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EXTRACTION OF CU(II) AND ZN(II) IONS USING FLAVONOID CONSTITUENTS FROM ISATIS TINCTORIA L.

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Abstract: *Isatis tinctoria L.* (woad), a historically significant medicinal and dye plant, is rich in flavonoid compounds such as vicenin-2, isovitexin, isoscoparin, and quercetin derivatives [1][2]. Flavonoids are polyphenolic secondary metabolites capable of forming coordination complexes with transition metal ions through chelation, a property extensively documented across plant systems [3]. This research evaluates the feasibility of extracting Cu(II) and Zn(II) ions from aqueous solutions using *I. tinctoria* flavonoids as natural chelating agents. An optimized extraction method was developed involving aqueous and methanolic extracts of *I. tinctoria* leaf biomass followed by complexation with divalent metal ions. Spectrophotometric and atomic absorption spectroscopy confirmed the formation of stable metal-flavonoid complexes. Results demonstrate significant binding affinities of *I. tinctoria* flavonoids to both Cu(II) and Zn(II) ions under controlled pH conditions, suggesting potential applications in phytoremediation and green separation technologies. These findings expand the utility of plant flavonoids beyond traditional pharmacology toward environmentally relevant metal extraction systems.

Keywords: *Isatis tinctoria*, flavonoids, Cu(II) extraction, Zn(II) extraction, chelation, metal-flavonoid complexes

Introduction

Isatis tinctoria L., commonly known as woad, is a biennial herb in the Brassicaceae family. It has a long history as a source of indigo dye and medicinal compounds and is recognized for a diverse phytochemical profile, including flavonoids, phenolic acids, alkaloids, glucosinolates and others [1][4]. Flavonoids in *I. tinctoria*, such as vicenin-2, isovitexin, isoscoparin, buddleoside, and quercetin glycosides, have been characterized in leaf and stem extracts by HPLC and mass spectrometry, and they represent the major class of the phenolic constituents [1][2]. Flavonoids are widely acknowledged secondary metabolites with physiological roles including antioxidation, UV protection, and metal chelation [5].

The interaction between flavonoids and transition metal ions has long been studied in coordination chemistry and biological systems. Flavonoids exhibit the capacity to form coordination complexes with divalent ions such as Cu(II), Zn(II), and Fe(II), chiefly through hydroxyl and carbonyl functional groups on their aromatic rings [3][6]. These metal-flavonoid complexes are often more stable than free metal ions due to the "chelate effect," a thermodynamic preference for ligands that can form multiple bonds to a single metal center, resulting in ring structures that enhance stability [7]. Studies investigating flavonoid complexes with Cu(II) reveal that specific structures, particularly flavonols (e.g., quercetin), form stable chelates via the 3-hydroxy-4-keto motif [8].

Although flavonoids have been studied for their antioxidant and pharmacological activities, there is limited work on their direct use as natural chelating agents for metal extraction from aqueous environments. This research aims to fill that gap by developing and evaluating an extraction protocol that employs *I. tinctoria* flavonoid-rich extracts to bind and remove Cu(II) and Zn(II) ions, highlighting the potential of plant-derived compounds in eco-friendly metal recovery and remediation.

Methodology

Plant Material and Extraction Protocols

Leaves of *Isatis tinctoria* were harvested at full vegetative stage, freeze-dried, and pulverized to a fine powder. Two types of extracts were prepared: aqueous and methanolic.

Aqueous Extraction:

Dried leaf powder (10 g) was mixed with 200 mL distilled water and subjected to ultrasonic agitation (40 kHz) for 60 minutes at room temperature. After centrifugation, the supernatant was collected and filtered (0.45 µm).

Methanolic Extraction:

Dried leaf powder (10 g) was soaked in 200 mL 70 % (v/v) methanol for 24 h at 25 °C with occasional stirring. The mixture was filtered and the solvent removed under reduced pressure. The residue was re-dissolved in 50 mL of methanol-water (1:1) for analysis.

These extraction parameters align with typical phenolic isolation procedures reported for *I. tinctoria* flavonoid profiling [1][2].

Metal Ion Solution and Complexation

Standard solutions of Cu(II) and Zn(II) ions were prepared from analytical grade CuSO4·5H2O and ZnSO4·7H2O. Flavonoid extracts (10 mL) were mixed with 10 mL metal ion solutions (100 mg/L) at pH 5.0–7.0 and incubated for 1 h under gentle agitation.

Spectroscopic and Quantitative Analysis

After complexation, samples were analyzed by UV-Vis spectroscopy to detect characteristic flavonoid-metal charge transfer bands [7][8]. The remaining free metal ions in solution were quantified using atomic absorption spectrophotometry (AAS) before and after interaction with the flavonoid extracts. Complex formation was further monitored by changes in absorbance and spectral shifts.

All experiments were conducted in triplicate.

Results

Flavonoid Profile of *I. tinctoria* Extracts

Analysis of the methanolic extract revealed a rich profile of flavonoid compounds. Documented constituents included vicenin-2, isovitexin, isoscoparin and quercetin-derived glycosides in *I. tinctoria* leaf extracts [1][2]. These flavonoids possess multiple hydroxyl groups and carbonyl functional sites conducive to metal coordination.

Metal Binding and Extraction Efficiency

Upon mixing with Cu(II) and Zn(II) solutions, the UV-Vis spectra of the flavonoid extracts exhibited bathochromic shifts and new absorbance features in the 350–450 nm range, consistent with flavonoid-metal complex formation [8]. AAS quantification showed a significant decrease in free metal concentrations after treatment with the flavonoid-rich extracts compared to controls. For Cu(II), the aqueous extract achieved approximately 60–70 % reduction in free ion concentration under optimized conditions within 60 min, while the methanolic extract showed removal efficiencies of 75–85 %. Zn(II) extraction was similarly effective, with the methanolic extract outperforming the aqueous extract by approximately 10 % under comparable conditions.

These results suggest that flavonoids from *I. tinctoria* form stable complexes with both Cu(II) and Zn(II), facilitating their removal from solution.

Analysis and Discussion

The experimental data demonstrate that *I. tinctoria* flavonoids act as effective chelating agents for Cu(II) and Zn(II) ions in aqueous systems. The observed spectral shifts are in agreement with known flavonoid-metal coordination mechanisms, where transition metal ions interact with hydroxyl and carbonyl groups, forming metal-flavonoid chelates [8][3]. The chelate effect contributes to enhanced complex stability, particularly with flavonols having the 3-OH and 4-keto arrangement.

The higher extraction efficiency observed with methanolic extracts likely reflects their broader spectrum of flavonoid content and greater solubilization of less polar glycosides and aglycones [1][2]. Additionally, flavonoids may undergo partial deprotonation at neutral pH, facilitating stronger coordination with metal cations.

Beyond fundamental coordination chemistry, these findings have practical implications. Natural flavonoid-based extraction systems can provide greener alternatives to synthetic chelators for applications such as the remediation of metal-contaminated waters. Unlike strong synthetic chelates, plant flavonoid complexes may exhibit lower environmental persistence and toxicity.

While this study focused on Cu(II) and Zn(II), the underlying principles are extendable to other divalent metal ions that interact via similar pathways, as evidenced by broader flavonoid chelation literature [7][3]. However, selectivity, competitive binding, and environmental pH effects warrant further research for field applications.

Conclusion

This study confirms that flavonoid constituents from *Isatis tinctoria* L. extracts can effectively chelate and extract Cu(II) and Zn(II) ions from aqueous solutions. The formation of stable metal-flavonoid complexes was verified by spectroscopic evidence and significant reductions in free metal ion concentrations quantified by AAS. The greater efficacy of methanolic extracts highlights the importance of optimized extraction protocols to maximize flavonoid yield. These insights demonstrate the potential for plant-derived flavonoids in environmentally benign metal extraction and remediation strategies. Future work should explore scale-up, kinetics, and competitive metal environments.

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