3rd International Conference on Chemo and BioInformatics
Kragujevac, September 25-26, 2025, Serbia

ICCBIKG 2025
International Conference
on Chemo and BioInformatics

Book of Proceedings

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3rd International Conference on Chemo and BioInformatics

ICCBIKG 2025

BOOK OF PROCEEDINGS

September 25-26, 2025

Kragujevac, Serbia

Sponsored by

3rd International Conference on Chemo and BioInformatics, Kragujevac, September 25-26, 2025, Serbia.

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Press:

„Grafo Ink“, Kragujevac

Impression:

10 copies

Publisher:

Institute for Information Technologies, University of Kragujevac, Serbia, Jovana Cvijića bb, 2025

CIP - Каталогизација у публикацији Народна библиотека Србије, Београд

54:004(048)(0.034.2)
57+61]:004(082)(0.034.2)

INTERNATIONAL Conference on Chemo and BioInformatics (3 ; 2025 ; Kragujevac)
Book of Proceedings [Elektronski izvor] / 3rd International Conference on Chemo and BioInformatics, ICCBIKG 2025, September 25-26, 2025, Kragujevac, Serbia ; [editors Igor Saveljić, Nenad Filipović]. - Kragujevac : University, Institute for Information Technologies, 2025 (Kragujevac : Grafo Ink). - 1 USB fleš memorija ; 1 x 2 x 3 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Tiraž 10. - Bibliografija uz svaki rad.

ISBN 978-86-82172-05-5

a) Хемија -- Информациона технологија -- Зборници b) Биомедицина -- Информациона технологија -- Зборници

COBISS.SR-ID 176312073

Organized by:

Institute for Information Technologies, University of Kragujevac, **Organizer**

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Synergistic antibacterial effect of copper complex and *Lythrum salicaria* plant extract

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DOI: 10.46793/ICCBIKG25.453K

Abstract: The increasing resistance of *Pseudomonas aeruginosa* to conventional antibiotics necessitates the exploration of novel antimicrobial strategies. This study aimed to evaluate the potential synergistic effects of three copper-based metal complexes (**Cu1–Cu3**) in combination with an extract of *Lythrum salicaria* against *P. aeruginosa*. The antimicrobial efficacy of the individual agents and their combinations was assessed through determination of minimum inhibitory concentrations (MICs) and calculation of the fractional inhibitory concentration index (FICI). The results demonstrated that all tested combinations exhibited an indifferent interaction (FICI between 0.5 and 4), indicating neither synergistic nor antagonistic effects. These findings suggest that, under the tested conditions, the combined application of copper complexes and *L. salicaria* extract does not enhance antibacterial efficacy against *P. aeruginosa*, although each agent retains individual antimicrobial potential.

Keywords: copper(II), synergistic effect, plant extract, antimicrobial activity

1. Introduction

Medicinal inorganic chemistry provides unique opportunities for designing therapeutic agents by exploiting the diverse coordination, redox, and kinetic properties of metal ions and their ligands [1]. The constant misuse of antibiotics has led to the emergence of resistant bacterial strains that no longer respond to conventional antibiotics. Consequently, the scientific community is increasingly focused on exploring new antimicrobial compounds, such as coordination complexes of transition metals [2].

Antibacterial properties of copper have been recognized since ancient civilizations. Its mechanism of action is based on the generation of reactive oxygen species (ROS), which irreversibly damage microbial cell membranes. Complexation between copper

and various organic ligands can enhance its antimicrobial activity, efficiency, and selectivity [2]. Ferrocene derivatives represent such a class of ligands due to their stability in biological environments, reversible redox properties, lipophilic nature, and low toxicity [3].

Pseudomonas aeruginosa is an opportunistic Gram-negative bacteria associated with a wide range of healthcare-associated infections, particularly in immunocompromised individuals. Its intrinsic resistance mechanisms, high adaptability, and remarkable ability to acquire additional resistance genes make it one of the most challenging pathogens to treat with conventional antibiotics. The emergence of multidrug-resistant and extensively drug-resistant strains has further limited available therapeutic options, posing a serious threat to global public health [4]. Therefore, the development and evaluation of novel antimicrobial strategies are crucial for overcoming current treatment limitations and mitigating the spread of antibiotic resistance.

Muteeb et al. [5] were the first to report the antibacterial effect of combining metal complexes with aqueous extracts derived from the aerial parts and roots of *Salvia pratensis*. Building upon their findings, the present study investigates the synergistic antibacterial effect of combining metal complexes with aqueous extract from *Lythrum salicaria* in pursuit of enhanced antimicrobial activity.

2. Methodology

Synergy between the tested complexes **Cu1-Cu3** (which are shown in Figure 1) and *L. salicaria* aqueous extract of the aerial part. (**EX**) against *P. aeruginosa* was studied by the checkerboard assay method [6] with modification described in detail by Muruzović et al. [7].

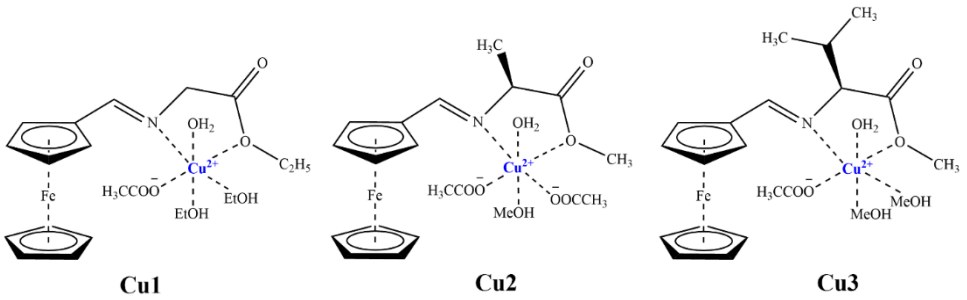

Figure 1. Structural formulas of copper complexes with ferrocene-based ligands.

In this research, three iminoester ligands with ferrocene were used. Ligands were synthesized by the condensation reaction between ferrocenecarbaldehyde and aminoester hydrochlorides [8]. With ferrocene-containing iminoesters as ligands, three novel copper complexes were synthesized. The complexes were synthesized by stirring equimolar amounts of (CH3COO)2Cu • H_2O and ligands in methanol for **Cu2** and **Cu3**, while ethanol was used for **Cu1**, followed by refluxing overnight.

In vitro interactions between antimicrobial agents were determined and quantified by calculating the fractional inhibitory concentration (FIC) index using the following formula:

FIC index = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone).

Interpretation of the FIC index (FICI) was as follows:

FICI ≤ 0.5 synergy (S);

FICI > 0.5 – 1 additive (Ad);

FICI 1 - 4 indifference (I), and

FICI > 4 antagonism (An).

The action of antimicrobial agents is considered to be

- synergistic if their joint effect is stronger than the sum of effects of individual agents
- additive if their joint effect is equal to the sum of effects of individual agents
- indifferent if their joint effect is equal to the effect of either individual agent
- antagonistic if their joint effect is weaker than the sum of the effects of the individual agents or weaker than the effect of either individual agent.

3. Results and Discussion

This study for the first time investigated the potential synergistic antibacterial activity of three copper complexes (**Cu1–Cu3**) in combination with an extract of *L. salicaria* against *P. aeruginosa*. The antimicrobial interactions between the compounds were assessed using the fractional inhibitory concentration index (FICI), calculated based on the minimum inhibitory concentrations (MICs) of the individual agents and their combinations.

The FICI values, summarized in Table 1, indicated that all tested combinations exhibited an indifferent interaction (FICI between 1 and 4) against *P. aeruginosa*. These findings suggest that while both the metal complexes and the plant extract possess individual antimicrobial properties, their combined application does not result in enhanced (synergistic) or reduced (antagonistic) efficacy under the tested conditions. The lack of synergy may be attributed to differing mechanisms of action, potential chemical interactions that reduce bioactivity, or concentration-dependent effects not captured in the current setup.

Table 1. Interaction between tested complexes and *L. salicaria* root extract (EX) on *P. aeruginosa*

Combination/species	MIC	FIC index
Cu1+EX	500+10000	2 (I)
Cu2+EX	500+10000	2 (I)
Cu3+EX	500+2500	1.25 (I)

*MIC values of the most effective combinations given as μg/ml;
FIC index - fractional inhibitory concentration index

4. Conclusions

In this study, the combined antibacterial activity of copper complexes (**Cu1–Cu3**) and *L. salicaria* extract against *P. aeruginosa* was evaluated. The results demonstrated an indifferent interaction between the tested agents, indicating no synergistic or antagonistic effects under the applied conditions. While each agent showed individual antimicrobial activity, their combination did not enhance overall efficacy.

Future research should focus on evaluating their activity against other clinically relevant pathogens. Investigating the mechanisms of action and potential effects in biofilm-forming conditions or *in vivo* models may provide further insight into their therapeutic potential. Additionally, structural modifications of the metal complexes or use of alternative plant extracts may yield more promising synergistic interactions.

Acknowledgment

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants: No. 451-03-136/2025-03/200378 and 451-03-136/2025-03.

References

[1] A. AlAli, M. Alkanad, K. Alkanad, A. Venkatappa, N. Sirawase, I. Warad, S. A. Khanum., *A comprehensive review on anti-inflammatory, antibacterial, anticancer and antifungal properties of several bivalent transition metal complexes,* Bioorganic Chemistry, 160 (2025) 108422.

[2] K. Ngece, V. Khwaza, A.M. Paca, B.A. Aderibigbe., *The Antimicrobial Efficacy of Copper Complexes: A Review*, Antibiotics 14 (2025) 516.

[3] M.F.R. Fouda, M.M. Abd-Elzaher, R.A. Abdelsamaia, A.A. Labib., *On the medicinal chemistry of ferrocene*, Appl. Organometal. Chem, 21 (2007) 613-625.

[4] S. Qin, W. Xiao, C. Zhou, Q. Pu, X. Deng, L. Lan, H. Liang, X. Song, M. Wu, *Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics.*, Signal Transduction and Targeted Therapy, 7 (2022) 199.

[5] G. Muteeb, M.T. Rehman, M. Shahwan, M. Aatif., *Origin of Antibiotics and Antibiotic Resistance, and Their Impacts on Drug Development: A Narrative Review*, Pharmaceuticals, 16 (2023) 1615.

[6] K.P. Satish, R.C. Moellering, G.M. Eliopoulus, *Antimicrobial Combinations. In*: Lorian V. ed. *Antibiotics in Laboratory Medicine*. Lippincott Williams & Wilkins. (2005)

[7] M. Muruzović, K. Mladenović, O. Stefanović, T. Žugić Petrović, Lj. Čomić., *In vitro interaction between Agrimonia eupatoria L. extracts and antibiotic.,* Kragujevac Journal of Science, 39 (2017) 169–176.

[8] Xiao Xu, Longzhu Bao, Lu Ran, Zhenyan Yang, Dingce Yan, Chun-Jiang Wang, Huailong Teng, *Synthesis of Bioactive Fluoropyrrolidines via Copper(I)-Catalyzed Asymmetric 1,3-Dipolar Cycloaddition of Azomethine Ylides*, Chem. Sci. 13 (2022) 1398.

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INTRODUCTION

The alarming rise of antimicrobial resistance, particularly among Gram-negative pathogens such as *Pseudomonas aeruginosa*, poses a significant threat to public health and limits the effectiveness of conventional antibiotics. Inorganic copper complexes and plant extracts have attracted increasing attention due to their proven individual antibacterial properties. Investigating potential synergistic effects between these agents may contribute to the development of novel, more effective therapeutic approaches against multidrug-resistant infections.

OBJECTIVE

To investigate whether the combination of three copper complexes (Cu1-Cu3) and *Lythrum salicaria* extract exhibits synergistic, additive, indifferent, or antagonistic effects against *P. aeruginosa*.

RESULTS

Combination	MIC (µg/ml)	FICI	Effect
Cu1 + EX	500 + 10000	2.00	Indifferent
Cu2 + EX	500 + 10000	2.00	Indifferent
Cu3 + EX	500 + 2500	1.25	Indifferent

METHODOLOGY

Checkerboard method - assessment of MIC (minimum inhibitory concentrations) and calculation of FICI (Fractional Inhibitory Concentration Index).

Formula for FICI:

$$FICI = \frac{MIC(A)\ in\ combination}{MIC(A)\ alone} + \frac{MIC(B)\ in\ combination}{MIC(B)\ alone}$$

Interpretation of the FIC index (FICI) was as follows:
FICI ≤ 0.5 synergy (S);
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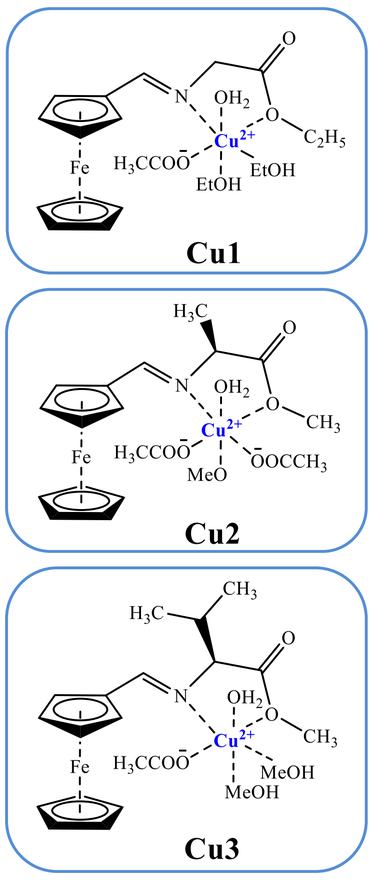
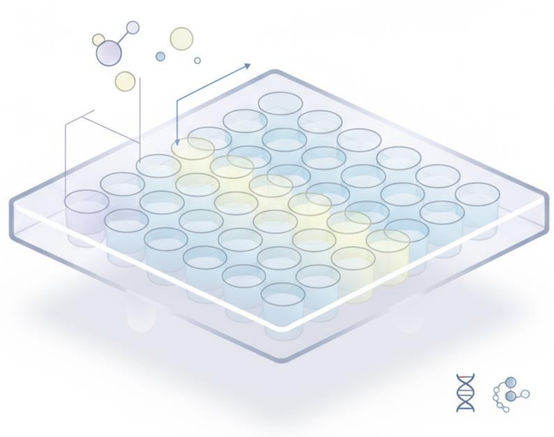


Lythrum salicaria



Checkerboard assay
Drug combination synergy

CONCLUSION

The combination of copper complexes and *L. salicaria* extract did not enhance antimicrobial activity against *P. aeruginosa*.

Each agent individually demonstrated proven potential, but their joint action was not superior to the individual effects.

Future studies should examine similar combinations on other pathogens and in more complex models (biofilm, *in vivo*).

ACKNOWLEDGMENT

This research was supported by the Ministry of Education and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (project numbers: 451-03-136/2025-03/200378 and 451-03-136/2025-03).

REFERENCES

[1] K. Ngece, V. Khwaza, A.M. Paca, B.A. Aderibigbe., Antibiotics 14 (2025) 516.

[2] M. Muruzović, K. Mladenović, O. Stefanović, T. Žugić Petrović, Lj. Čomić., Kragujevac Journal of Science, 39 (2017) 169–176.