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Data analysis regarding the behavior against different kinds of anaerobic germs of three first-row transition metal complex compounds

Research article

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Abstract
As most kinds of pathogenic agents tend to develop new resistance mechanisms to the common medicines, innovative solutions for counteracting this alarming situation are lately required.
Within this context, the current paper aims to propose as potential antibacterial drugs three complex compounds formed by first-row transition divalent metal ions with a Schiff base, presenting the results of a study regarding the behavior of the Schiff base itself, compared to the ones of these three substances, when tested against different kinds of anaerobic germs.
The work has been performed by using Kirby-Bauer disk diffusion method, the results being afterwards computationally processed by appealing to the Python multi-paradigm programming language.

Keywords: complex compounds, Schiff bases, anti-bacterial activity, Kirby-Bauer disk diffusion method, Python programming language

1. INTRODUCTION

An increasingly grave threat to the worldwide public health is the resistance lately developed by pathogenic agents, which obviously requires novel countering solutions.

As classic drugs seem to quickly exhaust their structural available variations, it becomes self-evident that there is an urgent need for innovative alternatives to circumvent this danger.

Therefore, many scientists have undergone remarkable efforts in this respect in the recent years, biological activity of complex compounds being constantly investigated, because it was proved that, frequently, they could be taken into account as potential drugs [1-6].

2. MATERIALS AND METHODS

2.1. Chemical compounds used for the study

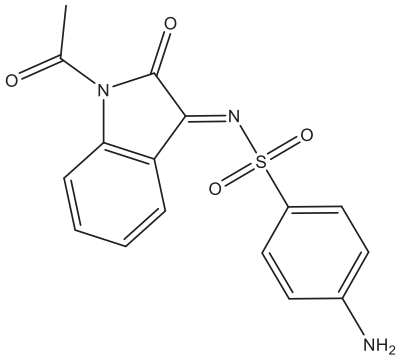
As formerly stated [7], sulfanilamide-derived compounds on one side and isatin-derived ones on the other side are likely to demonstrate a good antibacterial activity for themselves and an even better activity against pathogenic germs upon coordination to different transitional metals.

This is why we dealt with a Schiff base that was obtained by the condensation of sulfanilamide (4-aminobenzene-sulfonamide) with N-acetylisatin (1-acetylindoline-2,3-dione), which is therefore susceptible to exhibit a satisfactory biological activity for itself and an improved one when acting as ligand – *i.e.*, chelated with transitional metals.

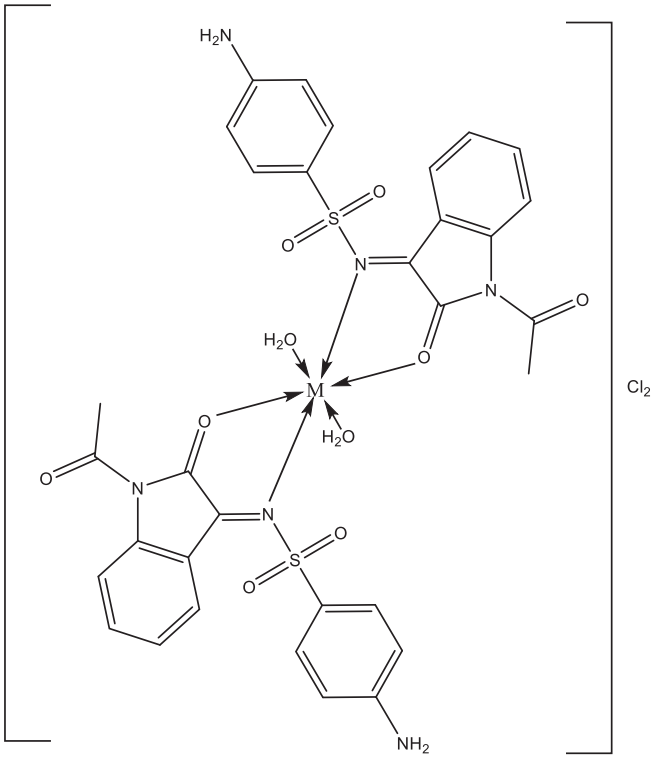
This ligand, the IUPAC name of which is N-(1-acetyl-2-oxoindolin-3-ylidene)-4-aminobenzene-sulfonamide and which will be denoted by L in all what follows, was formerly described [7], in terms of synthesis and characterization, being proved that its structural formula is the one presented below (the first structural formula).

Thus, acting as a bidentate ligand, this Schiff base was also earlier proved [7] to lead – after reacting with Co(II), Ni(II) and Zn(II)

chlorides, within ethanol solutions – to complex compounds exhibiting the following general structural formula, with an octahedral geometry, also presented below (the second structural formula).



L = (*E*)-*N*-(1-acetyl-2-oxoindolin-3-ylidene)-4-aminobenzenesulfonamide



$[ML_2(H_2O)_2]Cl_2$; M = Co(II)/Ni(II)/Zn(II), where L = (*E*)-*N*-(1-acetyl-2-oxoindolin-3-ylidene)-4-aminobenzenesulfonamide

These complex compounds were also described, in terms of synthesis and characterization, in a former paper of ours [7], within which we had already stated that the Schiff base that was obtained by the condensation of sulfanilamide with N-acetylisatin is susceptible to exhibit good antibacterial activity and that a study regarding this matter was in progress, with the intention for it to represent the object of a future paper.

2.2. Bacteria used for the study

As it is well known, three important criteria are used in classifying bacteria [8].

One of these main classification criteria express whether or not the germs need the presence of air to live, grow and replicate themselves; according to this criterium, the ones that need the air are called "aerobic", whereas the other ones are obviously called "anaerobic" (when the air even gets to cause harm for their development, they are called "strictly anaerobic").

Another classification criterium is the one named "Gram staining", involving an interesting bacteriological laboratory technique proposed by the Danish scientist Hans Christian Gram, which mainly consist in treating different bacterial cultures with a primary dye (gentian violet), adding a mordant (iodine tincture), then a decolorizer (ethanol/acetone) and finally a counter stain (safranin/fuchsin) and noticing that bacteria genera act according to the properties of their cell walls: the ones that, although initially become purple, do not stay so (getting decolorized afterwards and finally turning pink or red) are called "Gram-negative", whereas the ones that remain purple all the way are called "Gram-positive" [8].

The last main criterium regards the shape, also dividing bacteria into two important classes: "bacilli" (rod-shaped) and "cocci" (sphere-shaped).

The current paper deals with different kinds of anaerobic germs, belonging to forth classes according to the last two criteria presented above: Gram-negative bacilli, Gram-negative cocci, Gram-positive bacilli and Gram-positive cocci. In order to preserve a clear order of ideas, we

have selected one strain of each kind, whose names are presented in Table 1, together with their strain designations [9].

Table 1. *Germs' genera and strain designations*[*]

Shape/type / Gram staining/color[**]	rod/bacillus	sphere/coccus
Gram-negative/pink	*Bacteroides fragilis* NCTC 9343	*Veillonella parvula* NCTC 11810
Gram-positive/purple	*Clostridium botulinum* NCTC 2916	*Anaerococcus octavius* NCTC 9810

[*]according to the National Collection of Type Cultures (NCTC)

[**]in presence of gentian violet, according to the technique proposed by H. Ch. Gram

All these germs' genera were isolated from clinical specimens. More precisely, the Gram-negative bacillus, *Bacteroides fragilis (NCTC 9343)* [10], was isolated from human intestinal tract; the Gram-negative coccus, *Veillonella parvula* (*NCTC 11810)* [11], was isolated from human oral cavity; the Gram-negative bacillus, *Clostridium botulinum (NCTC 2916)* [12], was isolated from human faces; Gram-negative coccus, *Anaerococcus Octavius (NCTC 9810)* [13], was isolated from human nasal polyps (with maxillary sinusitis).

2.4. Performing the experimental analysis

The disc-diffusion method [14] was performed.

More precisely, the experimental analysis was performed as follows: in the first phase, the culture medium was inoculated on Petri plates having 15 cm in diameter, until the volume of the inoculum was considered sufficient for the germ to be distributed as to obtain an uniform coverage of the entire surface of the Petri plate [14].

We have used thirty-two Petri plates (two for each of the sixteen parts of the analysis – *i.e.*, for each combination substance-bacterium), as to minimize the errors. Within each plate, 10 - 12 discs of 6 mm in diameter were placed, each of them being impregnated with one substance involved in the current study. Consequently, 20 - 24 results were recorded in each of the sixteen cases.

The inhibition zone diameters recorded in each case were given as an array, which was further submitted to the data analysis that will be presented in what follows.

2.5. Performing the data analysis

For the mathematical applications used in developing the data analysis, we have appealed to Python multi-paradigm programming language [15]. More precisely, we have firstly loaded and then cleaned the data, after which we have used Jupyter Notebook – which is a web application used to create and afterwards share different documents that might contain codes, texts or visualizations, this particular application being used for data science, statistical modeling, machine learning and much more, as it is able to combine mathematical details with charts [16].

3. RESULTS AND DISCUSSION

3.1. Experimental findings

All the experimental findings will be herby presented, in a form appropriate for the goal of this paper, *i.e.*, carrying out the data analysis. Maintaining the letters G–/G+ to designate Gram-negative/Gram-positive bacteria and b/c to designate bacilli/cocci, sixteen arrays can be reported:

```
L_G-_b = np. array([10.9, 11.1, 11.5, 11.6, 11.5, 10.6, 10.8,
11.8, 11.1, 11.2, 11.3, 11.5, 11.6, 11.9, 11.3, 10.8, 10.9,
11.4, 11.4, 11.3, 10.8, 11.2])
L_G-_c = np. array([11.9, 12.0, 11.5, 11.6, 12.3, 11.8, 11.9,
12.3, 11.5, 11.6, 11.5, 11.6, 11.8, 11.8, 12.1, 12.2, 11.9,
12.0, 11.4, 11.3])
L_G+_b = np. array([12.3, 12.9, 12.1, 12.4, 11.8, 12.8, 11.7,
12.1, 12.7, 12.6, 12.8, 12.9, 12.8, 12.6, 12.8, 12.7, 12.1,
12.2, 12.9, 12.7, 12.3, 12.6])
L_G+_c = np. array([11.9, 12.6, 12.7, 12.7, 12.8, 12.2, 12.4,
12.4, 12.9, 12.6, 12.6, 11.8, 12.7, 12.1, 12.9, 12.6, 12.9,
12.3, 12.8, 12.8])
Co_G-_b = np. array([17.0, 16.3, 16.3, 17.7, 16.7, 16.6, 17.0,
16.2, 16.8, 17.4, 16.5, 16.8, 17.1, 16.7, 17.2, 17.7, 17.6,
17.2, 16.8, 17.3, 17.5, 16.7])
Co_G-_c = np. array([19.9, 19.3, 19.4, 19.4, 19.7, 20.2, 20.4,
19.9, 19.2, 19.2, 20.2, 19.2, 19.3, 19.4, 19.7, 19.9, 19.4,
20.0, 19.7, 19.9])
```

```
Co_G+_b = np. array([15.3, 15.0, 14.5, 15.6, 15.5, 15.6, 15.8,
14.8, 15.1, 15.0, 15.2, 15.5, 15.6, 14.9, 15.3, 14.9, 15.5,
15.0, 15.9, 15.4, 15.4])
Co_G+_c = np. array([17.1, 17.5, 16.6, 17.5, 17.6, 16.8, 16.8,
17.1, 17.6, 16.3, 17.8, 17.2, 17.9, 17.4, 17.3, 17.5, 16.4,
17.0, 16.8, 17.0])
Ni_G-_b = np. array([12.8, 12.1, 12.9, 13.0, 12.5, 12.6, 12.8,
12.8, 12.9, 13.2, 12.3, 12.6, 12.9, 12.5, 13.2, 13.5, 13.0,
13.4, 12.6, 13.1])
Ni_G-_c = np. array([13.5, 13.0, 13.1, 14.1, 14.3, 14.0, 13.6,
13.5, 13.6, 13.8, 14.3, 14.6, 14.0, 13.5, 13.8, 13.9, 14.2,
14.2, 14.5, 14.4, 13.4, 13.5, 13.6])
Ni_G+_b = np. array([13.8, 14.5, 13.8, 14.1, 13.7, 13.6, 13.8,
14.3, 14.1, 13.6, 13.9, 13.5, 13.8, 14.2, 14.0, 13.9, 13.6,
13.7, 13.5, 13.9, 13.4, 13.4, 13.0, 13.3])
Ni_G+_c = np.array([14.1, 14.5, 13.9, 14.6, 14.5, 13.6, 13.8,
14.8, 14.0, 14.6, 14.9, 14.3, 14.4, 14.3, 14.0, 14.3, 14.5,
13.8, 13.8, 13.9, 14.4, 14.2])
Zn_G-_b = np. array([16.9, 17.1, 17.5, 16.6, 17.5, 17.6, 16.8,
16.8, 17.1, 17.6, 16.9, 16.3, 17.8, 17.2, 17.9, 17.4, 17.3,
17.5, 16.4, 17.3, 16.8, 17.2])
Zn_G-_c = np.array([18.4, 18.3, 17.8, 18.1, 18.2, 17.9, 17.0,
17.2, 17.7, 17.8, 17.9, 17.9, 18.4, 17.2, 18.3, 18.2, 18.2,
18.1, 18.0, 17.9, 17.4])
Zn_G+_b = np. array([17.1, 17.3, 16.9, 17.5, 16.8, 17.1, 17.6,
17.5, 17.6, 16.8, 17.3, 17.6, 16.9, 16.5, 16.8, 17.9, 17.2,
17.2, 17.5, 17.4, 17.4, 16.5, 16.6])
Zn_G+_c = np. array([15.8, 15.5, 15.0, 15.1, 15.7, 15.6, 15.8,
16.3, 16.1, 15.6, 15.9, 15.5, 15.0, 15.2, 15.2, 15.9, 15.6,
15.7, 15.5, 15.9, 16.4, 16.0, 15.3, 15.3])
```

3.2. Data analysis results

For each of the sixteen array reported above, by using Python multi-paradigm programming language, we have determined the following parameters, representative in order to minimize the reading errors for the inhibition zone diameter:

- the mean of the array;
- the median of the array (its second quartile);
- the standard deviation of the array;
- the empirical standard deviation rule interval of the array;
- the variance of the array;
- the confidence interval of the array.

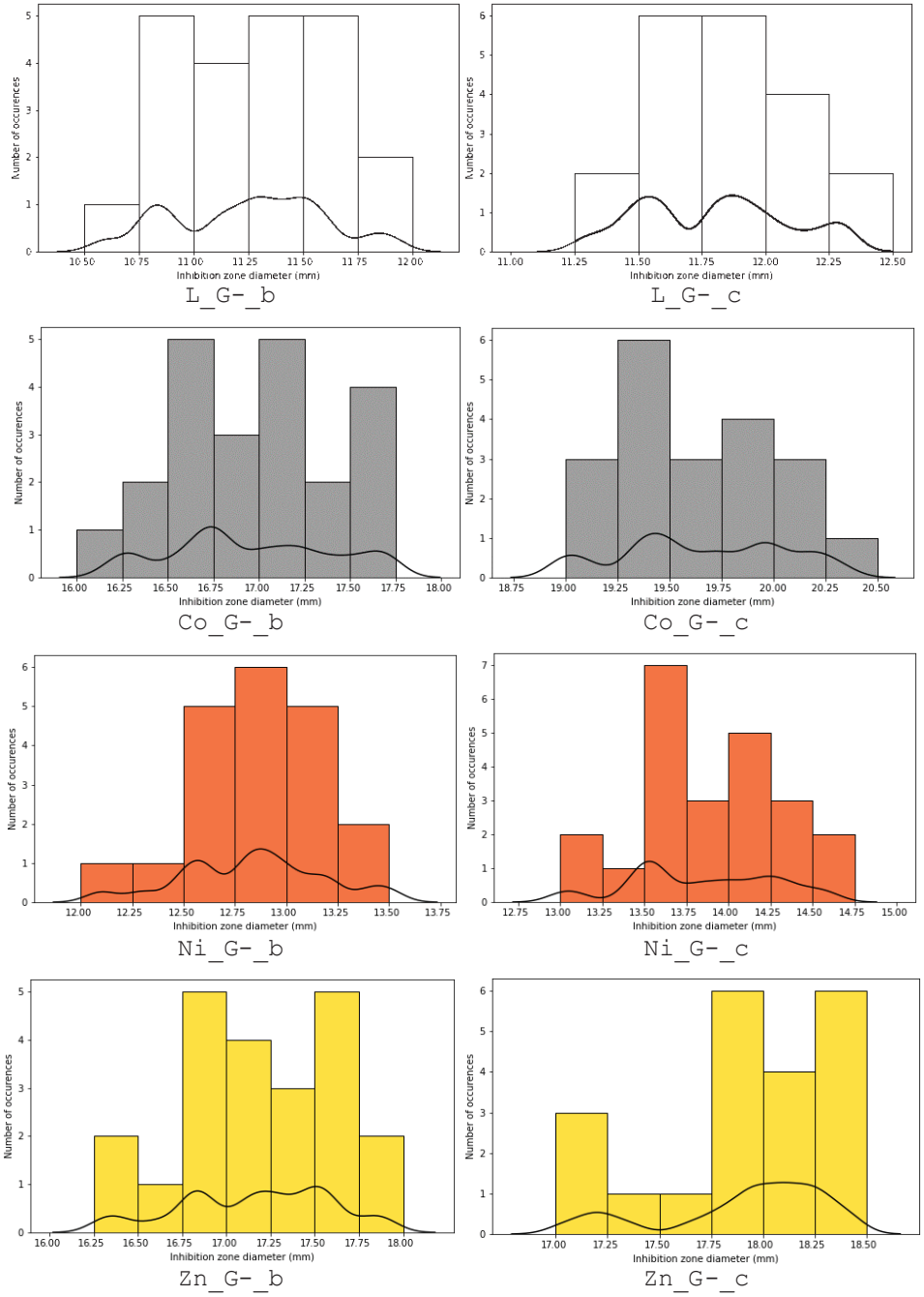


Figure 1. Results of the statistical analysis performed on the arrays containing the results of the experiments in which the ligand L and its complex compounds with Co(II), Ni(II) and Zn(II) were tested against the chosen Gram-negative bacteria (within all plots, the x axis presents the inhibition zone diameter, whereas the y axis presents the number of occurrences)

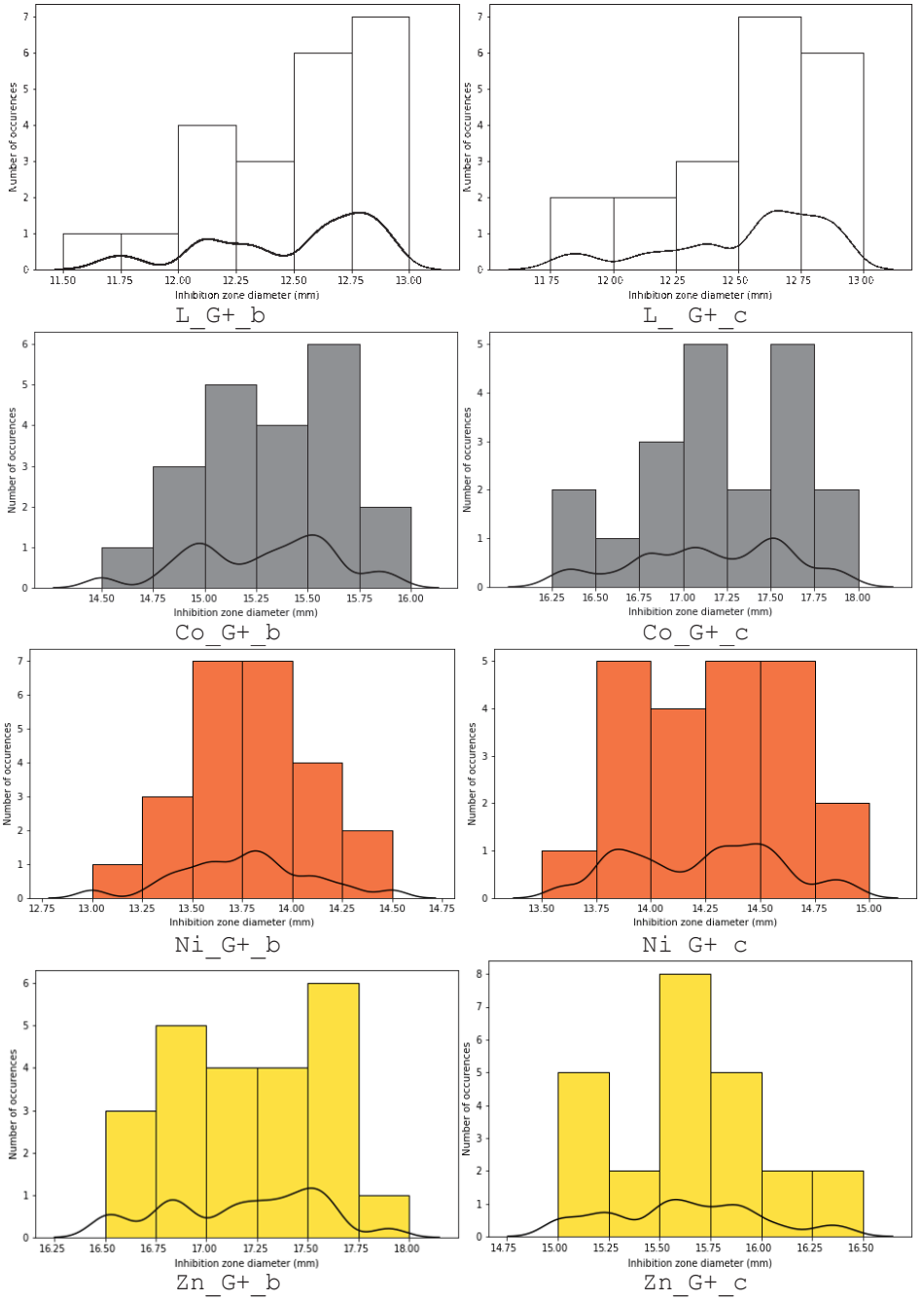


Figure 2. Results of the statistical analysis performed on the arrays containing the results of the experiments in which the ligand L and its complex compounds with Co(II), Ni(II) and Zn(II) were tested against the chosen Gram-positive bacteria (within all plots, the x axis presents the inhibition zone diameter, whereas the y axis presents the number of occurrences)

The confidence interval is considered the most significant, this being defined as an interval computed from the statistics of the sample such that, if the sampling procedure generating the data was repeated, and the confidence interval was re-computed for each random realization, the fraction of such intervals which contains the true population parameter would tend towards 95%. Figures 1 and 2 show the charts thus obtained.

Within all the previous charts, the provided curve represents the kernel density estimate (usually denoted as „kde"), which is a metric showing the true distribution of the data across the total value range of the sample points, being independent on the number of bins chosen for plotting [17].

The results obtained by data analysis for the ligand are the following:

L_G-_b

- **mean: 11.25**
- **median (the second quartile): 11.3**
- **standard deviation: 0.35**
- **empirical standard deviation rule interval: [10.55, 11.95]**
- **variance: 0.12**
- **confidence interval: [11.13, 11.37]**

L_G-_c

- **mean: 11.8**
- **median (the second quartile): 11.8**
- **standard deviation: 0.3**
- **empirical standard deviation rule interval: [11.2, 12.4]**
- **variance: 0.09**
- **confidence interval: [11.71, 11.89]**

L_G+_b

- **mean: 12.49**
- **median (the second quartile): 12.6**
- **standard deviation: 0.36**
- **empirical standard deviation rule interval: [11.77, 13.21]**
- **variance: 0.13**
- **confidence interval: [12.36, 12.62]**

L_G+_c

- **mean: 12.54**
- **median (the second quartile): 12.6**
- **standard deviation: 0.33**
- **empirical standard deviation rule interval: [11.88, 13.2]**
- **variance: 0.1**

- **confidence interval: [12.44, 12.64]**

The similar results obtained by data analysis for the complex compound with Co(II) are the following:

Co_G-_b
- **mean: 16.96**
- **median (the second quartile): 16.9**
- **standard deviation: 0.45**
- **empirical standard deviation rule interval: [16.06, 17.86]**
- **variance: 0.21**
- **confidence interval: [16.75, 17.17]**

Co_G-_c
- **mean: 19.67**
- **median (the second quartile): 19.7**
- **standard deviation: 0.37**
- **empirical standard deviation rule interval: [18.93, 20.41]**
- **variance: 0.14**
- **confidence interval: [19.53, 19.81]**

Co_G+_b
- **mean: 15.28**
- **median (the second quartile): 15.3**
- **standard deviation: 0.36**
- **empirical standard deviation rule interval: [14.56, 16]**
- **variance: 0.13**
- **confidence interval: [15.15, 15.41]**

Co_G+_c
- **mean: 17.16**
- **median (the second quartile): 17.15**
- **standard deviation: 0.45**
- **empirical standard deviation rule interval: [16.26, 18.06]**
- **variance: 0.2**
- **confidence interval: [16.96, 17.36]**

The results for the complex compound with Ni(II) are the following:

Ni_G-_b
- **mean: 12.84**
- **median (the second quartile): 12.85**
- **standard deviation: 0.35**
- **empirical standard deviation rule interval: [12.14, 13.54]**
- **variance: 0.13**
- **confidence interval: [12.71, 12.97]**

Ni_G-_c
- **mean: 13.84**
- **median (the second quartile): 13.8**
- **standard deviation: 0.44**
- **empirical standard deviation rule interval: [12.96, 14.72]**
- **variance: 0.12**

- confidence interval: [13.72, 13.96]

Ni_G+_b

- mean: 13.77
- median (the second quartile): 13.8
- standard deviation: 0.34
- empirical standard deviation rule interval: [13.09, 14.45]
- variance: 0.12
- confidence interval: [13.65, 13.89]

Ni_G+_c

- mean: 14.24
- median (the second quartile): 14.3
- standard deviation: 0.35
- empirical standard deviation rule interval: [13.54, 14.94]
- variance: 0.13
- confidence interval: [14.11, 14.37]

Finally, the results for the complex compounds with Zn(II) are the following:

Zn_G-_b

- mean: 17.16
- median (the second quartile): 17.2
- standard deviation: 0.43
- empirical standard deviation rule interval: [16.3, 18.02]
- variance: 0.19
- confidence interval: [16.97, 17.35]

Zn_G-_c

- mean: 17.9
- median (the second quartile): 18
- standard deviation: 0.4
- empirical standard deviation rule interval: [17.1, 18.7]
- variance: 0.16
- confidence interval: [17.74, 18.06]

Zn_G+_b

- mean: 17.17
- median (the second quartile): 17.2
- standard deviation: 0.39
- empirical standard deviation rule interval: [16.39, 17.95]
- variance: 0.15
- confidence interval: [17.02, 17.32]

Zn_G+_c

- mean: 15.62
- median (the second quartile): 15.6
- standard deviation: 0.38
- empirical standard deviation rule interval: [14.86, 16.38]
- variance: 0.18
- confidence interval: [15.44, 15.8]

3.3. Evaluating the antibacterial activity

As it is well known [8], the susceptibility of the germ to the action of a particular substance can be evaluated.

More exactly, a bacterium is to be called "sensitive/susceptible" to the action of the chemical compound if it exhibits a big inhibition zone diameter (*i.e.*, the substance, if used as medicine, is more probable to eradicate the infection, when administrated regularly); it is to be called „intermediary sensitive/ susceptible" if it exhibits a medium inhibition zone diameter (*i.e.*, the substance may be effective by administration in high concentrations in the tissues/organs where the infection is localized) and, finally, it is to be called "resistant" if it exhibits a small inhibition zone diameter (*i.e.*, the substance is more probable not to be able to eradicate the infection). Nonetheless, we must stress out that the limits to define the classification for the susceptibility depends on the type of bacteria and also on the medicine concentration, being labeled in literature [8].

Table 2 resumes the main results of the study, regarding the antibacterial activities of the ligand and its complex compounds realized with Co(II), Ni(II) and Zn(II).

Table 2. *Synthesis of the main data analysis results regarding the antibacterial activities*

Bacteria type / Chemical compound	Inhibition zone diameter (mm): mean / confidence interval			
	Gram-negative		Gram-positive	
	bacillus	coccus	bacillus	coccus
L	11.25 / [11.13, 11.37]	11.8 / [11.71, 11.89]	12.49 / [12.36, 12.62]	12.54 / [12.44, 12.64]
$[CoL_2(H_2O)_2]Cl_2$	16.96 / [16.75, 17.17]	19.67 / [19.53, 19.81]	15.28 / [15.15, 15.41]	17.16 / [16.96, 17.36]
$[NiL_2(H_2O)_2]Cl_2$	12.84 / [12.71, 12.97]	13.84 / [13.72, 13.96]	13.77 / [13.65, 13.89]	14.24 / [14.11, 14.37]
$[ZnL_2(H_2O)_2]Cl_2$	17.16 / [16.97, 17.35]	17.9 / [17.74, 18.06]	17.17 / [17.02, 17.32]	15.62 / [15.44, 15.8]

By analysing this table, one can see that an enhanced antibacterial effect was obtained by chelation of the Schiff base that we dealt with, *i.e.*, N-(1-acetyl-2-oxoindolin-3-ylidene)-4-aminobenzene-sulfonamide.

Overall, as far as the antibacterial activity is concerned, the zinc divalent ion seemed to be the most reliable for complexation in the cases of bacilli, whereas the cobalt divalent ion seemed to be the most reliable for complexation in the cases of cocci.

4. CONCLUSION

As a conclusion of this work, one may observe that, as expected, all the substances involved in the current study exhibited certain biological activity when submitted to the test against the chosen germs' genera. Moreover, the effect was proved to increase upon coordination in all cases.

Nevertheless, the data analysis showed that no confidence interval managed to reach the value of 20 mm inhibition zone diameter (which is the minimum value obtained for the classical recommended medicines), so that, unfortunately, the tested complex compounds did not prove themselves to be actual competitors for the already used antibiotics, especially taking into account their toxicity (which might result in potential severe adverse effects), but also the higher cost required for their synthesis and commercialization.

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