UNIVERSITY OF KRAGUJEVAC

FACULTY OF AGRONOMY IN ČAČAK

SYMBIOTECH

4th INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY

12–13 March 2026

Faculty of Agronomy in Čačak, University of Kragujevac, Serbia

- PROCEEDINGS -

4th INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY

XXXI Savetovanje o biotehnologiji sa međunarodnim učešćem

- PROCEEDINGS -

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Print-run: 30

Printed by

Ofis servis Čačak, Rajićeva 5-7, 32000 Čačak

ISBN 978-86-87611-99-3

Year of publication: **2026**

4th INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY
12-13 March 2026, Čačak

Original scientific paper
DOI: 10.46793/SBT26.785K

SYNERGISTIC INTERACTIONS OF A COPPER(II) COMPLEX AND A NATURAL EXTRACT WITH HSA WITH IBUPROFEN AND EOSIN Y

Ana Kesić[1], Andrija Gigić[2], Jovana Bogojeski[2],Dragana Stevanović[2], Jovana Bugarinović[2]*

Abstract: The interactions of a copper(II) complex and a natural extract from *Salvia pratensis* root with human serum albumin (HSA) were studied using fluorescence spectroscopy, focusing on their synergistic effects. Binding studies in the presence and absence of site markers, ibuprofen and eosin Y, assessed binding affinity and potential sites. The copper(II) complex showed moderate interaction with HSA, while the extract had stronger binding. The combined system exhibited enhanced, marker-independent binding, indicating a synergistic effect. These findings suggest the extract influences the interaction between the copper complex and HSA, affecting its transport and biodistribution.

Keywords: copper(II) complex, human serum albumin, synergistic effect, binding interactions, *Salvia pratensis*.

Introduction

The design of therapeutic agents through inorganic medicinal chemistry leverages the unique coordination, redox, and kinetic properties of metal ions and their ligands (Palermo, et al. 2021) The effectiveness of metal-based drugs depends on their ability to interact with biological macromolecules, particularly plasma proteins such as human serum albumin (HSA), which serves as the primary carrier for many drugs and bioactive compounds in the blood (Sharmin, et al. 2021).

Copper complexes are a promising class of inorganic compounds with significant therapeutic potential. They have shown value in biological research due to their low toxicity and potent, selective effects, including notable

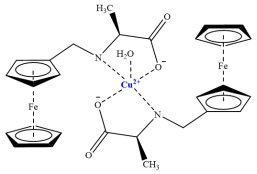
[1]University of Kragujevac, Institute for Information Technologies, Liceja Kneževine Srbije 1A, 34000 Kragujevac, Serbia; Ana Kesić (akesic@uni.ac.kg.rs): https://orcid.org/0000-0002-8729-8748;
[2]University of Kragujevac, Faculty of Science, R. Domanovica 12, 34000 Kragujevac, Serbia; Andrija Gigić: https://orcid.org/0009-0000-3242-0657; Jovana Bogojeski: https://orcid.org/0000-0002-3433-7774; Dragana Stevanović: https://orcid.org/0000-0003-3199-0775; Jovana Bugarinović: https://orcid.org/0000-0002-3561-6732

antifungal, antibacterial, and anticancer activities (Ji, et al. 2021). Ferrocene derivatives are particularly valuable as ligands because of their stability in biological environments, reversible redox properties, lipophilicity, and low toxicity, making them ideal scaffolds for developing new metal-based therapeutics (Mohammad, et al. 2007). This unique properties make ferrocene useful platforme in bioorganic chemistry for the synthesis of novel bioactive compounds. A great number of bioactive ferrocene-containing compounds is known. Among them, the most significant are ferrocifen, potential drug for breast cancer and ferroquine, candidate drug for malaria (Sharma and Kumar, 2021). It has been noticed that redox activity of iron in ferrocene unit induces anitumor activity and has important role in malaria treatment with feroquine (Hillard, et al. 2006). Understanding how metal complexes bind to HSA is essential for predicting drug bioavailability, distribution, metabolism, and potential drug interactions. The strength and specificity of a compound′s binding to serum albumin directly affect its pharmacokinetics and biological activity.

Natural plant extracts, such as those from *Salvia pratensis*, contain diverse bioactive compounds with antioxidant and biological properties. Combining these extracts with metal complexes may produce synergistic effects that improve protein binding and therapeutic potential (Nikola, et al. 2022). This study is the first to investigate the synergistic interaction between a novel ferrocene-based copper(II) complex and an aqueous extract of *Salvia pratensis* roots in their binding to human serum albumin, aiming to uncover the molecular mechanisms of complex-protein interaction and identify specific binding sites that may enhance pharmacological activity.

Materials and methods

Synergy between the tested complex **Cu1** (Figure 1) and the aqueous root extract of *Salvia pratensis* (**SPR**) in interaction with human serum albumin (HSA) was studied by fluorescence spectroscopy (Malihe, et al. 2020).



Cu1

Figure 1. Structural formula of copper complex with ferrocene-based ligand

A copper(II) complex with a ferrocene-based aminoester ligand was synthesized as follows. The ligand was prepared via a condensation reaction between ferrocenecarbaldehyde and aminoester hydrochlorides [Xiao, et al. (2021)]. The novel copper complex was obtained by stirring a methanolic solution containing 1 mol of $(CH_3COO)_2Cu \cdot H_2O$ and 2 mol of the ligand, followed by overnight reflux.

Interactions between the complex in the presence of the natural extract (**SPR**) and human serum albumin (HSA) were investigated using fluorescence spectroscopy. The excitation wavelength was set at 295 nm to selectively excite tryptophan residues, while fluorescence spectra were recorded in the range of 300-500 nm. The examined complex was added to the HSA solution (2 μM) in increasing concentrations (0-4 μM), and fluorescence spectra were recorded after each addition. Fluorescence titration was performed for three systems: (i) the **Cu1** complex alone (0-4 μM); (ii) the **Cu1** complex combined with the **SPR** extract (0-4 μM **Cu1**, 0-20 μg/L **SPR**); and (iii) the **SPR** extract alone. Additionally, binding affinity experiments were performed in the presence of site marker molecules (ibuprofen and eosin Y) to identify potential binding sites and mechanisms. The Stern-Volmer constant (K_{SV}) values were calculated from the linear relationship between I_0/I and [Q] using the Stern-Volmer equation, serving as a measure of binding affinity.

Results and discussion

This study, for the first time, investigated the potential synergistic interaction between the copper complex **Cu1** in combination with an aqueous root extract of *S. pratensis* and human serum albumin (HSA). Fluorescence titration experiments revealed a concentration-dependent reduction in fluorescence intensity upon incremental addition of the complex to the HSA solution. This reduction in fluorescence intensity is attributed to alterations in the tertiary structure of the protein, induced by changes in the microenvironment surrounding the tryptophan residue within serum albumin upon binding of the complex.

The fluorescence emission spectra resulting from the interaction of the **Cu1** complex, **SPR** extract, and their combination (**Cu1-SPR** system) with HSA were recorded and presented in Figure 2, while the corresponding binding constants (K_{SV}) derived from the Stern-Volmer analysis are listed in Table 1. The **Cu1** complex alone exhibited moderate binding affinity toward HSA. However,

when combined with the **SPR** extract, the binding affinity increased significantly, representing a substantial enhancement compared to the **Cu1** complex alone. This marked increase in binding affinity strongly suggests a synergistic effect between the copper complex and the bioactive compounds present in the plant extract. The **SPR** extract alone demonstrated high affinity for HSA, comparable to the **Cu1-SPR** system, indicating that the plant extract actively participates in protein binding.

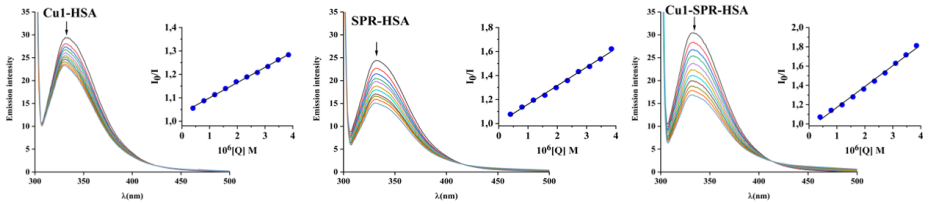


Figure 2. Emission spectra of HSA in the presence of the **Cu1** complex and natural extract, **SPR**.

Table 1. Interaction constants for the examined complex **Cu1** with HSA and site markers in the presence of natural extract, **SPR**

	HSA	HSA-eosine Y	HSA-ibuprofen
	$10^5 K_{sv}$ [M^{-1}]	$10^5 K_{sv}$ [M^{-1}]	$10^5 K_{sv}$ [M^{-1}]
Cu1	0.65 ± 0.1	0.26 ± 0.1	0.92 ± 0.1
Cu1-SPR	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.1
SPR	2.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.1

To elucidate the binding mechanisms and identify specific binding sites on HSA, fluorescence titration experiments were performed in the presence of site marker molecules eosin Y (Figure 3) and ibuprofen (Figure 4). The results revealed differential binding behavior depending on the site marker used. In the presence of eosin Y a Site I marker (subdomain IIA), the binding affinity decreased, suggesting that the complex and its combination with **SPR** preferentially avoid Site I (subdomain IIA) am binding. Conversely, in the presence of ibuprofen a Site II (subdomain IIIA), the binding affinity increased, indicating that both species favor binding at Site II (subdomain IIIA) region of the protein. The **SPR** extract alone showed similar binding preferences, with lower affinity in the presence of eosin Y and higher affinity with ibuprofen. These findings indicate that the **Cu1** complex, whether alone or in combination with the **SPR** extract, preferentially binds to the Site II (subdomain IIIA) region

of human serum albumin. The synergistic enhancement of binding affinity observed in the **Cu1-SPR** system, combined with the specific binding site localization, suggests that the bioactive compounds in the plant extract facilitate and enhance the interaction between the copper complex and HSA, potentially through complementary binding mechanisms or protein conformational changes that promote complex-protein interactions.

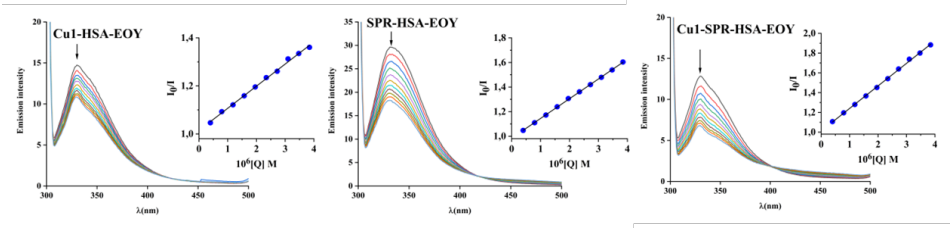


Figure 3. Emission spectra of HSA and eosin Y in the presence of the **Cu1** complex and natural extract, **SPR**

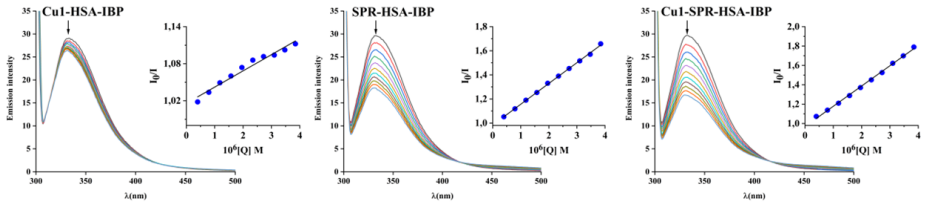


Figure 4. Emission spectra of HSA and ibuprofen in the presence of the **Cu1** complex and natural extract, **SPR**

Conclusion

The results of this study demonstrate that the copper(II) complex **Cu1** exhibits relatively moderate binding toward HSA on its own. In contrast, the presence of the aqueous root extract of *Salvia pratensis* (**SPR**) markedly enhances its interaction with the protein, indicating an apparent synergistic effect. The **SPR** extract itself shows a pronounced affinity for HSA, and site marker experiments reveal that both **Cu1** and the **Cu1-SPR** system preferentially bind to specific regions of the protein associated with Site II (subdomain IIIA), rather than Site I (subdomain IIA). These findings highlight the important role of plant-derived bioactive components in modulating metal complex-protein interactions and suggest that combining metal-based therapeutics with natural

extracts may represent a promising strategy for tuning their pharmacokinetic and biological properties. Future studies should focus on evaluating the potential antimicrobial and anticancer properties of this **Cu1-SPR** system.

Acknowledgement

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.

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4th INTERNATIONAL SYMPOSIUM ON BIOTECHNOLOGY
(31st SYMPOSIUM ON BIOTECHNOLOGY
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University of Kragujevac, Faculty of Agronomy in Čačak

12–13 March 2026, Čačak, Republic of Serbia

CERTIFICATE OF ATTENDANCE

Ana Kesić, Andrija Gigić, Jovana Bogojeski, Dragana Stevanović, Jovana Bugarinović

SYNERGISTIC INTERACTIONS OF A COPPER(II) COMPLEX AND A NATURAL EXTRACT WITH HUMAN SERUM ALBUMIN IN THE PRESENCE OF IBUPROFEN AND EOSIN Y

Chair of Organizing Committee
Prof. dr Pavle Mašković, Ph.D.

Chair of Scientific Committee
Prof. dr Vladimir Kurćubić, Ph.D.

SYNERGISTIC INTERACTIONS OF A COPPER(II) COMPLEX AND A NATURAL EXTRACT WITH HSA WITH IBUPROFEN AND EOSIN Y

Ana Kesić[1,*], Andrija Gigić[2] , Jovana Bogojeski[2], Dragana Stevanović[2], Jovana Bugarinović[2]

[1] University of Kragujevac, Institute for Information Technologies, Kragujevac, Serbia.

[2]University of Kragujevac, Faculty of Science, Kragujevac, Serbia.

SUMMARY

Inorganic medicinal chemistry exploits the unique coordination, redox, and kinetic properties of metal ions to design therapeutic agents capable of interacting with biological macromolecules. Human serum albumin (HSA), as the primary drug carrier in blood, plays a crucial role in determining the bioavailability and pharmacokinetics of metal-based drugs. Copper complexes represent a particularly promising class of therapeutic agents, demonstrating notable antifungal, antibacterial, and anticancer activities with relatively low toxicity. Ferrocene derivatives are highly valuable ligands due to their chemical stability, reversible redox properties, lipophilicity, and low toxicity, making them ideal scaffolds for developing novel metal-based therapeutics. Among the most significant ferrocene-containing compounds are ferrocifen, a potential drug for breast cancer, and ferroquine, a candidate drug for malaria, where the redox activity of the iron center plays a key role in their biological mechanisms. Natural plant extracts, such as those from *Salvia pratensis*, contain diverse bioactive compounds that may produce synergistic effects when combined with metal complexes, potentially enhancing protein binding and therapeutic outcomes.

MATERIALS AND METHODS

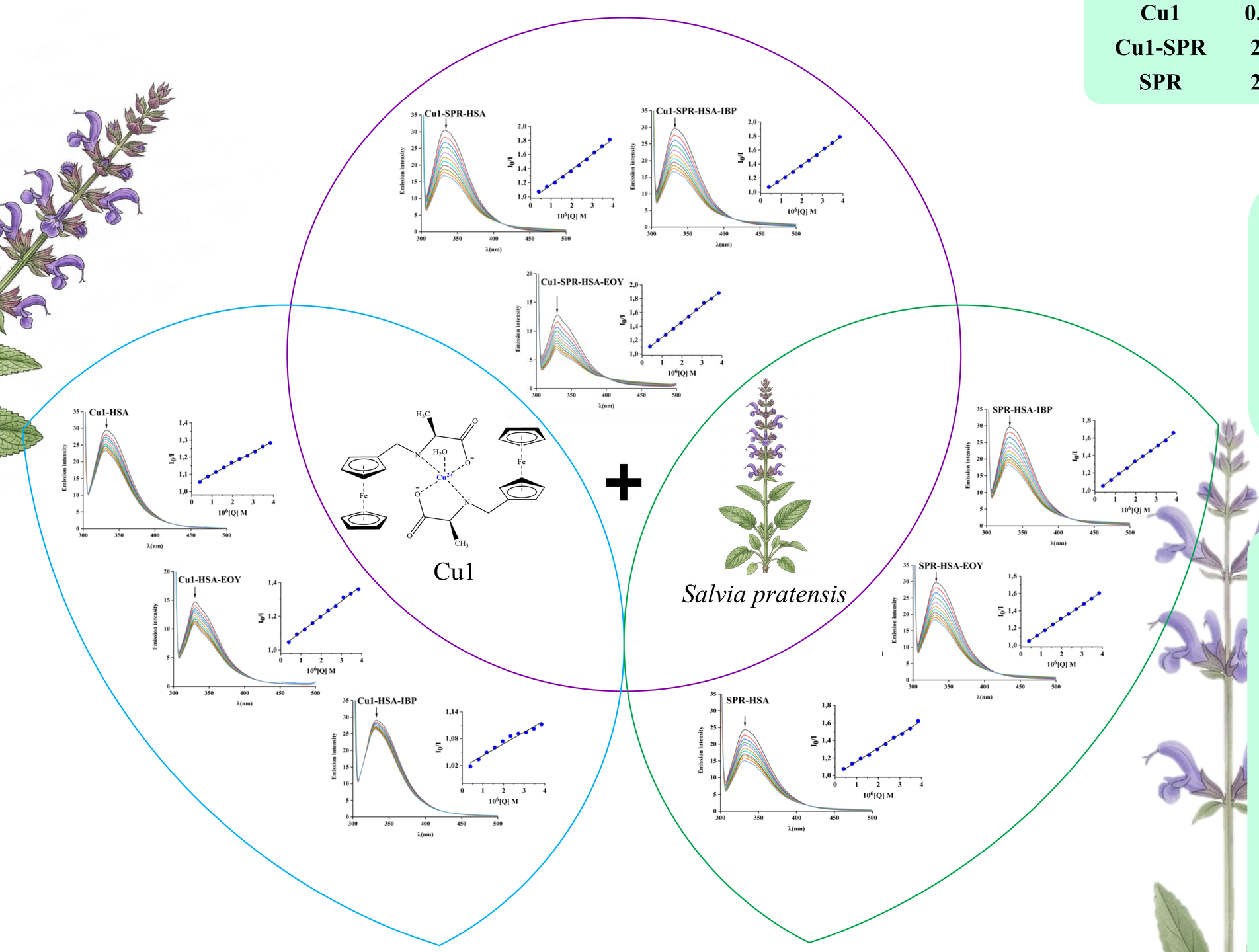
Synergy between the tested complex Cu1 and the aqueous root extract of *Salvia pratensis* (SPR) in interaction with human serum albumin (HSA) was studied by fluorescence spectroscopy. The excitation wavelength was set at 295 nm to selectively excite tryptophan residues, while fluorescence spectra were recorded in the range of 300-500 nm. The examined complex was added to the HSA solution (2 μM) in increasing concentrations (0-4 μM), and fluorescence spectra were recorded after each addition. Fluorescence titration was performed for three systems: (i) the Cu1 complex alone (0-4 μM); (ii) the Cu1 complex combined with the SPR extract (0-4 μM Cu1, 0–20 μg/L SPR); and (iii) the SPR extract alone. Additionally, binding affinity experiments were performed in the presence of site marker molecules (ibuprofen and eosin Y) to identify potential binding sites and mechanisms. The Stern-Volmer constant (K_{SV}) values were calculated from the linear relationship between I_0/I and [Q] using the Stern-Volmer equation, serving as a measure of binding affinity.

OBJECTIVE

This study investigates the synergistic interaction between a novel ferrocene-based copper(II) complex and an aqueous extract of *Salvia pratensis* roots in binding to HSA, aiming to elucidate the molecular mechanisms and identify binding sites that may enhance pharmacological activity.

RESULTS

Combination	HSA 10^5 K_{SV} [M^{-1}]	HSA-eosin Y 10^5 K_{SV} [M^{-1}]	HSA-ibuprofen 10^5 K_{SV} [M^{-1}]
Cu1	0.65 ± 0.1	0.26 ± 0.1	0.92 ± 0.1
Cu1-SPR	2.2 ± 0.1	2.1 ± 0.1	2.3 ± 0.1
SPR	2.5 ± 0.1	1.7 ± 0.1	1.6 ± 0.1



Graphical representation of Stern-Volmer plots used for the calculation of K_{SV} constants for Cu1, SPR, and Cu1–SPR upon interaction with HSA, HSA–eosin Y, and HSA–ibuprofen

KEYWORDS

- **Copper(II) complex**
- **Ferrocene-based ligand**
- **Human serum albumin (HSA)**
- **Fluorescence spectroscopy**
- **Stern-Volmer equation**
- **Synergistic effect**
- ***Salvia pratensis***

CONCLUSION

- **SPR extract significantly enhances Cu1 binding to HSA, indicating a pronounced synergistic effect.**
- **Both Cu1 and the Cu1-SPR system preferentially bind to Site II (subdomain IIIA) of HSA.**
- **Combining metal-based therapeutics with natural extracts is a promising strategy for modulating pharmacokinetic properties, warranting further investigation into the antimicrobial and anticancer potential of the Cu1-SPR system.**

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ACKNOWLEDGMENT

This research is funded by the Ministry of Education and Ministry of Science, Technological Development and Innovation, Republic of Serbia, Grants: No. 451-03-33/2026-03/200378 and 451-03-136/2025-03.