td>
<td>(10.84)</td>
<td></td>
</tr>
<tr>
<td>[Co(L)_2]</td>
<td>Brown</td>
<td>300 &gt;</td>
<td>82</td>
<td></td>
<td>6.81</td>
<td>55.42</td>
<td>5.54</td>
<td>12.93</td>
<td>11.08</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(5.92)</td>
<td>(4.22)</td>
<td>(11.85)</td>
<td>(10.75)</td>
<td></td>
</tr>
<tr>
<td>[Ni(L)_2]</td>
<td>Reddish orange</td>
<td>300 &gt;</td>
<td>87</td>
<td></td>
<td>6.70</td>
<td>55.49</td>
<td>5.54</td>
<td>12.94</td>
<td>11.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.11)</td>
<td>(4.68)</td>
<td>(11.83)</td>
<td>(10.91)</td>
<td></td>
</tr>
<tr>
<td>[Cu(L)_2]</td>
<td>Deep brown</td>
<td>300 &gt;</td>
<td>82</td>
<td></td>
<td>7.34</td>
<td>55.10</td>
<td>5.51</td>
<td>12.85</td>
<td>11.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.77)</td>
<td>(5.11)</td>
<td>(11.84)</td>
<td>(10.81)</td>
<td></td>
</tr>
<tr>
<td>[Zn(L)_2]</td>
<td>Brown</td>
<td>300 &gt;</td>
<td>80</td>
<td></td>
<td>7.45</td>
<td>55.04</td>
<td>5.50</td>
<td>12.84</td>
<td>11.01</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(6.81)</td>
<td>(4.95)</td>
<td>(11.93)</td>
<td>(10.74)</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1: 1H-NMR spectrum for azo ligand (L).

Dyeing estates from generated compounds have been tasted as well applied during cotton fabric such as (1% shade). Fabric dye was formative in (15-20) Co) on (1 hr), as well at pH (10).

3. Results and Discussion

Rudy with azo ligands (L) joined from 3,5-dimethylphenol for suitable diazotized at alkaline solution has been performance. Synthesized ligand has been specified through spectral studies (1HNMR, FT-IR, UV-Vis) and microelemental analysis (C, H, N, O). Aqueous-ethanol solutions have been permanently obtained to the study for interaction of metal salts with the produced ligand.

1H-NMR spectra

Azo ligand (L) spectrum at dimethylsulfoxide (Fig. 1) display various signals at (δ=6.49-7.68) ppm refers into aromatic protons. Signals acquired on (δ=8.56) as well (δ=6.38) ppm lead into protons of amide and phenol. Finding, signals on (δ=1.90) as well as (δ=1.40) ppm were specified to δ(CH3) of phenol and (N-(CH3))2, signals at (δ=2.07, 2.69) and (δ=2.5) ppm which were assigned to (CH2) group and DMSO-d6

Mass spectra

The mass spectrum of the azo ligand (L) showed peak centered at m/z = 405 due to the formula C20H25N4O3Cl. The general pattern of fragmentation are summarized in Scheme-3, see Fig. 2. Mass spectra of the complexes displays peaks centered at m/z = 865 and 871 due to the formulas C40H48N8O6Cl2Ni and C40H48N8O6Cl2Cu respectively. The general pattern of fragmentation are summarized in Schemes-4 and 5, see Fig. 3 and 4.

Fig. 2: Mass spectrum for azo ligand (L)

Calibration curve

Varied molar concentration (10-5-10-3 M/L) for mixed aqueous ethanol ligand as well metal ions, only reach (1-3×10-4 M/L) condensation followed beer’s law as well displayed evident intense color. Best straight lines fit have been taken for correlation factor R>0.9980 such as assigned on Fig. 6.
Scheme - 3: Fragmentation pattern for azo.

Fig. 3: Mass spectrum for $[\text{Ni}(\text{L})_2]$ complex

Scheme - 4: Fragmentation pattern for $[\text{Ni}(\text{L})_2]$ complex.

Fig. 4: Mass spectrum for $[\text{Cu}(\text{L})_2]$ complex

Scheme - 5: Fragmentation pattern for $[\text{Cu}(\text{L})_2]$ complex.
Calibration curve

Varied molar concentration (10^{-5}–10^{-3} M/L) for mixed aqueous ethanol ligand as well metal ions, only reach (1-3x10^{-4} M/L) condensation followed Beer’s law as well displayed evident intense color. Best straight lines fit have been taken for correlation factor R>0.9980 such as assigned on Fig. 6.

![Fig. 6: Linear relationship between molar concentration and absorption.](image)

Model conditions

For search out interaction between produced ligand as well as metal ions under education at preparation of compounds, spectrum from combining solutions at ligand as well metal ions into attain for optimum pH as well condensation, also firm wave length (\(\lambda_{\text{max}}\)) have been the first studies .As well as mole ratio metal to ligand (M:L) has been defined into prepare compounds. Perfect condensation has been option of compound solution based on this solution gives the highest absorption at fixed (\(\lambda_{\text{max}}\)) with diverse pH, as well as results are depicted on Table 2. Trial results evidence such absorbance with all prepared compounds are maximum as well fixed on buffer solution from NH\(_4\)OOCCH\(_3\) on pH extent (4-9).All prepared compounds were found to have a perfect pH like is shown at Fig. 7.

![Fig. 7: Effect of pH at absorption (\(\lambda_{\text{max}}\)) to the metal chelates.](image)

Metal to ligand ratio

Appoint for the complexes in solutions have been tested by mole ratio and job techniques. At both situations outcomes spread 1:2 (metal to ligand) ratio. Picked plot is shown at Fig. 8. Table 2 synopsizes outcomes gated, and specification into making compounds.

![Fig. 8: Mole ratio and Job manners to the compounds solutions.](image)

Physical estates

Solid metal chelates at ratio (1:2) (Metal:Ligand) have been produced by interaction of the ligand melted in ethanol with the metal ions melted in perfect pH. The outcome of elemental analysis and the metal import from compounds were in real identical as well calculated values. Conductivity from compounds melted at dimethylsulfoxide (10^{-3} M/L) display non-electrolytic type\(^{(17)}\) data are included at Table 3.

Determination for stability constant and gibbs free energy

Constant (K) of stability to the (1:2) metal to ligand compound can be computed according to the equations.

\[
K = \frac{1 - \alpha}{4\alpha C^2} ; \quad \alpha = \frac{A_{\text{m}} - A_s}{A_{\text{m}}}
\]

Where \(c\) = condensation to the compound solution at mole/ L \(\alpha\) = degree for decomposition, \(A_s\) = Absorption in solution containing same amount of

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ligand and metal ion and Am= the absorption for solution including selfsame quantities for metal as well surplus for ligand. High values with (K) pointed into high stability for produced compounds. Thermodynamic parameters for Gibbs free energy (ΔG) have been as well studied. ΔG data were reckoned of the equation (19).

\[ \Delta G = -RT \ln k \]

Where; \( R = \) gas constant = 8.314 J.mol\(^{-1}\).K, \( T = \) absolute temperature (Kelvin). Negative value for (ΔG) due to the reaction between azo dye ligand (L) as well metal ions understudy are automatic, see Table 2.

Table 2 : Stability constant and Gibbs free energy with produced compounds.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>( \alpha )</th>
<th>( \alpha_m )</th>
<th>( \alpha )</th>
<th>( k )</th>
<th>( \ln k )</th>
<th>ΔG kJ.mol(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Co}(L)_2])</td>
<td>0.083</td>
<td>0.166</td>
<td>0.500</td>
<td>16.13x10(^5)</td>
<td>16.596</td>
<td>-41.117</td>
</tr>
<tr>
<td>([\text{Ni}(L)_2])</td>
<td>0.033</td>
<td>0.122</td>
<td>0.729</td>
<td>11.78x10(^5)</td>
<td>16.281</td>
<td>-40.337</td>
</tr>
<tr>
<td>([\text{Cu}(L)_2])</td>
<td>0.099</td>
<td>0.177</td>
<td>0.440</td>
<td>43.07x10(^5)</td>
<td>17.578</td>
<td>-43.550</td>
</tr>
<tr>
<td>([\text{Zn}(L)_2])</td>
<td>0.136</td>
<td>0.211</td>
<td>0.355</td>
<td>58.63x10(^5)</td>
<td>15.488</td>
<td>-38.372</td>
</tr>
</tbody>
</table>

**Electronic spectra**

UV-Vis spectra to the produced compounds melted on ethanol (10\(^{-3}\) M/L) have been gauged as well datum formed are listed on Table 3. UV-Vis spectral into azo ligand (Fig. 9) shows peaks on 263, 312 and 386 nm were appointed into mild energy (π-π*) transition. The spectrum for Co\(^{II}\) complex (Fig. 9) displays peaks in 266,311,383 nm whom were qualified into ligand field, and peak in 480 nm was specified into charge transfer. Else three peaks at 765,862 and 977 nm whom were referred into electronic transition type \( ^4A_2(F) \rightarrow ^4T_{1g}(F) \), \( ^4A_2(F) \rightarrow ^4T_{2g}(F) \) and \( ^4A_2(F) \rightarrow ^4T_{2g}(F) \) respectively, also the value from magnetic moment on 4.82 B.M may be possessed as extra confirmation to tetrahedral geometry. Ni\(^{II}\) complex showed peaks in 271,309,391 and 477 nm consequent into ligand field and charge transfer sequences. Another peaks in 783,877 and 974 nm whom were appointed into electronic transition style \( ^3T_{1g}(F) \rightarrow ^3T_{1g}(P), ^3T_{1g}(F) \rightarrow ^3A_{2g}(F) \) and \( ^3T_{1g}(F) \rightarrow ^3T_{2g}(F) \) continuity. The magnetic moment for this complex was discovered in 3.88 B.M whom was much close to the tetrahedral perimeter. The electronic spectrum for Cu\(^{II}\) complex appears peaks at 311,383 and 468 nm due to ligand field and charge transfer, the peak in 977 nm whom was qualified into electronic transition style \( ^2T_{2g} \rightarrow ^2E \). Magnetic moment for complex has been found on 1.96 B.M whom was much close to the tetrahedral environment. Electronic spectral for Zn\(^{II}\) complex do offer charge transfer, and the magnetic susceptibility seemed the complex has diamagnetic moments, result to (d-d) transition are not likely hence electronic spectrum did not confer any productive datum, on fact this outcome is a good agreement for former work from tetrahedral geometry.
Table 3: Conditions from produced compounds and UV-Visible, magnetic susceptibility as well conductance menstruations datum.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Optimum pH</th>
<th>Optimum Molar Conc. x 10^4</th>
<th>M:L Ratio</th>
<th>(λ_max) nm</th>
<th>ABS</th>
<th>ε_max (L.mol^(-1).cm^(-1))</th>
<th>Λ_{eff} (S.cm^2.mol^(-1)) in DMSO</th>
<th>μ_{eff} (B.M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand(L)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>263</td>
<td>312</td>
<td>386</td>
<td>1.207 1.919</td>
<td>1207 1919</td>
</tr>
<tr>
<td>[Co(L)_2]</td>
<td>7</td>
<td>2.5</td>
<td>1:2</td>
<td>266</td>
<td>311</td>
<td>383</td>
<td>380 65 862 977</td>
<td>1315 365</td>
</tr>
<tr>
<td>[Ni(L)_2]</td>
<td>4</td>
<td>2.0</td>
<td>1:2</td>
<td>271</td>
<td>309</td>
<td>391</td>
<td>366 783 877 974</td>
<td>1366 85</td>
</tr>
<tr>
<td>[Cu(L)_2]</td>
<td>7</td>
<td>2.0</td>
<td>1:2</td>
<td>311</td>
<td>383</td>
<td>468</td>
<td>477 977 1415 2214</td>
<td>1366 85</td>
</tr>
<tr>
<td>[Zn(L)_2]</td>
<td>7</td>
<td>2.5</td>
<td>1:2</td>
<td>266</td>
<td>311</td>
<td>385</td>
<td>385 472 1633 2098</td>
<td>1341 317</td>
</tr>
</tbody>
</table>

FTIR spectra

FTIR spectra into azo ligand as well their metal chelates have been collated, and the datum has been listing on Table 4. The broad band at spectral for ligand on 3371 cm^(-1), that was described into stretching vibration from υ(OH) phenol, the demise for band in spectra with all produced compounds pointed out deprotonation with phenol group to the coordination for metal ion(25). Spectrum presented bands in 3219 and 1739 cm^(-1) because of υ(NH) and υ(C=O) vibration, no significant change in these bands were noticed, the possibility that coordination occur via the donating atom in these groups were excluded(26). Band for azo group at 1570 cm^(-1) displaced to the lower wave number with change during shape in spectra with all produced compounds(27). The bands at the range (1371-1585 cm^(-1)) due to bending frequency from (6CH_3) as well stretching vibration from υ(C=C)(28). Stretching frequency bands to metal-nitrogen on other than metal-oxygen more(29,30) confirmed during presence into bands at rate 449-598 cm^(-1).
Antimicrobial screening result

The ligand (L) and its complexes reported here were evaluated for antibacterial activity against (Staphylococcus aureus, Staphylococcus epidermidis and Psedomonas Aeruginaso), (G+ev) and (Streptococcus sp., Escherichia coli and Klebsiella sp.), (G-ev) and antifungal activity such as Candida albicans (Yeast) by employing disk diffusion method. The data of antibacterial and antifungal activities of zone inhibition were measured in millimeter of ligand and its complexes with are given in Table (5) and (Fig.10).

As can be seen from the antibacterial activity and antifungal activity of ligand and metal complexes in (Fig.10), among all these complexes the ZnL compound show the higher antibacterial activity with inhibition zone of 13mm against Staphylococcus epidermidis, Streptococcus sp. and Escherichia coli compared with ligand and other metal complexes. In case of ZnL compound the antifungal activity of Candida albicans shows higher inhibition area of 15mm is higher activity from ligand and rest metal complexes.

Dyeing properties

Dyeing performance of the prepared compounds was defined at cotton fabric. Dyes have been article into light and detergent constancy. So whole dyes appeared the dyeing is highly excellent holding and depth on the fabric. The dyeing was referred on Fig.11.

Table 5: Biological activity results of Ligand (L) and its complexes showed the inhibition circle diameter in millimeter for the bacteria after 24h

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Staphylococcus aureus (G+ev)</th>
<th>Staphylococcus epidermidis (G+ev)</th>
<th>Psedomonas Aeruginaso (G+ev)</th>
<th>Streptococcus sp. (G-ev)</th>
<th>Escherichia coli (G-ev)</th>
<th>Klebsiella sp. (G-ev)</th>
<th>Candida albicans (Yeast)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (DMSO)</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Ligand(L)</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>CoL</td>
<td>12</td>
<td>12</td>
<td>12</td>
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<tr>
<td>NiL</td>
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<td>12</td>
<td>11</td>
<td>11</td>
<td>11</td>
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<td>9</td>
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<td>CuL</td>
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<td>ZnL</td>
<td>12</td>
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<td>12</td>
<td>13</td>
<td>13</td>
<td>12</td>
<td>15</td>
</tr>
</tbody>
</table>

As = asymmetry, s = symmetry, br = broad, sh = sharp, s = strong, w = weak.
Fig. 10: Graphical presentation of antibacterial and antifungal activity of ligand and its complexes, measuring the inhibition zone (mm). DMSO: dimethyl sulfoxide.

4. Conclusion

In current work, the metal ions compounds were prepared for ligand. Willing compounds are depicted during melting point, spectroscopic studies, as well conductivity and magnetic quantifications. Exploration from antimicrobial efficiencies has been uploaded out opposite the tested organism. Dye as well as their produced compounds were used at cotton fabric depend on the outcome datum a tetrahedral structure proposed with prepared compounds.

5. Conflicts of interest

“There are no conflicts to declare”.

5. References


[13]. Iniama, G.E., Nfor, E.N., Okon, E.D. and Iorkpiligh, I.T. 2014. Antimicrobial activities of synthesized Zinc(II) mixed ligand complexes derived from 2-acetylpyridine-4-phenylsemicarbazone and nitrogen-


