Journal of Chemistry of Goods and Traditional Medicine

J Chem Good Trad Med, Volume 5, Issue 2, 2026 Chemistry of Goods

# PREPARATION OF BIO-LIGANDS FROM POMEGRANATE (PUNICA GRANATUM L.) PEEL AND THEIR APPLICATION IN THE SYNTHESIS OF CU AND ZN COMPLEX MICRONUTRIENT FERTILIZERS

***Ibrohimjon R. Asqarov***
Andijan State University Professor of the Department of Chemistry, Doctor of Chemistry, Honored Inventor of Uzbekistan, Chairman of the TABOBAT Academy of Uzbekistan
https://orcid.org/0000-0003-1625-0330

***Jasurbek M. Jalolov***
Fergana State Technical University, Doctoral student in the Department of Chemical Engineering.
https://orcid.org/0009-0009-2926-2219

***Farhod S. Abdugapparov***
Andijan State University Associate Professor of Chemistry, Doctor of Philosophy in Chemistry (PhD)
https://orcid.org/0000-0002-4213-8396
E-mail: farhodabdugaffarov@mail.ru

DOI: https://doi.org/10.55475/jcgtm/vol5.iss2.2026.643

**Keywords:** pomegranate peel, bio-ligand, Cu-biochelate, micronutrient fertilizer, FTIR spectroscopic analysis, polyphenols, coordination complex, eco-friendly fertilizer.

**Annotation**

In this study, the preparation of bio-ligands from pomegranate (Punica granatum L.) peel and the synthesis of a Cu-based biochelate in their presence were investigated. The results of FTIR spectroscopic analysis confirmed that phenolic and carboxyl functional groups formed coordination bonds with $Cu^{2+}$ ions. The appearance of new Cu–O bonds indicated the formation of a coordination complex. The obtained biocomplex was evaluated as an environmentally safe micronutrient fertilizer.

**How to Cite:**

Jalolov, J., Asqarov, I., & Abdugapparov, F. PREPARATION OF BIO-LIGANDS FROM POMEGRANATE (PUNICA GRANATUM L.) PEEL AND THEIR APPLICATION IN THE SYNTHESIS OF Cu AND Zn COMPLEX MICRONUTRIENT FERTILIZERS. Journal of Chemistry of Goods and Traditional Medicine, 5(2). https://doi.org/10.55475/jcgtm/vol5.iss2.2026.643

**Introduction.** It is well known that the supply of micronutrients plays a crucial role in achieving high crop productivity and improving product quality in agriculture. Micronutrients are also essential for enhancing plant growth and increasing resistance to adverse environmental conditions and various plant diseases. Among these elements, copper (Cu) and zinc (Zn) play an important role in plant physiology, as they participate in enzymatic processes, photosynthesis, oxidation–reduction reactions, the synthesis of proteins and nucleic acids, and the maintenance of the antioxidant defense system in plants [1].

In particular, zinc (Zn), as a micronutrient, serves as a cofactor for more than 300 enzymes and is involved in DNA synthesis as well as in the metabolism of various plant hormones. Deficiency of this element leads to chlorosis in plant leaves and results in reduced growth rates [3].

Copper (Cu) present in plant tissues is a structural component of plastocyanin and oxidoreductase enzymes. Plastocyanin plays a key role in photosynthesis by facilitating electron transport and thereby contributing to energy production. Oxidoreductase enzymes regulate redox processes, ensuring proper metabolic activity and antioxidant defense in plants. This enhances plant tolerance to environmental stresses. However, excessive amounts of copper may exert toxic effects on plants. Therefore, not only the quantity of these micronutrients but also the chemical form in which they are applied is of great importance.

Currently, in agricultural practice, copper (Cu) and zinc (Zn) are mainly applied in the form of sulfate salts ($CuSO_4$ and $ZnSO_4$). However, under alkaline and carbonate soil conditions, these compounds tend to convert into poorly soluble forms, which significantly reduces their availability for plant uptake [4]. In addition, the application of these salts at high concentrations may cause phytotoxic effects on plants. Therefore, in recent years, there has been increasing interest worldwide in organic and chelated micronutrient fertilizers that are more easily absorbed by plants and exhibit lower toxicity.

For example, it has been established that Zn–amino acid chelates exhibit higher efficiency than $ZnSO_4$ in improving grain quality and mineral composition in wheat [5]. Furthermore, organic chelates have been reported to demonstrate superior performance in carbonate soils compared with conventional mineral salts [6].

From this perspective, considerable attention has been devoted by many researchers to the development of new types of micronutrient fertilizers based on bio-ligands. Bio-ligands are natural organic substances capable of forming coordination complexes with metal ions. Such compounds include biologically active substances such as amino acids, organic acids, humic substances, flavonoids, and tannins [7,8].

Bio-ligands stabilize incorporated metal ions, facilitate their efficient uptake by plants, and reduce their potential toxic effects.

In studies conducted by various authors, biochelates have been highly evaluated as an environmentally favorable alternative to the synthetic chelates currently used in agricultural practice [9].

Therefore, the extraction of bio-ligands from natural raw materials and the development of micronutrient fertilizers based on them represent a promising research direction.

Among such natural sources, pomegranate peel (Punica granatum L.) is of particular interest. Pomegranate peel constitutes a significant portion of the fruit mass and is usually discarded as a waste product in the food industry. However, it represents a rich source of biologically active compounds.

Scientific studies have revealed that pomegranate peel contains punicalagin, ellagic acid, gallic acid, and various flavonoids [10,11].

These compounds contain numerous hydroxyl (–OH) and carboxyl (–COOH) functional groups capable of forming coordination complexes with metal ions. In this regard, pomegranate peel can be considered an inexpensive and biodegradable raw material suitable for use as a bio-ligand.

The technologies used for the extraction of polyphenols from pomegranate peel directly influence their effectiveness as bio-ligands. Experimental studies have demonstrated that polyphenols can be efficiently obtained using extraction methods such as hydroalcoholic (water–ethanol) extraction, ultrasound-assisted extraction, and fermentation techniques [12,13].

Variations in extraction conditions lead to changes in both the composition and concentration of phenolic compounds, which in turn affect the subsequent complex formation process. Therefore, controlling the chemical composition of bio-ligands is considered one of the key tasks.

Polyphenols possess the ability to form complexes with metal ions, and this process occurs through the dissociation of phenolic groups followed by their coordination with metal ion.

Various authors have emphasized that polyphenols possess a pronounced ability to bind metal ions [14].

Furthermore, researchers have highlighted that carboxyl and hydroxyl groups play a key role in metal binding [15].

It has been established that copper (Cu) is capable of forming stable complexes with organic compounds, which has been confirmed by experimental studies [16].

These findings theoretically substantiate that the polyphenols present in pomegranate peel are capable of forming stable complexes with $Cu^{2+}$ and $Zn^{2+}$ ions.

The interaction of pomegranate peel with metal ions has been confirmed by a number of experimental studies [17,18,19,20].

These studies demonstrate that pomegranate peel extract is capable of chemically interacting with metal ions and forming stable complexes.

Numerous studies on chelate-structured micronutrient fertilizers based on organic compounds have confirmed their high agrobiological efficiency. In particular, Zn–amino acid chelates have demonstrated high effectiveness in wheat cultivation. Organic fertilizers based on Cu have also shown positive effects on photosynthesis and crop productivity. This indicates that incorporating Cu and Zn ions into an organic matrix plays an important role in enhancing their efficiency [21].

Bio-ligands derived from pomegranate peel possess additional advantages, as they not only participate in complex formation but also exhibit antioxidant properties. This suggests that Cu and Zn complexes based on pomegranate peel may enhance plant resistance to environmental stress factors [22,23].

At the same time, an analysis of the available literature indicates the presence of a significant scientific gap. Although the polyphenols of pomegranate peel and their interactions with metal ions have been well studied, studies focused on the development of Cu and Zn complex micronutrient fertilizers based on these compounds and the evaluation of their agrobiological efficiency are scarce. This highlights the scientific novelty of the present research direction.

**Results and Discussion.**

Infrared (IR) spectroscopy was employed to determine the structure of the Cu-biochelate synthesized based on pomegranate peel extract and to identify the functional groups involved in the complex formation process. In this analysis, the spectra of the bio-ligand (pomegranate peel extract) and the resulting Cu complex were comparatively examined. (Figure 1)

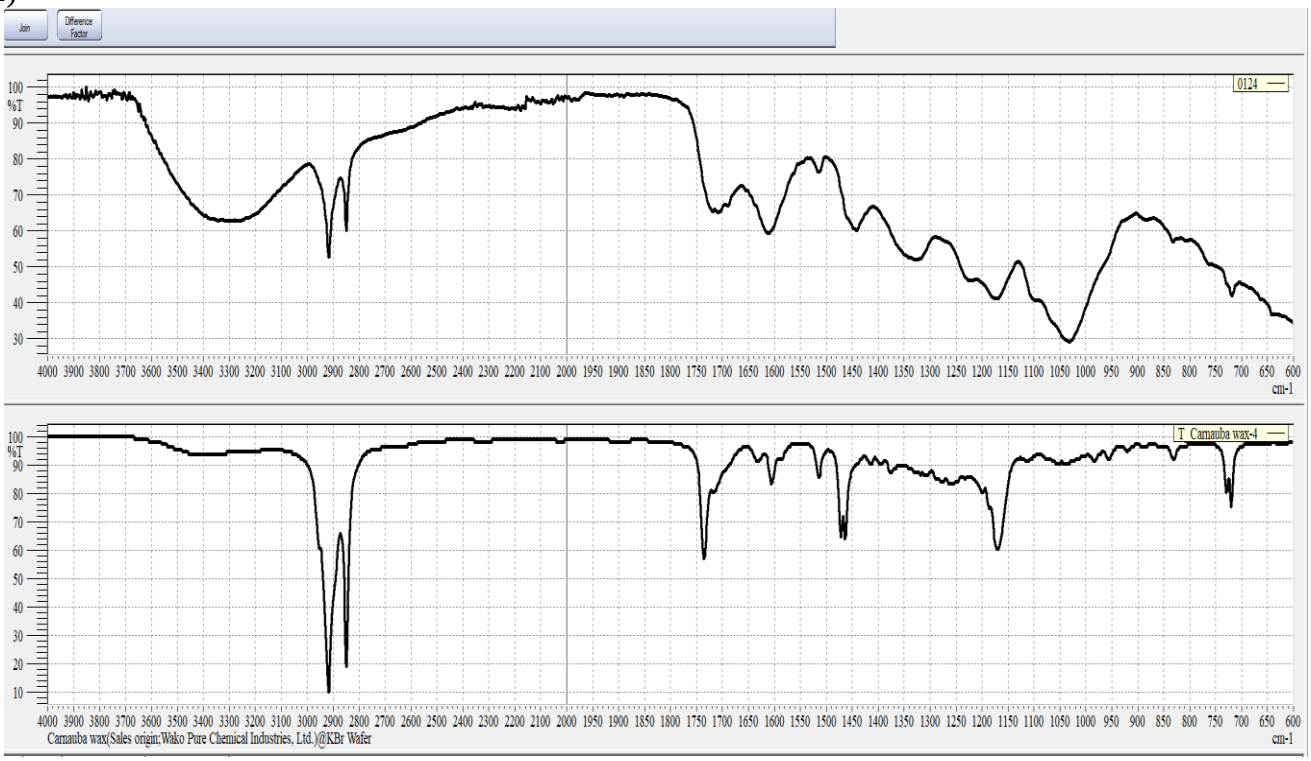



Figure 1. FTIR spectra of the bio-ligand and the Cu complex.

In the spectrum of the pomegranate peel extract, broad and intense absorption bands were observed in the range of 3200–3600 $cm^{-1}$, which are characteristic of phenolic and hydroxyl (–OH) groups. After complex formation with $Cu^{2+}$ ions, a decrease in the intensity of these bands and a shift toward lower frequencies were observed. This phenomenon indicates the deprotonation of hydroxyl groups and the formation of coordination bonds with metal ions.

Absorptions corresponding to C–H stretching vibrations in the region of 2920–2850 $cm^{-1}$ were observed in both spectra, and no significant changes were detected in this region. This suggests that the aliphatic part of the bio-ligand remained largely unchanged during the complex formation process.

The absorption bands in the range of 1700–1600 $cm^{-1}$ correspond to the vibrations of carbonyl (C=O) groups and aromatic rings, which were clearly observed in the spectrum of the bio-ligand. After the formation of the complex, a shift of this band toward lower frequencies and a change in absorption intensity were detected. This indicates that carboxyl and aromatic groups participate in the formation of coordination bonds with $Cu^{2+}$ ions.

Significant changes were also observed in the absorption bands within the 1600–1400 $cm^{-1}$ region. This range is typically associated with the asymmetric and symmetric stretching vibrations of carboxylate ($COO^-$) groups. The observed changes in these absorption bands confirm that –COOH groups interact with the metal ions and are converted into the $COO^-$ form during complex formation.

Changes in the intensity of absorption bands corresponding to C–O and C–O–C groups were also observed in the 1300–1000 $cm^{-1}$ region. This indicates that phenolic and ether groups also participate in the complex formation process.

As the most significant result, new absorption bands appeared in the 600–400 $cm^{-1}$ region in the spectrum of the Cu-biochelate. These absorptions are characteristic of Cu–O bonds and serve as direct evidence of the formation of coordination bonds between the metal and the bio-ligand.

Thus, the results of the FTIR spectroscopic analysis indicate that the phenolic and carboxyl groups present in the pomegranate peel extract form a stable biocomplex with $Cu^{2+}$ ions. The shifts in spectral absorption bands and the appearance of new signals corresponding to Cu–O bonds provide clear confirmation of the complex formation process.

**Experimental Section**

**Reagents and Equipment.**

Reagents: 1% NaOH solution, 3% $H_2O_2$ solution, pomegranate peel, distilled water.

Equipment: Laboratory drying oven (Model: ShS 80-01 SPU), laboratory mill (Model: KIKA M20 WERKE), magnetic stirrer (Model: MC-7-X-550-PRO).

***Preparation of Pomegranate Peel.***

In this study, pomegranate (Punica granatum L.) peel was used as a source of bio-ligand. The raw material was obtained from a local market, thoroughly washed several times with distilled water, and cleaned of external impurities. The washed peels were then placed in a laboratory drying oven (Model: ShS 80-01 SPU) and dried at a temperature of 60–70 °C for 6–8 hours.

The dried material was ground using a laboratory mill (Model: KIKA M20 WERKE) to obtain particles with a size of 0.5–1 mm. The resulting material was stored in airtight containers for subsequent experiments**.**

***Alkaline Extraction of the Bio-Ligand.***

An alkaline extraction method was employed to activate the polyphenolic compounds present in pomegranate peel. For this purpose, the ground biomass was mixed with a 1% NaOH solution at a solid-to-liquid ratio of 1:10 (w/v).

The resulting mixture was stirred using a magnetic stirrer (Model: MC-7-X-550-PRO) at a temperature of 55–60 °C for 2 hours at a stirring speed of 300 rpm. During this process, the activity of organic compounds containing phenolic, flavonoid, and other functional groups present in the pomegranate peel was enhanced.

After completion of the extraction process, the mixture was filtered, and the solid biomass remaining on the filter was washed several times with distilled water to remove excess alkali residues.

***Activation of Biomass Using an Oxidizing Agent.***

To enhance the reactivity of functional groups, the biomass was treated with hydrogen peroxide ($H_2O_2$). For this purpose, the biomass remaining on the filter was mixed with a 3% $H_2O_2$ solution at a ratio of 1:10.

The mixture was stirred on a magnetic stirrer at a temperature of 50–55 °C for 1 hour at a stirring speed of 300 rpm. As a result of this oxidative treatment, the phenolic and carboxyl functional groups present in the biomass were activated, thereby increasing their ability to form complexes with metal ions.

After completion of the process, the mixture was filtered and the solid phase was washed several times with distilled water. The material was then dried at 60 °C for 4–5 hours. As a result, a bio-ligand capable of forming coordination bonds with metal ions was obtained.

***Synthesis of Cu-Biochelate.***

The synthesis of the Cu-based biochelate was carried out by reacting the obtained bio-ligand with a copper sulfate solution. For this purpose, the bio-ligand was mixed with a 1% $CuSO_4$ solution at a ratio of 1:20.

The resulting mixture was stirred on a magnetic stirrer at a temperature of 50–60 °C for 1–1.5 hours at a stirring speed of 300 rpm. During this process, the hydroxyl (–OH) and carboxyl (–COOH) functional groups present in the bio-ligand formed coordination complexes with $Cu^{2+}$ ions.

After completion of the reaction, the mixture was filtered and the solid phase was separated as Cu-biochelate. The obtained product was washed with distilled water and dried at 60 °C. The product yield was 43%.

***Infrared Spectroscopic Analysis.***

Fourier-transform infrared spectroscopy (FTIR) was used to analyze the structure of the obtained bio-ligand and Cu-biochelate. The spectra were recorded in the range of 4000–400 $cm^{-1}$. The spectral analysis revealed that coordination bonds with the metal were formed involving functional groups such as –OH, –COOH, and C–O, and the formation of Cu–O bonds was also confirmed.

**Conclusion.** The results of this study demonstrate that the polyphenolic compounds present in pomegranate (Punica granatum L.) peel are capable of forming stable biochelates with $Cu^{2+}$ ions through coordination bonding. FTIR spectroscopic analysis confirmed the active involvement of hydroxyl and carboxyl functional groups in the complex formation process and verified the formation of Cu–O bonds. The obtained biocomplex exhibits high bioavailability, low toxicity, and environmentally safe characteristics, making it a promising candidate for application as an efficient micronutrient fertilizer in modern agrochemistry.

## References

1. Ahmed, N.; Zhang, L.; Li, J. Micronutrients and their effects on horticultural crop quality, productivity and sustainability. Scientia Horticulturae, 2024, 318, 112600.
2. Neocleous, D.; Koukounaras, A.; Siomos, A. Impact of chelated or inorganic zinc applications in hydroponics. Agronomy, 2020, 10(6), 881.
3. Kumar, V.; Pandita, S.; Sidhu, G.P.S. Copper bioavailability, uptake, toxicity and tolerance in plants. Chemosphere, 2021, 262, 127810.
4. Rahman, N.; Arshad, M.; Afzal, M. Bioavailability, speciation, and crop responses to copper, zinc, and boron fertilization. Agronomy, 2022, 12(8), 1837.
5. Ghasemi, S.; Khoshgoftarmanesh, A.H.; Afyuni, M. Effectiveness of zinc-amino acid chelates in wheat. European Journal of Agronomy, 2013, 45, 68–74.
6. Souri, M.K. Beneficial effects of foliar application of organic chelate fertilizers. Advances in Horticultural Science, 2018, 32, 321–330.
7. López-Rayo, S.; Hernández, D.; Lucena, J.J. EDDS ligand as a soil solubilizer of Fe, Mn, Zn and Cu. Environmental Research, 2023, 216, 114613.
8. Yin, F.; Li, J.; Wang, Y. Biodegradable chelating agents for enhancing phytoremediation. Ecotoxicology and Environmental Safety, 2024, 281, 116113.

9. Leporino, M.; De Mastro, F.; Santamaria, P. Biochelates as eco-friendly alternatives to synthetic chelates. Scientia Horticulturae, 2025, 330, 112901.
10. Mo, Y.; Zhao, Y.; Wang, L. Pomegranate peel as a source of bioactive compounds. Frontiers in Nutrition, 2022, 9, 887113.
11. Singh, J.; Kaur, H.P.; Verma, A. Pomegranate peel phytochemistry and applications. ACS Omega, 2023, 8, 35452–35469.
12. Huang, Z.; Foo, S.C.; Choo, W.S. Extraction of polyphenols from pomegranate peel. Food & Function, 2025, 16, 210–225.
13. Buenrostro-Figueroa, J.J.; et al. Extraction of polyphenols from pomegranate peel by fermentation. Fermentation, 2023, 9(6), 530.
14. Schmidt, M.A.; Martinez, C.E. Metal mobilization by natural polyphenols. Chemosphere, 2013, 93, 2255–2262.
15. Pan, J.; Li, X.; Zhang, Y. Adsorption mechanism of copper and zinc by biosorbents. Science of the Total Environment, 2022, 805, 150312.
16. Steinberg, S.M.; et al. Copper complexation by dissolved organic matter. Environments, 2018, 5(11), 125.
17. Ben-Ali, S.; Jaouali, I.; Ouederni, A. Adsorption of copper using pomegranate peel. Journal of Cleaner Production, 2017, 142, 3893–3900.
18. El-Ashtoukhy, E.S.Z.; Amin, N.K.; Abdelwahab, O. Removal of copper ions using pomegranate peel. Desalination, 2008, 223, 162–173.
19. Mohammadi, A.; et al. Green synthesis of ZnO nanoparticles using pomegranate peel. Scientific Reports, 2025, 15, 20853.
20. Bouricha, M.; et al. Green synthesis of CuO nanoparticles using pomegranate peel extract. Inorganic Chemistry Communications, 2024, 152, 110598.
21. Ma, J.; et al. Copper and iron foliar fertilizers in grape cultivation. Scientia Horticulturae, 2019, 246, 440–447.
22. Saparbekova, A.A.; et al. Phenolic compounds from pomegranate peel. Molecules, 2022, 27, 9827.
23. Shabir, I.; et al. Bioactive potential of punicalagin. Food Research International, 2024, 180, 113785.