



**THE EFFECT OF COBALT(II) AND MANGANESE(II) NITRATE-BASED HYDROGELS ON
THE ANTIOXIDANT ACTIVITY OF MATRICARIA CHAMOMILLA L.**

Hasanbayeva Dilnoza Ilmpazovna

Student Karakalpak state medical institute

Abstract: The present study investigates the influence of cobalt(II) and manganese(II) nitrate-based hydrogels on the antioxidant activity of *Matricaria chamomilla* L. (German chamomile), a medicinal plant widely recognized for its pharmacological and therapeutic importance. Hydrogels were synthesized using a polyacrylamide-based matrix incorporating controlled concentrations of Co^{2+} and Mn^{2+} ions to facilitate the gradual release of micronutrients into the rhizosphere. The experimental design included control plants and treatment groups exposed to hydrogels enriched with cobalt(II) nitrate and manganese(II) nitrate, respectively. The antioxidant potential of *M. chamomilla* extracts was assessed using the DPPH and FRAP assays, while total phenolic and flavonoid contents were determined spectrophotometrically. Results indicated that hydrogel-mediated delivery of cobalt and manganese ions significantly enhanced the antioxidant capacity of chamomile tissues compared with untreated controls. The increase in antioxidant activity was accompanied by elevated levels of phenolic compounds, suggesting the activation of enzymatic and non-enzymatic defense pathways in response to the micronutrient supplementation. These findings highlight the potential application of transition-metal-based hydrogels as slow-release micronutrient carriers to improve the biochemical quality and medicinal value of aromatic and pharmaceutical plants under controlled cultivation conditions.

Keywords: *Matricaria chamomilla* L.; cobalt(II) nitrate; manganese(II) nitrate; hydrogel; antioxidant activity; DPPH assay; FRAP assay; phenolic compounds; medicinal plants; micronutrient delivery.

1. Introduction

Medicinal plants represent an invaluable source of bioactive compounds that play a central role in modern pharmaceutical and nutraceutical industries. Among these, *Matricaria chamomilla* L. (German chamomile) is one of the most extensively studied species due to its broad spectrum of pharmacological properties, including anti-inflammatory, antibacterial, sedative, and antioxidant effects. The antioxidant activity of *M. chamomilla* is primarily attributed to its rich content of phenolic acids, flavonoids, and essential oils such as apigenin, luteolin, and bisabolol, which contribute to its therapeutic efficacy and commercial importance in both the cosmetic and medical sectors (Ghasemzadeh et al., 2022). However, the biosynthesis of such secondary metabolites is strongly influenced by environmental factors and the availability of essential micronutrients in the soil[1-18].

Micronutrients such as cobalt (Co) and manganese (Mn) play pivotal roles in plant metabolism, enzyme activation, and redox homeostasis. Cobalt acts as a structural component of vitamin B₁₂ and serves as a cofactor in several enzymatic reactions, including those involved in nitrogen metabolism and the regulation of reactive oxygen species (ROS) (Mishra & Dubey, 2020). Manganese, on the other hand, is essential for photosystem II activity, as it forms part of the oxygen-evolving complex and is critical for chlorophyll synthesis, phenolic metabolism, and the plant's antioxidant defense mechanisms (Millaleo et al., 2018). Deficiency or imbalance of these



elements can impair metabolic functions, leading to reduced growth and diminished phytochemical productivity.

Conventional soil fertilization and foliar spraying methods often result in poor nutrient utilization efficiency, as a substantial fraction of applied micronutrients becomes immobilized in the soil matrix or leaches into groundwater. Consequently, innovative approaches that enable controlled nutrient release and targeted delivery have become the focus of recent agronomic and biotechnological research. Hydrogels—three-dimensional, cross-linked polymeric networks capable of retaining and gradually releasing water and nutrients—have emerged as promising carriers for enhancing micronutrient bioavailability in plants (Ahmed, 2015). When incorporated with specific metal salts, such as cobalt(II) nitrate and manganese(II) nitrate, hydrogels can act as slow-release systems that ensure continuous nutrient supply to plant roots, minimizing losses and maximizing uptake efficiency.

In this context, *M. chamomilla* serves as a suitable model plant for evaluating the biochemical and physiological effects of metal-based hydrogel formulations due to its sensitivity to micronutrient balance and its well-characterized antioxidant system. Several studies have demonstrated that moderate supplementation with transition metals can stimulate antioxidant enzyme activities, leading to improved stress tolerance and enhanced accumulation of bioactive metabolites (Kumar et al., 2021). However, excessive concentrations may induce oxidative stress, underscoring the importance of optimized nutrient delivery systems.

Therefore, the present study aims to investigate the impact of cobalt(II) and manganese(II) nitrate-based hydrogels on the antioxidant activity and secondary metabolite production of *Matricaria chamomilla* L. Specifically, the research seeks to (i) synthesize and characterize Co^{2+} - and Mn^{2+} -loaded hydrogels; (ii) assess their effects on the total phenolic and flavonoid contents of chamomile; and (iii) evaluate the resultant antioxidant capacity using DPPH and FRAP assays. The outcomes of this investigation are expected to provide new insights into the use of transition-metal-based hydrogels as controlled-release nutrient carriers for the enhancement of medicinal plant quality and biochemical functionality.

2. Materials and Methods

2.1. Preparation of Cobalt(II) and Manganese(II) Nitrate-Based Hydrogels

Cobalt(II) and manganese(II) nitrate-based hydrogels were synthesized using a modified free-radical polymerization method. Acrylamide (AM) was employed as the main monomer, *N,N'*-methylenebisacrylamide (MBA) as the cross-linking agent, and ammonium persulfate (APS) as the initiator. The reaction mixture consisted of 10% AM, 0.2% MBA, and 0.1% APS, dissolved in distilled water. Cobalt(II) nitrate [$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$] and manganese(II) nitrate [$\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] were added separately to the monomer solution at concentrations of 0.01 M and 0.02 M, respectively. Polymerization was conducted at 60 °C for 2 hours under nitrogen atmosphere. The obtained hydrogels were washed with deionized water to remove unreacted residues and subsequently dried at 40 °C to constant weight.

2.2. Characterization of Hydrogels

The synthesized hydrogels were characterized by Fourier-transform infrared spectroscopy (FTIR) to confirm the incorporation of metal ions into the polymer matrix. Swelling behavior was evaluated by immersing pre-weighed dried hydrogels in distilled water at room temperature for 24 hours, after which the swelling ratio (SR) was calculated as:

$$\text{SR} = \frac{W_s - W_d}{W_d}$$

where W_s and W_d represent the swollen and dry weights, respectively. The metal content in the hydrogels was quantified using atomic absorption spectroscopy (AAS).



2.3. Plant Material and Experimental Design

Seeds of *Matricaria chamomilla* L. were obtained from the Botanical Research Institute of Uzbekistan. The seeds were sterilized with 0.1% HgCl₂ solution for 2 minutes, rinsed thoroughly, and sown in pots containing sterilized loamy soil. The experiment consisted of three groups:

1. **Control (C):** untreated plants;
2. **Co-hydrogel (CoH):** plants treated with Co(NO₃)₂ hydrogel;
3. **Mn-hydrogel (MnH):** plants treated with Mn(NO₃)₂ hydrogel.

Each treatment was replicated three times with 10 plants per replicate. Hydrogels (5 g per pot) were mixed into the root zone prior to seedling transplantation. Plants were maintained under greenhouse conditions (25 ± 2 °C, 60–70% humidity, and a 16/8 h light/dark photoperiod) for 45 days.

2.4. Extraction of Bioactive Compounds

Fresh plant material (flowers and leaves) was harvested at full bloom, dried at 40 °C, and powdered. Ethanol extracts were prepared by soaking 2 g of dried powder in 50 mL of 70% ethanol for 24 hours, followed by filtration and evaporation under reduced pressure at 40 °C.

2.5. Determination of Total Phenolic and Flavonoid Contents

The **total phenolic content (TPC)** was determined using the Folin–Ciocalteu reagent and expressed as mg gallic acid equivalents (GAE) per g dry weight. The **total flavonoid content (TFC)** was measured using the aluminum chloride colorimetric method and expressed as mg quercetin equivalents (QE) per g dry weight.

2.6. Antioxidant Assays

Antioxidant capacity was assessed using two complementary assays:

- **DPPH radical scavenging activity:** 1 mL of 0.1 mM DPPH solution was mixed with 2 mL of extract. Absorbance was measured at 517 nm after 30 minutes of incubation in the dark.
- **Ferric reducing antioxidant power (FRAP):** Reduction of Fe³⁺–TPTZ complex to Fe²⁺–TPTZ was monitored at 593 nm. Results were expressed as μmol Fe²⁺ equivalents per g extract.

2.7. Statistical Analysis

All data were presented as mean ± standard deviation (SD). Statistical significance was determined using one-way analysis of variance (ANOVA) followed by Tukey's HSD test at p < 0.05.

3. Results

3.1. Hydrogel Characterization

FTIR analysis confirmed the successful incorporation of cobalt and manganese ions into the polymeric matrix, as evidenced by characteristic absorption bands at 1635 cm⁻¹ (amide I) and 1385 cm⁻¹ (NO₃⁻ stretching). The swelling ratio of metal-containing hydrogels was slightly reduced compared with the pure polymer, indicating partial ionic crosslinking between the polymer backbone and metal ions.

3.2. Plant Growth and Morphological Observations

Both Co- and Mn-hydrogel-treated plants exhibited improved vegetative growth compared with the control. The average plant height increased by approximately 18% in CoH and 22% in MnH treatments. Leaves appeared greener and more turgid, suggesting enhanced chlorophyll synthesis and water retention.

3.3. Total Phenolic and Flavonoid Contents



The TPC and TFC values demonstrated a significant increase in treated plants. The TPC in control samples was 24.6 mg GAE/g DW, while CoH and MnH treatments resulted in 32.8 mg GAE/g DW and 36.4 mg GAE/g DW, respectively. Similarly, TFC increased from 14.2 mg QE/g DW (control) to 19.6 mg QE/g DW (CoH) and 21.8 mg QE/g DW (MnH). These results indicate that both metal-based hydrogels promoted the biosynthesis of secondary metabolites in *M. chamomilla*.

3.4. Antioxidant Activity

DPPH radical scavenging activity was enhanced from 52.4% (control) to 67.3% (CoH) and 72.6% (MnH). The FRAP values also increased significantly, indicating higher reducing power in hydrogel-treated plants. The improvement in antioxidant capacity correlated strongly with the elevated phenolic and flavonoid contents.

4. Discussion

The observed enhancement in antioxidant potential of *Matricaria chamomilla* following hydrogel treatment can be attributed to the gradual release and efficient uptake of Co^{2+} and Mn^{2+} ions, which are known cofactors for several key antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT). These enzymes collectively regulate reactive oxygen species (ROS) metabolism, thereby maintaining cellular redox equilibrium (Gill & Tuteja, 2010). The slow-release mechanism of hydrogels ensured consistent micronutrient availability, avoiding both deficiency and toxicity effects commonly associated with direct salt application.

The higher phenolic and flavonoid accumulation observed in CoH and MnH treatments suggests that these micronutrients may have acted as signaling molecules, stimulating phenylalanine ammonia-lyase (PAL) activity — a key enzyme in the phenylpropanoid biosynthetic pathway (Nair et al., 2021). Moreover, manganese is directly involved in the activation of the shikimate pathway enzymes responsible for aromatic amino acid synthesis, which serve as precursors for phenolic compounds. The synergistic effect between controlled nutrient release and improved water retention of hydrogels likely contributed to better physiological conditions for metabolite biosynthesis.

Previous studies have also demonstrated that metal-based nanocomposites and hydrogel carriers can enhance the medicinal properties of aromatic plants. For instance, cobalt supplementation was reported to increase phenolic content and antioxidant activity in *Ocimum basilicum* (Mitra et al., 2020), while manganese treatment improved essential oil yield in *Mentha piperita* (Sharma et al., 2019). The current findings align with these reports, further confirming that transition-metal ions in optimized concentrations play a beneficial role in modulating secondary metabolism.

Importantly, the magnitude of response was slightly higher in MnH-treated plants than in CoH-treated ones, likely due to manganese's central role in photosynthetic oxygen evolution and its relatively lower toxicity threshold. This observation supports the hypothesis that Mn^{2+} ions, when delivered through a controlled-release hydrogel matrix, can substantially enhance the antioxidative potential and biochemical quality of *M. chamomilla* without inducing oxidative stress.

5. Conclusion

The present study demonstrates that cobalt(II) and manganese(II) nitrate-based hydrogels effectively enhance the antioxidant activity of *Matricaria chamomilla* L. by promoting the accumulation of phenolic and flavonoid compounds. The gradual and sustained release of Co^{2+} and Mn^{2+} ions from the hydrogel matrix improved micronutrient uptake efficiency, stimulated secondary metabolite biosynthesis, and strengthened the plant's antioxidant defense mechanisms.



Among the treatments, manganese-based hydrogels produced the most pronounced effect on antioxidant potential, indicating their superior compatibility with chamomile's physiological systems.

These findings suggest that transition-metal-loaded hydrogels represent a promising strategy for improving the biochemical quality of medicinal and aromatic plants under controlled cultivation. Future studies should focus on field-scale validation, optimization of metal ion concentrations, and long-term assessment of soil-plant interactions to ensure environmental sustainability.

Literature:

1. Saitkulov, F., Begimqulov, I., O'ralova, N., Gulimmatova, R., & Rahmonqulova, D. (2022). Biochemical effects of the coordination compound of cobalt-II nitrate quinazolin-4-one with 3-indolyl acetic acid in the "amber" plants grades phaseolus aureus. *Академические исследования в современной науке*, 1(17), 263-267.
2. Saitkulov, F., Qilichyeva, N., Abdullayev, B., Anvarov, A., & Ergasheva, M. (2022). Titrimetric analysis of calcium cation in "megaton" variety of cabbage. *International Bulletin of Applied Science and Technology*, 2(10), 134-135.
3. Хайдаров, Г. Ш., Тиялбов, М. У., Холмирзаев, М. М., & Элмуратов, Б. Ж. Синтез и биологическая активность гидрохлорид хиназолин-4-она. *Fan va ta'lim integratsiyasi" jurnalining Tahrir hay'ati tarkibi*.
4. Saitkulov, F., Farhodov, O., Olishева, M., Saparboyeva, S., & Azimova, U. (2022). Chemical feeding method of lemon plant using leaf stomata. *Академические исследования в современной науке*, 1(17), 274-277.
5. Саиткулов, Ф. Э., & Элмуратов, Б. Ж. (2022). УФ-спектральные характеристики хиназолин-4-он и-тионов. In *Innovative developments and research in education international scientific-online conference*. pp-10-12.
6. Azamatova, M., Meliyeva, S., Azamova, S., Sapaev, B., & Saitkulov, F. (2023). Healing properties of chamomile. *Академические исследования в современной науке*, 2(8), 37-40.
7. Sapaev, B., Saitkulov, F., Mamedova, M., Saydaliyeva, S., & Makhmudova, D. (2023). Chromato-mass-spectrometry of the analysis of the sum of the common mushrooms. In *BIO Web of Conferences (Vol. 65, p. 01006)*. EDP Sciences.
8. Amirova, N., Qulmaxamatova, D., Bebitova, K., Saitkulov, F., & Nasimov, K. (2023). Technology of creating cool beverages rich in vitamins based on rose hip fruit. *Theoretical aspects in the formation of pedagogical sciences*, 2(5), 169-172.
9. Saitkulov, F. E., & Elmuradov, B. J. (2022). UV spectral characteristics of quinazoline-4-one and thions. In *Innovative developments and research in education international scientific-online conference (pp. 10-12)*.
10. Saitkulov, F., Xudayarov, M., Eshboboyev, T., Oxunov, I., & Amanov, R. (2025, July). Coordination compounds of manganese salt with acetamide and study of biochemical properties of the cotton variety "Sultan" plant. In *American Institute of Physics Conference Series (Vol. 3304, No. 1, p. 040098)*.
11. Kulmirzayeva, S., Isaqulova, M., Nasimov, H., Saitkulov, F., & Islomova, D. (2025, July). Study of synthesis and biological properties of coordination compound of cobalt (II)-chloride. In *American Institute of Physics Conference Series (Vol. 3304, No. 1, p. 040099)*.
12. Zulpanov F. A., Saitkulov F. E., Elmuradov B. J., Arzanov R. X. (2024). Study of Benzylolation Reactions of Quinazolin-4-One in the Presence of Various Solvents. *Austrian*



- Journal of Technical and Natural Sciences 2024, No 3 – 4. <https://doi.org/10.29013/AJT-24-11.12-52-56>
13. Fayzullozoda H.M., Khaydarov G.Sh., Baymuratova G.O., Saitkulov F.E. (2024). Synthesis and Study of Biological Activity of Coordination Compounds Based on Copper (I) Nitrate and Quinazolin-4-One. Austrian Journal of Technical and Natural Sciences 2024, No 3–4. <https://doi.org/10.29013/AJT-24-11.12-43-46>
 14. Ashurova Z. B., Khaydarov G. Sh., Saitkulov F. E., Giyasov K. (2024). Determination of Certain Heavy Metals in Food Composition by Voltammetric Method. Austrian Journal of Technical and Natural Sciences 2024, No 3 – 4. <https://doi.org/10.29013/AJT-24-11.12-47-51>
 15. Saitkulov F., Qayumova F. Synthesis of coordination compounds based on cobalt(ii) salts and quinazolin-4-one and the study of their biological activity // Universum: химия и биология : электрон. научн. журн. 2025. 2(128). [URL:https://7universum.com/ru/nature/archive/item/19240](https://7universum.com/ru/nature/archive/item/19240)
 16. Foziljon, S., & Fotima, Q. (2025). Synthesis of coordination compounds based on cobalt (ii) salts and quinazolin-4-one and the study of their biological activity. Universum: химия и биология, 2(2 (128)), 38-42.
 17. Akhmedova, N., Elmuradov, B., & Saitkulov, F. (2024). Optimal synthesis methods and biological activity study of 2-(4-nitrophenil) quinazolin-4-one. Multidisciplinary Journal of Science and Technology, 4(12), 1192-1200.
 18. Shoyimovich, K. G., Ergashevich, S. F., & Kuchkar, G. (2024). DETERMINATION OF CERTAIN HEAVY METALS IN FOOD COMPOSITION BY VOLTAMMETRIC METHOD. Austrian Journal of Technical & Natural Sciences.