A new Schiff base ligand, N-hydroxy-N'-salicylaldenediene-urea was synthesized through the condensation of salicylaldehyde with hydroxyurea. The copper(II) complex of the Schiff base has been also obtained. Their structure has been proven using spectral methods such as UV-Vis, FT-IR, 1H-NMR and elemental analysis. The antimicrobial activity of the copper(II) complex was evaluated through comparison to the activity of the Schiff base on various bacterial strains. All tested compounds were very active against both gram-positive and gram-negative bacteria.

Keywords: Schiff base, coordinative complex, antimicrobial activity, UV-Vis, FT-IR and 1H-NMR experimental techniques

Synthesis and Biological Evaluation of a New Schiff Base and its Cu(II) Complex

Compounds with the azomethine group (-C=N-) in their structure have been known as Schiff bases. They are usually synthesized through condensation of primary amines with compounds with active carbonyl groups [1]. The biological activity of Schiff bases has been attracting the attention of organic chemists and medical researchers for many years. Nowadays, Schiff bases have well known representatives in the groups of anticancer [2], antimicrobial [3, 4], anti-inflammatory [5, 6], antiviral [7], analgesic [8], pesticidal [9] and antioxidant agents [10, 11].

Based on the above-mentioned applications of Schiff bases, this study presents the synthesis, physico-chemical characterization and antimicrobial effects of a new Schiff base and its Cu(II) complex.

Experimental part
Materials and methods
All chemicals and solvents were analytical reagent grade and they were supplied by Merck (Germany) and Chimopar (Romania). The melting points were determined using a Boethius apparatus without correcting the result. The IR spectra (from KBr pellets) were recorded on a FTS-135 BIO-RAD spectrometer. The UV-Vis spectra was obtained on a Hewlett-Packard 8453 UV-Vis spectrophotometer. Elemental analysis of C, H and N was carried out with an Elemental Vario Analyzer. The quantitative determination of Cu(II) was performed using the AAS-IN Carl-Zeiss-Jena spectrometer.

Synthesis and characterization of N-hydroxy-N'-salicylidene-urea
0.01mol (0.76g) of hydroxyurea dissolved in 10mL of methanol was mixed with 0.01mol (1.06mL) of salicylaldehyde dissolved in 30mL of methanol and then it was refluxed for 2-4 h [12-14]. The reaction mixture was concentrated in vacuo and after the addition of ethyl ether, a brown solid precipitate was collected. It was washed with a 2:1 ether/ethanol mixture and then it was crystallized from a diethyl ether.

The ligand was a brown crystalline powder, stable at room temperature, insoluble in water, soluble in ethanol and methanol, very soluble in acetone and dimethylformamide (DMF). Yield 75.7%; m.p. 158-160°C. UV-VIS \( \lambda_{\text{max}} \) (DMF) nm (\( \epsilon \), mol\(^{-1}\)·L\(^{-1}\)·cm\(^{-1}\)): 325 (3.27), FT-IR (KBr), cm\(^{-1}\): \( \nu_{\text{max}} \) 3390 (-OH aril), 1665 (C=O), 1064 (C-N), 1280 (OH aryl). \( \lambda_{\text{max}} \) (CDCl\(_3\)) nm (s, 2H, OH): 7.26-7.49 (m, 3H, H-Ar), 8.51 (s, 1H, CH=N), 5.45 (s, 1H, NH). Anal. Calcd. for C\(_8\)H\(_6\)N\(_2\)O\(_3\): C, 53.34; H, 4.48; N, 15.55. Found: C, 53.98; H, 4.75; N, 15.69.

Synthesis and characterization of Cu(II) complex
The Cu(II) complex was synthesized using the general procedure reported previously [15,16]. 1.99g (0.01mol) of Cu (OAc)\(_2\) · H\(_2\)O dissolved in 25mL methanol was added drop wise to 1.8g (0.01mol) of ligand dissolved in advance in 25mL methanol. The mixture was stirred at room temperature for 4 hours and then it was evaporated at 90°C, until the solution darkened; sparkling black micro-crystals were filtered, washed with a mixture of ethanol-water (1:1, v/v) and then with ethyl ether. The Cu(II) complex was a green crystalline powder that was stable at room temperature, insoluble in water, ethanol, benzene or chloroform, soluble in methanol, DMSO and DMF. Yield: 65.3%; m.p. 179-180°C. UV-VIS \( \lambda_{\text{max}} \) (DMF) nm (\( \epsilon \), mol\(^{-1}\)·L\(^{-1}\)·cm\(^{-1}\)): 8.09·10\(^4\), Ks = 6.91·10\(^5\); solubility (mol/L): 5.08·10\(^{-3}\), \( \nu_{\text{max}} \) (KBr), cm\(^{-1}\): 325 (3.27). FT-IR (KBr), cm\(^{-1}\): \( \nu_{\text{max}} \) 3390 (-OH aril), 1665 (C=O), 1064 (C-N), 1280 (OH aryl). \( \lambda_{\text{max}} \) (CDCl\(_3\)) nm (s, 2H, OH): 7.26-7.49 (m, 3H, H-Ar), 8.51 (s, 1H, CH=N), 5.45 (s, 1H, NH). Anal. Calcd. for C\(_8\)H\(_8\)N\(_2\)O\(_3\): C, 53.34; H, 4.48; N, 15.55. Found: C, 53.98; H, 4.75; N, 15.69.

Evaluation of antimicrobial activity
The antimicrobial activity of the ligand and the copper (II) complex were evaluated against Gram-positive bacteria: Staphylococcus aureus ATCC 25923 (Sa), Bacillus cereus ATCC 14579 (Bc), Bacillus subtilis ATCC 6633 (Bs), and Gram-negative bacteria: Escherichia coli ATCC 25922 (Ec), Pseudomonas aeruginosa ATCC 9027 (Pa). Chloramphenicol and Ampicillin were used as reference substances.

The qualitative antimicrobial assay of the compounds was performed using the agar diffusion method according to standard accepted disk sensitivity criteria of the National Committee for Clinical Laboratory Standards [17-20]. The agar dish diffusion procedure is a method approved by the National Committee for Clinical Laboratory Standards.
Results and discussions

The structures of the ligand and its complex was confirmed using spectroscopic methods and elemental analysis. The UV-Vis spectrum of the ligand included a large absorption peak at 282nm that shifted to 355nm in the UV-Vis spectrum of its Cu(II) complex due to the ligand's coordination with the metallic ion.

The IR spectrum of the ligand included a characteristic band at 1665cm⁻¹ which was due to vibration of C=O group. The shifting of that group to a lower frequency (1610cm⁻¹) in the spectrum of Cu(II) complex implied the coordination of the metal ion through the oxygen atom of the azomethine group. It was expected that the coordination of nitrogen to the metal atom would reduce the electron density in the azomethine group and thus lower the C=N band at 1685cm⁻¹ which was due to vibration of C=N group.

The UV-Vis spectrum of the ligand included an absorption band at 280nm which corresponded to the π-π* transition of the aromatic ring. The Cu(II) complex showed a higher absorbance in this region, indicating a stronger electronic transition due to the ligand's coordination with the metal ion.

The 'H-NMR spectrum of the complex possessed significant modifications due to the coordination process in reference to that of the ligand. The proton signal of the -OH (11.45ppm) and the -NH (5.45ppm) groups from the structure of the ligand disappeared upon complexation with Cu(II). The aromatic protons did not seem to register significant changes as a result of the coordination process.

The results of the elemental analysis of ligand and its complex were found to be in good agreement with the values that had been theoretically calculated.

Table 1 summarized the antimicrobial activity of tested compounds against Gram-positive and Gram-negative reference strains, in comparison to Ampicillin and Chloramphenicol.

The Cu(II) complex showed a higher antimicrobial action than the free ligand and that effect was evident against all reference bacteria tested. The Cu(II) complex was more active than the ligand because of the coordination involving the -OH and the nitrogen atom of C=N group.

The association of the ligand with Cu(II) substantially increased the *in vitro* susceptibility of Gram-positive and Gram-negative tested bacteria. Against *Staphylococcus aureus* ATCC25923, both ligand and its Cu(II) complex exerted the lowest degree of antimicrobial activity when compared to sporulated bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*.

Both tested substances showed at concentrations of 10µg/mL an antimicrobial profile similar to that of ampicillin and chloramphenicol (30µg/disc) against *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. On the contrary, *Staphylococcus cereus* and *Bacillus subtilis* were less sensitive to these compounds than *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The data of table 1 shows large inhibition zones of microbial growth. The differences in activity probably reflected the differences in the mode of action of their chemical structures against the bacterial cell.

Conclusions

The research study reported the successful synthesis and antimicrobial activity evaluation of a new Schiff base and its Cu(II) complex. Both substances were physically and chemically characterized through elemental analysis, 'H-NMR, UV-Vis and IR analysis, the ratio of metal/ligand combination, the melting point and the solubility. The IR spectrum confirmed the hypothesis of the formation of
the complex by coordination of copper ions to the azomethinic nitrogen and to the phenolic oxygen. The tested compounds were active against both gram-positive and gram-negative bacteria. The antimicrobial activity of the complex resembled that of Ampicillin and Chloramphenicol. Also, all tested bacteria revealed a lower sensitivity against the ligand than against the complex.

References

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