SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF COMPOSITE COMBINATIONS OF Cu(II), Fe(III) AND Mn(III) WITH α-KETOGLUTARIC ACID AND 1-(O-TOLYL) BIGUANIDE

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They were synthesized and characterized by standard physico-chemical methods (elemental chemical analysis, IR spectroscopy, UV-Vis-NIR electron spectroscopy and molar conductance) three complex combinations of Mn (III), Fe (III) and Cu (II), having as ligands α-ketoglutaric acid (HA) and 1- (o-tolyl) biguanide (TB). The formulas thereof are: [Cu(TB)(HA)(ClO₄)•5H₂O (C₁), [Fe(TB)(HA)₂]ClO₄•1,5C₆H₅OH (C₂) and [Mn₂(TB)(HA)₂(OH(H₂O))₄(CI₂)₃ (C₃), where HA is deprotonated H₂A.

The new complexes inhibit the adherence to the inert substrate of bacterial strains Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853, which recommend them for possible therapeutic applications. For C₁ and C₂ complexes was observed a moderate influence on development and metabolic activity of HeLa cells in culture.

Keywords: α-ketoglutaric acid, 1-(o-tolyl)biguanide, Cu(II), Fe(III), Mn(III) complexes, biological activity.

1. Introduction

Biguanides are compounds which show important biological properties, such as: antibacterial, antifungal, antimalarial, antitumoral and hypoglycemic activity [1-6]. There are known many coordination compounds having as a ligand a biguanide, which exhibit biological properties, therefore the study of these compounds it is of interest.

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The literature reports complexes of Cu(II) and Zn(II) having as a ligand chlorhexidine (CHX): \([\text{CuZn(CHX)(NO}_3\text{)}_2\text{Cl}_2\cdot2\text{C}_2\text{H}_5\text{OH}, \text{Cu(CHX)SO}_4\cdot\text{C}_2\text{H}_5\text{OH}, \text{Cu}_2(\text{CHX})(\text{NO}_3\text{)}_3], \text{[CuZn(CHX)CH}_3\text{COO]Cl}_2\cdot2\text{C}_2\text{H}_5\text{OH}, which have antibacterial and antifungal properties [7-9]. Also, there were synthesized and characterized complexes of Fe(III), Ni(II) and Cu(II) with ligands N, N-dimethylbiguanide and derivatives thereof [10-12]. The [FeO(DMGB)]\text{Cl}_2 and [Fe(DMGB)\text{Cl}\cdot0.5\text{H}_2\text{O complexes, where HDMBG = N, N-dimethylbiguanide, have antibacterial properties against S. aureus and E. coli species [11].}

Some manganese complexes (IV) with biguanide ligands were synthesized and studied: \([\Delta]\text{-Mn(bigH)}_3(\text{ClO}_4\text{)}_2\cdot\text{H}_2\text{O}, \text{[Mn(bigH)}_3\text{SO}_4(\text{NO}_3\text{)}_6\cdot3\text{H}_2\text{O, [14], [Mn(bigH)}_3(\text{NO}_3\text{)}_6, \text{[15], where bigH: biguanide, C}_2\text{H}_5\text{N}_5, and [Mn(C}_{10}\text{H}_{24}\text{N}_{10})_2(\text{OH})_2(\text{OH})_2\cdot2\text{H}_2\text{O, [16], where C}_{10}\text{H}_{24}\text{N}_{10} it is hexamethylene dibiguanide.}

The \(\alpha\)-ketoglutaric acid has an essential biological role in the Krebs cycle and is a very important agent for the metabolism of lipids and carbohydrates, in the synthesis and degradation of amino acids and proteins [17]. There were synthesized complexes with lanthanides in which the ligand is \(\alpha\)-ketoglutaric acid [18]. A series of complexes with the formulas: \([\text{M}_{\text{III}}\text{M}_{\text{II}}\text{L}_6(\text{NO}_3\text{)}_6(\text{OH})_2\text{H}_2\text{O})_6(\text{NO}_3\text{)}, where M(III) = Ce and M(II) = Cu, Co, Ni, and L is \(\alpha\)-ketoglutaric acid, have antibacterial properties and antioxidant activity [19].

Given the biochemical role of copper, manganese and iron, as well as biological properties of \(\alpha\)-ketoglutaric acid and 1-(o-tolyl) biguanide, we report in this paper the preparation and characterization of three new complex combinations of these metals with mentioned ligands.

2. Experimental part

Materials and methods

For the synthesis of the three complexes were used: \(\alpha\)-ketoglutaric acid - C\(_5\)H\(_6\)O\(_5\) (Alfa Aesar), 1-(o-tolil)biguanide - C\(_9\)H\(_{13}\)N\(_5\), Cu(ClO\(_4\))\(_2\)•6H\(_2\)O, Fe(ClO\(_4\))\(_3\), Mn(ClO\(_4\))\(_2\)•6H\(_2\)O (Sigma Aldrich) and C\(_2\)H\(_5\)OH (Chemical Company).

Complexes synthesis

The first stage in the synthesis of C1-C3 complexes consisted in dissolution of the substances used (the two ligands and the metal salt) in ethanol, followed by the actual chemical reaction and washing with ethanol and ethyl ether of the obtained complexes. The molar ratio of metal salt: \(\alpha\)-ketoglutaric acid: 1-(o-tolyl) biguanide was 1:1:1, using 1 mmol of each substance. The resulting compounds were pure, therefore no further purification was needed.
Chemical and spectral analysis

Elemental analysis to determine the content of carbon, nitrogen and hydrogen was carried out by microcombustion, with an organic analyzer Elemental Analyzer Flash 2000. For the determination of metal content an atomic absorption spectrophotometer Perkin Elmer Analyst 400 was used.

Electronic spectra were recorded with a Jasco V670 spectrophotometer by the diffuse reflection method at room temperature, in the range of 200-1500 nm wavelength, using MgO as a standard.

For FTIR spectra a Nicolet IS 50 FT-IR spectrophotometer was used, the working range being 4000-200 cm\(^{-1}\).

The molar electric conductivity was determined at 25\(^\circ\)C, in a DMF solution having a concentration of 0.001 mol/L with a CyberScan PCD 6500 conductometer.

Biological activity

For the biological activity of the C1-C3 complexes and \(\text{H}_2\text{A}\) and TB ligands, the MTT test was carried out on HeLa cells, at a concentration of 500 \(\mu\)g/mL, incubation time 24 h at a temperature of 37 \(^\circ\)C, in the atmosphere of 5% \(\text{CO}_2\). The test was also performed for the solvent used in dilution - DMSO, (HeLa control).

The method principle: The cellular oxidoreductases NAD(P)H dependent (NAD(P)H = low phosphate nicotine-adenine dinucleotide), under certain conditions, may reflect the number of cells viable from a culture. These enzymes reduce the reagent tetrazolium MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) to formazan, which is insoluble and has a purple color (Scheme 1). To evaluate the color intensity for formazan, the optical density was measured at 570 nm.

Scheme 1. MTT reduction to formazan
The tetrazolium dye reduction method is often used for cytotoxicity measurement of some compounds and cell proliferation. Since MTT reagent is sensitive to light, the reaction is performed in the dark.

Antimicrobial activity against bacterial strains *Staphylococcus aureus* and *Pseudomonas aeruginosa* was determined in vitro for both synthesized complexes, as well as for the ligands. In order to establish the minimum inhibitory concentration (MIC), a quantitative method was used, based on the generation of binary serial microdilutes in a liquid medium (broth) steriley distributed in 96-well plates. Thus, working solutions of 10 mg/mL in DMSO were prepared for each compound (the ligands and the three complexes). From these, eight serial solutions are prepared by binary serial dilutions with concentrations between 5 mg/mL and 0.0391 mg/mL. Eight wells were pipetted with 150 μL of liquid medium; 150 μL of compound 10 mg/mL of compound were first added, 150 μL of the first well in the second well, in the third 150 μL of the second well and so on to the eighth well.

After the microdilutions were made, a 20 μL volume of 0.5 McFarland microbial suspension was added to each well. Similarly, the working solvent (DMSO) was used. Each test was also run with a microbial culture witness (a row of wells containing exclusive culture medium inoculated with microbial suspension) and an environmental sterility witness. The seedlings were incubated for 24 hours at 37 °C.

After incubation, the inhibitory minimum concentration (MIC) value for each compound was determined macroscopically as its last concentration at which the appearance of microbial growth, *i.e.* the occurrence of environmental turbidity, but also by spectrophotometric reading of the microbial culture absorbance developed in the liquid medium at 620 nm. In the following wells, including the growth control well, the medium was cloudy as a result of microbial growth. The obligatory sterility blanket showed no bacterial growth, the liquid content remaining clear, transparent.

To determine the influence of the complexes and ligands on the adhesion of microbial biofilm to the inert substrate, microbial cells were grown in 96-well plates with nutrient broth and in the presence of compounds of interest. The preparation and concentration of the solutions in each compound (ligands, C1-C3 complexes and solvent - DMSO) was the same as for the determination of the minimum inhibitory concentration. The seedlings were incubated at 37 °C for 24 hours. After incubation, the plates were emptied and washed with sterile physiological water, then a fixation was made for 5 minutes of the adherent cells with 0.1 mL of 80% methanol. By overturning, the methanol solution has been removed and the adhered cells were colored with 1% purple crystal (hexamethyl pararosaniline chloride) 0.1 mL/well alkaline solution for 15 minutes. The staining solution was removed, the plates being washed under a stream of water.
Microbial biofilms formed on plates were resuspended by bubbling in 33% acetic acid. The color intensity of the suspension was evaluated by measuring the absorbance spectra at 492 nm.

In the determination of biological activity, a static incubator for microorganisms was used - Heratherm Microbiological Incubator - Thermo Scientific and to measure the absorbance at certain wavelengths a Max F5 Multi-Mode Filter spectrophotometer.

3. Results and discussions

Elemental Analysis

To establish the formulas of complex combinations, the synthesized elemental analysis was performed (Table 1), being observed a good correlation between carbon, nitrogen, hydrogen and metal percentages obtained experimentally and calculated ones.

<table>
<thead>
<tr>
<th></th>
<th>Nitrogen %</th>
<th>Carbon %</th>
<th>Hydrogen %</th>
<th>Metal %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>exp.</td>
<td>calc.</td>
<td>exp.</td>
<td>calc.</td>
</tr>
<tr>
<td>C1</td>
<td>11.42</td>
<td>11.88</td>
<td>28.72</td>
<td>28.53</td>
</tr>
<tr>
<td>C2</td>
<td>9.88</td>
<td>9.92</td>
<td>37.65</td>
<td>37.44</td>
</tr>
<tr>
<td>C3</td>
<td>7.53</td>
<td>7.43</td>
<td>24.47</td>
<td>24.21</td>
</tr>
</tbody>
</table>

The proposed formulas for complexes are:
CuC14H28N5O14Cl, molar mass 589.4 g/mol for C1
FeC22H32N5O15.5Cl, molar mass 705.81 g/mol for C2
Mn2C19H28N5O25Cl3, molar mass 942.7 g/mol for C3

UV-Vis-NIR Spectra

The stereochemistry of the C1-C3 complexes was proposed following the analysis of complex spectra in ultraviolet-visible near infrared compared to those of H2A and TB ligands. Electronic spectra of the three complexes are presented in Figs. 1-3. The C1-C3 complexes present absorption bands in the range 230-345 nm, which are assigned to the two organic ligands and are due to transitions π-π* and n-π*. As a result of ligand coordination to metal ions, these bands are slightly displaced. For the C1 complex, the spectral band from 545 nm (18350 cm⁻¹) was assigned to charge transfer, and those of 680 nm (14700 cm⁻¹) and 845 nm (11830 cm⁻¹) to transitions dₓz, dᵧz → dₓ²−y², respectively dz² → dₓ²−y², according to distorted octahedral symmetry (oblong).
The spectral band assignments for C2 are: band at 470 nm (21280 cm$^{-1}$) is attributed to $^6A_{1g}(S) \rightarrow ^4A_{1g}(G)$, $^4E_g(G)$ transition, band at 520 nm (19230 cm$^{-1}$) is attributed to $^6A_{1g}(S) \rightarrow ^4T_{2g}(G)$ transition and band of 925 nm (10810 cm$^{-1}$) is attributed to $^6A_{1g}(S) \rightarrow ^4T_{1g}(G)$ transition, the transitions being spin prohibited. Based on these assignments, the proposed symmetry for C2 is distorted octahedral symmetry. In the C3 complex spectrum, the band at 410 nm (24390 cm$^{-1}$) is assigned to the charge transfer, the band at 560 nm (17860 cm$^{-1}$) to the transitions transition $^5B_{1g} \rightarrow ^5E_g$, and the band at 685 nm (14600 cm$^{-1}$) to $^5B_{1g} \rightarrow ^5A_{1g}$ transition, in agreement with a tetragonal distorted octahedral symmetry [20].

![Fig.1. Electronic spectrum of [Cu(TB)(HA)(ClO$_4$)]•5H$_2$O](image1)

![Fig.2. Electronic spectrum of [Fe(TB)(HA)$_2$ClO$_4$•1,5C$_2$H$_5$OH](image2)
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**IR Spectra**

In order to determine the coordination of the ligands to the metal ion, the IR spectra of the C1-C3 complexes and ligands have been analyzed [21]. The characteristic spectral bands and their assignment for complexes and ligands are shown in Table 2. The IR spectrum of sodium α–ketoglutarate was also analyzed to determine the modulus of α–ketoglutaric acid coordination, the difference between the positions of the bands \( \nu(\text{COO}^-)_{\text{asym}} \) and \( \nu(\text{COO}^-)_{\text{sym}} \) being 180 cm\(^{-1}\).

In complexes spectra, a band shift is observed due to the valence vibration of the imine group, \( \nu(\text{C=N}) \), around 1610 cm\(^{-1}\); for C1 at 1590 cm\(^{-1}\), for C2 at 1620 cm\(^{-1}\) and for C3 at 1640 cm\(^{-1}\). This shift is in agreement with the coordination of the TB ligand to metal ions through the iminic nitrogen pair of electrons that are not involved [22]. The keto group in the \( \text{H}_2\text{A} \) ligand is involved in coordination in all the analyzed complexes. This is confirmed by the movement of the corresponding band, \( \nu(\text{C}=\text{O}) \), below 1720 cm\(^{-1}\) to approx. 1690 cm\(^{-1}\) in the C1-C3 spectra. In C2 and C3 complexes, the presence of the bands at 1078 cm\(^{-1}\), 620 cm\(^{-1}\), 1060 cm\(^{-1}\), and 619 cm\(^{-1}\), respectively, are attributed to ionic perchlorate.

Complex C1 has chelated bidentate perchlorate, which results from the assignment of appropriate bands: at 929 cm\(^{-1}\) \( \nu_1(\text{ClO}_4^-) \), at 357 cm\(^{-1}\) \( \nu_2(\text{ClO}_4^-) \), at 1310 cm\(^{-1}\), 1115 cm\(^{-1}\), 1087 cm\(^{-1}\) \( \nu_3(\text{ClO}_4^-) \) and at 619 cm\(^{-1}\), 601 cm\(^{-1}\), 563 cm\(^{-1}\) \( \nu_4(\text{ClO}_4^-) \). The bands from 1242 cm\(^{-1}\) and 931 cm\(^{-1}\) in the spectrum of the C3 complex are attributed to the deformation vibrations in the plane \( \delta(\text{OH}) \), indicating the presence of the bridged hydroxyl group, so this complex should be dinuclear. The presence of crystallization water in C1 and coordination in C3 is evidenced by the band of 3480 cm\(^{-1}\) and 3520 cm\(^{-1}\) which is attributed to \( \nu(\text{OH}_2) \) and the bands at 726 cm\(^{-1}\) and 561 cm\(^{-1}\) assigned \( \rho(\text{H}_2\text{O}) \) and \( \rho(\text{H}_2\text{O}) \). The band at 3440 cm\(^{-1}\) in the C2 spectrum is assigned to the crystallized ethyl alcohol.
The formation of the M-O and M-N bonds in the three complexes was highlighted by assigning bands in the ranges of 278-311 cm\(^{-1}\), respectively 319-440 cm\(^{-1}\) to 417 cm\(^{-1}\).

**Table 2**

<table>
<thead>
<tr>
<th>Assignments</th>
<th>1-(o-tolyl) biguanide</th>
<th>(\alpha)-ketoglutaric acid</th>
<th>C1 complex</th>
<th>C2 complex</th>
<th>C3 complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>(v(C=O)_{\text{sym}})</td>
<td>1720vs</td>
<td>1692s</td>
<td>1690s</td>
<td>1694s</td>
<td></td>
</tr>
<tr>
<td>(v(C=O)_{\text{asym}})</td>
<td>1692vs</td>
<td>1655s</td>
<td>1670s</td>
<td>1666s</td>
<td></td>
</tr>
<tr>
<td>(v(C=N))</td>
<td>1610vs</td>
<td>1590vs</td>
<td>1620vs</td>
<td>1640s</td>
<td></td>
</tr>
<tr>
<td>(v(COO^-)_{\text{sym}})</td>
<td>1544vs</td>
<td>1599vs</td>
<td>1534vs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\delta(NH_2))</td>
<td>1577s</td>
<td>1510m</td>
<td>1537s</td>
<td>1492m</td>
<td></td>
</tr>
<tr>
<td>(\delta(NH)^+)</td>
<td>1270m</td>
<td>1246m</td>
<td>1210m</td>
<td>1242m</td>
<td></td>
</tr>
<tr>
<td>(\delta(CH)^+)</td>
<td>1481vs</td>
<td>1460m</td>
<td>1495s</td>
<td>1460m</td>
<td></td>
</tr>
<tr>
<td>(v(COOH)_{\text{asym}})</td>
<td>1406s</td>
<td>1420m</td>
<td>1390s</td>
<td>1367m</td>
<td></td>
</tr>
<tr>
<td>(v(COO^-)_{\text{asym}})</td>
<td>1409s</td>
<td>1440s</td>
<td>1419s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\delta(OH))</td>
<td>1310m</td>
<td>1115s</td>
<td>1078vs</td>
<td>1060vs</td>
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<tr>
<td>(v_{f}(\text{ClO}_4^-))</td>
<td>1087vs</td>
<td>620s</td>
<td>619s</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(v_{f}(\text{ClO}_4^-))</td>
<td>929m</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(v_{f}(\text{ClO}_4^-))</td>
<td>619s</td>
<td>601m</td>
<td>563m</td>
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<tr>
<td>(\Delta=v_{\text{sym}}(\text{COO}^-)-v(\text{COO}^-))</td>
<td>357s</td>
<td></td>
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<tr>
<td>(v(M-N))</td>
<td>417w</td>
<td>319w</td>
<td>440w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(v(M-O))</td>
<td>278w</td>
<td>279w</td>
<td>311w</td>
<td></td>
<td></td>
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<tr>
<td>(v(OH)_{\text{alcohol}})</td>
<td>3440w</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(v(OH)_{\text{water}})</td>
<td>3480w</td>
<td>3520w</td>
<td></td>
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<td></td>
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<tr>
<td>(\rho_{f}\text{coord. water})</td>
<td>726m</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(\rho_{w}\text{coord. water})</td>
<td>561m</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(\Delta=v_{\text{sym}}(\text{COO}^-)-v(\text{COO}^-))</td>
<td>135</td>
<td>159</td>
<td>115</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Both \(H_2A\) and TB compounds works as bidentate ligands in the three complexes. The TB ligand coordinates to the metal ion through the imine nitrogen atoms, and the \(\alpha\) -ketoglutaric acid coordinates to the deprotonated metal ion through the oxygen atom from the ketone group in the alpha and the adjacent carboxyl groups (the structures of the two ligands are shown in Fig. 4). C3 complex is dinuclear, having a hydroxyl group in the deck.
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The molar conductivity was determined in a solution of N, N-dimethylformamide, at a concentration of $10^{-3}$ M at 25°C. For the C1 complex was obtained a molar conductance value of $35.5 \text{ S} \cdot \text{cm}^2 \text{mol}^{-1}$, for C2 $71.2 \text{ S} \cdot \text{cm}^2 \text{mol}^{-1}$, while C3 complex had a molar conductance of $212.7 \text{ S} \cdot \text{cm}^2 \text{mol}^{-1}$. These values indicate an electrolyte type 1:1 for C2, 1:3 for C3 and a non-electrolyte for C1 [23].

**Biological activity**

HeLa cells metabolism varies depending on the type of material used. Thus, the ligands and C3 complex exhibited a very low effect on HeLa tumor cells under the conditions tested, while complex combinations C1 and C2 have demonstrated a moderate cytotoxic effect on these tumor cells, reducing their viability by 20% and 17%, respectively. The percentages of viability were calculated based on untreated control. Fig. 5 represents the percent viability values for the samples analyzed (at a concentration of $500 \mu\text{g/mL}$ for 24 h incubation at 37°C) and untreated control indicating the metabolic activity of HeLa cell cultures.

Fig. 5. Values of viability for H$_2$A, TB, C1, C2, C3 and untreated control for HeLa cell cultures
Evaluation of antimicrobial activity of H\textsubscript{2}A and TB ligands and C1-C3 complexes was made on \textit{Staphylococcus aureus} ATCC 25923 and \textit{Pseudomonas aeruginosa} ATCC 27853 species. The solvent used in dilutions (DMSO) does not influence the antimicrobial activity of the tested compounds at the working concentrations.

For Gram positive \textit{Staphylococcus aureus}, the complex combination C3 has the best antimicrobial activity with a minimum inhibitory concentration (MIC) of 0.3125 mg/mL, while the ligands and the other two complexes have a MIC of 1.25 mg/mL. H\textsubscript{2}A ligand activity against Gram negative \textit{Pseudomonas aeruginosa}
has a lower antimicrobial activity than the other ligand TB and C1-C3 complexes. C3 complex has the lowest MIC value (0.3125 mg/mL), which means that it has the best antimicrobial activity among all the tested compounds. The minimum inhibitory concentrations for the compounds tested against the two bacterial strains are shown in Figs. 6 and 7.

Regarding the ability to inhibit the microbial biofilm adhesion to the inert substrate, both complexes and ligands inhibit this process depending on the dose, to the minimum biofilm eradication concentration (MBEC), 0.0391 mg/mL (H₂A, C1, C3), 0.0781 mg/mL (TB) and 0.01562 mg/mL (C2) in the case of Staphylococcus aureus. For Pseudomonas aeruginosa all the compounds have a biofilm inhibitory capacity up to a minimum biofilm eradication concentration of 0.0391 mg/mL (TB, C1, C3) and 0.0781 mg/mL (H₂A, C2).

The influence of ligands and complexes on the ability of adhesion to the inert substrate is shown in Figs. 8 and 9.

![Fig.8. Influence of H₂A, TB, C1, C2, C3 on the ability of adhesion to the inert substrate of S. aureus strain](image)
The antimicrobial activity of the complex C1 can be explained by its nature as the non-electrolyte type, who has high lipophilicity which allows passive diffusion through porines and inhibition of either the cytosolic enzymes, or of the microbial DNA. For complexes C2 and C3 (both of electrolyte type), their activity may come from the electrostatic interaction of the complex cation with the negatively charged components of the membrane and their inactivation. On the other hand, antimicrobial activity may be associated with stereochemistry, the valence of the metal, as well as the combined effect of the ligand and the metal ion in the inactivation of a certain component involved in the pathogenesis of the microorganism.

A weaker antimicrobial activity of the C2 complex can be attributed to the fact that two molecules of H₂A and only one of TB are coordinating to iron. C3 complex is dimer and its good antimicrobial activity can be attributed to the breakdown of the hydroxyl bridge.

4. Conclusions

Three complexes combinations of Cu (II), Mn (III) and Fe (III) have been synthesized and characterized, having α-ketoglutaric acid and 1-(o-tolyl) biguanide as ligands and based on the analyzes performed, their formulas have
been proposed. The biological activity of these complexes and also of the ligands used in the synthesis was tested on HeLa tumor cells. It has been found a better cytotoxic effect on HeLa cells of C1 and C2 complexes than the ligands.

Regarding ligands and complexes testing against Staphylococcus aureus and Pseudomonas aeruginosa species, C3 complex has the best antibacterial activity, which recommends it for possible therapeutic applications.

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REFERENCES


