Schiff bases can be synthesized from an aliphatic or aromatic amine and a carbonyl compound by nucleophilic addition forming a hemiaminal, followed by dehydration to generate an imine. Schiff bases are common ligands in coordination chemistry. The imine nitrogen is basic and to generate an imine. Schiff bases are common ligands in addition forming a hemiaminal, followed by dehydration aromatic amine and a carbonyl compound by nucleophilic

The paper presents the synthesis of a new complex combination of a Bis-Schiff base with Mn(II) ions with great potential for antimicrobial and anti-inflammatory activity. A new complex of the Salen-type ligand, 1-ethyl-salicylidene-bis-ethylene diamine was synthesized using Mn(II) ions. The chemical structure was confirmed through 1H-NMR and IR spectroscopy. The antimicrobial activities of the Bis-Schiff base and its complex were tested in comparison with Ampicillin, Chloramphenicol, Tetracycline, Ofloxacin and Nystatin. Those compounds were found to be active against Gram-positive or Gram-negative bacteria, and had an anti-inflammatory effect comparable to that of Indomethacin.

Keywords: Bis-Schiff base, Mn(II) complex, anti-inflammatory effect, antimicrobial effect.

Experimental part

The following reagents were used: MnSO₄ . H₂O, dimethyl sulfoxide (DMSO) sodium carboxymethyl cellulose (Na-CMC), and methanol. They were produced by Merck Germany or Chimopar Romania.

The melting points were determined using a Boetius apparatus. Elemental analysis was carried out using an Elemental Vario El Analyzer. The quantitative determination of Mn(II) ions from the synthesized complex was performed using the spectrometer AAS-IN Carl-Zeiss-Jena.

The DL50 values of new Bis-Schiff bases and their complexes with metallic ions, have also been established.

Fig. 1. Structure of 1-ethyl-salicylidene-bis-ethylene diamine (BSB)

The DL50 values of new Bis-Schiff bases and their complexes with metallic ions, have also been established.
Vis spectra have been obtained on a Hewlett-Packard 8453 spectrophotometer. The manganese (II) complex (Mn(BSB)) was synthesized according to the general method from the scientific literature [1, 25, 28]. Firstly, 25 mL of 0.0592M MnSO₄·H₂O solution prepared in 10⁻³M HCl was added to 10 M HCl was added to 50 mL of 0.014238M BSB solution prepared in anhydrous methanol while stirring at 40°C. The brown complex precipitated immediately. After cooling at room temperature, the precipitate was filtered and then washed with distilled water at first, and then with a methanol-water mixture and finally with ether. After drying in vacuum, a fine brown crystalline powder - Mn(BSB)₂ - was obtained and then it was analyzed.

The melting point of the Mn(BSB)₂, was 358-359°C. The crystalline powders proved to be stable at room temperature, insoluble in water, ethanol, benzene, and CHCl₃, but soluble in DMSO, methanol and dimethylformamide (DMF).

The experimental versus calculated results of the elemental analysis of Mn(BSB), were: 67.20% C (68.47); 5.78% H (6.56); 8.06% N (7.98) and 7.76% Mn (7.82).

Based on the characteristic absorbance of Mn(BSB)₂, [29], the formular weight was determined according to the following equation:

\[ F = \frac{a \cdot \epsilon}{V \cdot A} \]

where: \( F \) = formular weight of the complex, \( a \) = the amount of complex obtained; \( \epsilon \) = the molar absorption of the complex (for \( \lambda = 275 \) nm), \( A \) = absorbance determined experimentally, and \( V \) = volume of the solution.

The acute toxicity of the Bis-Schiff base and Mn(BSB), was estimated by orally administrating their 0.1% suspensions in Na-CMC to groups of 6-10 Swiss male mice, each weighing between 20 and 25 g, according to the classical laboratory methodology [30]. The animals received food and water ad libidum. Three hours before testing their access to water was discontinued.

Acute toxicity was evaluated using geometrically progressing doses in single administrations. The death of the animals and their behavioral reactions has been followed for 10 days. The testing was made in accordance with the international legislation and the internal regulations of the University of Medicine and Pharmacy concerning experiments using lab animals [14, 15].

Interpretation of the results was made by analyzing the regression lines and the data were submitted to ANOVA testing.

The qualitative antimicrobial assay of the compounds was performed by the agar diffusion method according to standard accepted disk sensitivity criteria of National Committee for Clinical Laboratory Standards [31, 32] using 10⁻³M methanolic solution of BSB, 10⁻³M methanolic solution of MnSO₄·H₂O, 1000 µg/mL BSB solution in DMSO and 1000 µg/mL Mn(BSB), solution in DMSO. The bacterial strains used on Sabouraud medium were: Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6533, Escherichia coli ATCC 25922, Candida albicans ATCC 10231, Kibesiella spp. The reference substances were used in very small quantities: 30 µg Chloramphenicol and Tetracycline, 10 µg Ampicillin, 5 µg Ofloxacin, and 100 µg Nystatin dissolved in DMSO and impregnated in discs of sterile paper.

For the qualitative assay, suspensions of the compounds, prepared in sterile peptone water from 24 h cultures of microorganisms were adjusted to 0.5 McFarland. Muller- Hinton Petri dishes of 90 mm were inoculated using those suspensions. The tested compounds were dissolved in DMSO and brought to 1000 µg/mL concentration levels. Chloramphenicol, Tetracycline, Ampicillin, Ofloxacin and Nystatin dissolved in DMSO were used as reference substances. The 6 mm discs impregnated with 10 µL solution of each compound were used as negative controls and placed in a circular pattern in each inoculated plate. Incubation of the plates was done at 37°C for 24 h. Evaluating the results was done by measuring the diameters of the inhibition zones generated by the tested substances. Toxicity tests of the DMSO solvent showed that the concentrations used in antibacterial activity assays did not interfere with the growth of the microorganisms [33, 34].

The anti-inflammatory activity was determined using male Wistar rats, weighing 180-200 g using carrageenan induced paw edema method [35-37]. The animals were randomly divided into groups of six. The standard drug (Indomethacin) and test compounds: BSB and Mn(BSB)₂ were administered p.o. as a suspension in 0.5% Na-CMC solution, one hour before the carrageenan injection. The control group received only 0.5% Na-CMC solution. The right hind paw edema was induced by sub-plantar injection of 0.2 mL of 2% carrageenan solution in saline (0.9%). The volume of paw edema (mL) was determined using plethysmometric method before and after 1, 2, 4, 6, 8 and 24 hours of carrageenan injection. The anti-inflammatory activity was evaluated as the variation of the volume of inflammation paw edema (mL).

The results were analyzed using one-way analysis of variance (ANOVA) and expressed as mean ± standard error of mean (S.E.M.).

**Results and discussions**

The complex combination was characterized from the physic-chemical and chemical point of view by elemental analysis, ¹H-NMR, UV-Vis, FT-IR techniques, which confirmed the structure and radio of metal/ BSB combination.

The elemental analysis of the complexes indicated the formation of the complexes in a 1:2 metal/ligand molar ratio. The formular weight of the complex was calculated using equation (1) based on the experimental data: \( a = 0.2100 \) mg; \( \epsilon = 10702.34 \) mol⁻¹ L cm⁻¹, \( V = 10 \) mL. By calculating the formular weight (F) of the Mn(BSB), complex, the theoretical (700.94) and the experimental values (702.34) obtained, confirmed the 1:2 molar ratio.

The ¹H-NMR spectra of BSB in CDCl₃ exhibited three singlets (δCH₃ = 12.80 ppm, δCH₃(Chloramphenicol) = 6.89 - 7.62 ppm, δCH₃(Mn(BSB)) = 2.15 ppm) and two doublets (δCH₃(CH₃) = 4.82 ppm). The ¹H-NMR spectra of the complex presented significant changes when compared to that of BSB, due to the coordination process. The -OH proton signal of the BSB disappeared upon complexation with Mn(II). The aromatic protons were shifted, while the methyl proton did not seem to presents a significant change because of the coordination.

The UV-Vis spectra of BSB in DMF showed two strong absorption bands in the 200-450 nm region, attributed to π-π* and n-π* transitions. The spectra of the complex presented modifications in the position and intensity of the bands characteristic to the free BSB, as well as the occurrence of new bands which were attributed to d-d or d-π* transition. The UV spectrum of BSB showed two maxima at 255(3.16) nm and 320(3.27) nm, but the latter suffers a bathochromic shifting at 275(2.99) nm and 460(5.50) nm in the spectrum of the complex which
suggested the involvement of the C=N group in the coordination reaction with Mn(II). In the spectrum of the complex, a small shoulder appeared at 425 nm, probably due to the coordination with the metallic ion.

The FT-IR spectra of the ligand showed major bands around 1618 cm\(^{-1}\) assigned to \(\nu_{C=N}\) which could also be found in the spectrum of the Mn(BBS)\(_2\), which suggested the involvement of the nitrogen atom from the C=N group in the coordination process. A more significant modification appeared in the 1040 cm\(^{-1}\) band, attributed to the -OH of the phenolic group, which was absent in the complex. That indicated the involvement of the oxygen anion into a δ bond with the metal cation. More than that, a peak appeared in the spectrum of the complex at 520 cm\(^{-1}\) that could be attributed to the metal-N bond, and another at 456 cm\(^{-1}\) attributed to the metal-O bond.

The recorded FT-IR spectrum confirmed the hypothesis of the formation of the complexes by the coordination of manganese to the azomethinic nitrogen and to the phenolic oxygen.

In order to evaluate the toxicity of the BSB and their complexes Mn(II), the following doses were tested: 100, 200, 400 and 800 mg/kg. At doses of 100 and 200 mg/kg, all compounds were nontoxic. At dose of 400 mg/kg, BSB and its complex induced central phenomena such as shaking and fast breathing. It was also noticed that, at 800 mg/kg dose, all compounds induced sudden death due to convulsive phenomena. In conclusion, BSB and the corresponding complexes are basically nontoxic.

The antimicrobial activity was estimated by measuring the diameter of the area inhibited by the tested compounds: BSB and its Mn(II) complex. The results from table 1 could be attributed to the structure of the tested compounds that seemed to be the main factor influencing the antibacterial activity. That was certainly correlated to the ability of a compound to diffuse through biological membranes to reach its site of action.

A good antimicrobial activity of the BSB was noticed when compared to Chloramphenicol, Tetracycline, Ampicillin, Ofloxacin and Nystatin on Candida albicans and Pseudomonas aeruginosa. The BSB did not have a great antimicrobial activity on Staphylococcus aureus, Bacillus subtilis, Bacillus cereus and Escherichia coli. The best antimicrobial activity was noticed against Candida albicans. The Mn(II) complex was efficient against Staphylococcus aureus, Bacillus subtilis, Escherichia coli Pseudomonas aeruginosa and Candida albicans. The cation involved in the complexes might intensify the antibacterial activity. The Mn(II) complex was most effective against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Candida albicans.

### Table 1

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>BBS 10 µg/mL</th>
<th>Mn(BBS)(_2) 10 µg/mL</th>
<th>Ampicillin 10 µg/mL</th>
<th>Tetracycline 30 µg/mL</th>
<th>Chloramphenicol 30 µg/mL</th>
<th>Ofloxacin 30 µg/mL</th>
<th>Nystatin 100 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>33.66±0.57</td>
<td>38.66±0.52</td>
<td>22.66±0.52</td>
<td>22.66±0.57</td>
<td>25.33±0.57</td>
<td>29.66±0.52</td>
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</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>16.33±0.52</td>
<td>20.32±0.52</td>
<td>34.33±0.52</td>
<td>29.66±0.52</td>
<td>34.66±0.52</td>
<td>29.66±0.52</td>
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</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>24.33±0.52</td>
<td>27.33±0.57</td>
<td>17.33±0.57</td>
<td>31.33±0.52</td>
<td>34.33±0.57</td>
<td>34.33±0.57</td>
<td>-</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>17.33±0.57</td>
<td>21.66±0.52</td>
<td>15.66±0.57</td>
<td>27.33±0.57</td>
<td>27.33±0.57</td>
<td>22.00±0.00</td>
<td>27.33±0.52</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>34.33±0.52</td>
<td>33.33±0.70</td>
<td>0</td>
<td>31.66±0.52</td>
<td>38.66±0.52</td>
<td>34.33±0.70</td>
<td>-</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>28.66±0.52</td>
<td>35.66±0.52</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.33±0.52</td>
</tr>
</tbody>
</table>

### Table 2

**IN VIVO ANTI-INFLAMMATORY ACTIVITY OF THE SYNTHESIZED COMPOUNDS IN CARRAGEENAN-INDUCED PAW EDEMA**

<table>
<thead>
<tr>
<th>Time</th>
<th>0</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>6h</th>
<th>8h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 19.05±1.02</td>
<td>28.26±1.62</td>
<td>32.25±1.02</td>
<td>38.66±1.32</td>
<td>38.66±1.32</td>
<td>23.87±1.15</td>
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<td></td>
</tr>
<tr>
<td>Indomethacin 10mg/kg 21.63±4.02</td>
<td>25.32±2.51</td>
<td>25.33±2.02</td>
<td>25.26±2.52</td>
<td>25.06±2.15</td>
<td>25.66±3.52</td>
<td>24.85±2.57</td>
<td></td>
</tr>
<tr>
<td>BBS 10mg/kg 17.33±0.52</td>
<td>26.35±1.57</td>
<td>28.53±0.55</td>
<td>29.33±0.62</td>
<td>29.33±2.67</td>
<td>28.83±1.57</td>
<td>25.52±0.67</td>
<td><strong>P&lt;0.01</strong></td>
</tr>
<tr>
<td>10µg/BBS2 10mg/kg 18.63±1.57</td>
<td>27.66±0.82</td>
<td>29.86±2.38</td>
<td>27.33±2.75</td>
<td>21.62±5.23</td>
<td>20.66±6.52</td>
<td>21.26±1.77</td>
<td><strong>P&lt;0.01</strong></td>
</tr>
<tr>
<td>10µg/BBS2 5mg/kg 19.43±0.72</td>
<td>26.63±0.65</td>
<td>27.05±1.32</td>
<td>31.66±0.52</td>
<td>30.66±6.52</td>
<td>28.35±8.70</td>
<td>21.83±0.27</td>
<td><strong>P&lt;0.01</strong></td>
</tr>
</tbody>
</table>

* P values compared with control group
** P values compared with the group receiving BSB
more active than BSB (28.83±1.57), and slightly higher than indomethacin (25.66±3.52). In the same conditions, the Mn(II) complex at a dose of 5mg/kg also presented 4h, 6h and 8h (P≤0.001), compared with the BSB (10mg/kg).

Conclusions
The research study reports the successful synthesis and antimicrobial activity of a new Schiff bases complex with Mn(II) ions. The complex was physic-chemically characterized through elemental analysis, UV-Vis and FT-IR analysis, and the ratio of metal/ligand combination, the melting point and the solubility were evaluated.

The antimicrobial activity of the complex was tested in comparison to the Bis-Schiff base against the Gram-positive and Gram-negative bacteria. The comparative study of the antimicrobial activity of a Bis-Schiff base and its new complex Mn(II)BSB), proved the fact that the BSB as well as its complex manifested an antimicrobial activity, similar to Chloramphenicol, Tetracycline, Ampicillin, Ofloxacin and Nystatin.

The study of the anti-inflammatory activity of the manganese(II) Bis-Schiff base complex proved that it induced effects comparable to that of Indomethacin. The anti-inflammatory effect of Mn(II) complex was stronger than the anti-inflammatory effect induced by the free Bis-Schiff base.

References
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