Frontiers in Environmental Research
Print ISSN: 2960-0251
Online ISSN: 2960-026X
DOI: https://doi.org/10.61784/fer3046

OPTIMIZATION OF NORMAL-PHASE CHROMATOGRAPHIC SEPARATION FOR QINGLONGYI AND PREDICTION OF ITS BIOACTIVITIES BASED ON PUBLIC CHEMICAL CONSTITUENT DATABASES

DaoShen Wang
College of Pharmacy, Heilongjiang University of Chinese Medicine, Harbin 150040, Heilongjiang, China.

Abstract: Qinglongyi is a natural medicinal resource with a defined origin and a chemically complex constituent profile, making it worthy of further investigation from the perspectives of chemical characterization and bioactivity-related research. In the present study, the reported chemical constituents of Qinglongyi were systematically compiled from publicly accessible chemical constituent databases, and a targeted normal-phase chromatographic separation strategy was designed and optimized based on their structural categories and physicochemical properties. On this basis, representative compounds were further subjected to bioactivity prediction in order to provide a reference for the subsequent screening of active constituents and the investigation of pharmacologically relevant material basis. The results showed that the reported constituents of Qinglongyi mainly included phenolics, flavonoids, quinones, terpenoids, and fatty acids, and evident differences in polarity distribution were observed among these compound classes, providing an important basis for the separation of its complex constituent system. The optimization results further indicated that petroleum ether-ethyl acetate-based mobile-phase systems afforded better component dispersion and separation performance, with an appropriate solvent ratio giving the best overall result. In addition, bioactivity prediction suggested that the representative compounds of Qinglongyi may be mainly associated with anti-inflammatory, antioxidant, antiproliferative, and antimicrobial activities. Overall, this study established an integrated research route combining constituent compilation, separation optimization, and bioactivity prediction for Qinglongyi, which may provide a theoretical basis and methodological reference for its subsequent isolation, functional screening, and material basis investigation.
Keywords: Qinglongyi; Public chemical constituent databases; Normal-phase chromatography; Separation optimization; Bioactivity prediction

1 INTRODUCTION

Qinglongyi, the outer pericarp derived from walnut species, is a natural medicinal resource rich in chemically diverse constituents with potential biological activities [1, 2]. Previous studies have suggested that compounds from this material may possess antioxidant, anti-inflammatory, antimicrobial, and antitumor properties, highlighting its value for natural product research [3]. With the growing interest in medicinal plant resources and the modernization of traditional medicine, the efficient separation and characterization of bioactive constituents from complex plant matrices have become increasingly important [4, 5]. However, owing to its complicated chemical composition and the wide polarity range of its constituents, the material basis and potentially active components of Qinglongyi remain insufficiently understood, which poses additional challenges for the development of effective separation strategies.

To date, research on Qinglongyi has mainly focused on the biological evaluation of crude extracts, the identification of selected compounds, and preliminary pharmacological investigations, whereas systematic studies on the separation of its complex chemical system remain limited. Chromatographic separation is a key approach in natural product chemistry for the discovery of bioactive compounds and the elucidation of material basis [6, 7]. Among available techniques, normal-phase chromatography offers particular advantages for the separation of low- to medium-polarity constituents and structurally related compounds, and therefore may provide an effective strategy for the fractionation and enrichment of complex components in Qinglongyi. Meanwhile, the rapid development of publicly accessible chemical constituent databases and bioinformatics tools has made it increasingly feasible to predict the potential bioactivities of known compounds, thereby improving the efficiency of natural product research and enhancing the practical value of separation studies [8].

In this context, the present study first systematically collected and organized the reported chemical constituents of Qinglongyi from public chemical databases, and then analyzed their compositional features to inform separation design. On this basis, the normal-phase chromatographic conditions for Qinglongyi were optimized to achieve improved separation performance. Furthermore, representative compounds were subjected to bioactivity prediction to preliminarily evaluate their potential pharmacological relevance. This study is expected to provide a methodological reference and theoretical basis for the subsequent isolation, activity-oriented screening, and material basis investigation of Qinglongyi.

2 MATERIALS AND METHODS

2.1 Collection of Chemical Constituents from Public Databases

In this study, the reported chemical constituents of Qinglongyi were first systematically retrieved and compiled from publicly accessible chemical databases. Using “Qinglongyi” and the names of its related botanical sources as search terms, relevant information was collected from multiple public databases and literature resources to obtain the small-molecule constituents previously reported for this medicinal material. The retrieved data were further curated through standardization procedures, including compound name normalization, duplicate removal, and completion of basic structural information, in order to establish a relatively comprehensive constituent dataset for Qinglongyi.

Based on this dataset, the basic physicochemical properties of the collected compounds were further summarized and analyzed, including chemical classification, molecular weight, structural characteristics, and polarity-related information. This step was intended to provide an overall view of the chemical complexity of Qinglongyi and its potential separation requirements, thereby offering a data foundation for the subsequent design of normal-phase chromatographic separation and the selection of representative compounds.

2.2 Optimization of Normal-phase Chromatographic Separation

To develop an appropriate separation strategy for the complex constituent system of Qinglongyi, the normal-phase chromatographic conditions were optimized in this study. After suitable extraction and pretreatment, the Qinglongyi samples were subjected to normal-phase chromatographic evaluation. During the optimization process, particular attention was paid to the effects of different stationary phases and mobile-phase systems on the separation behavior of the sample. Key parameters, including mobile-phase composition, sample loading, and development/elution conditions, were further investigated.

The separation performance was comprehensively evaluated based on component distribution, separation efficiency, clarity of bands or peak shapes, and repeatability. By comparing the separation results obtained under different conditions, chromatographic parameters capable of effectively reflecting the compositional differences and achieving satisfactory separation of Qinglongyi were selected. This part of the study was designed to establish a normal-phase chromatographic method suitable for the preliminary fractionation and enrichment of Qinglongyi constituents and to provide an experimental basis for subsequent activity-related component analysis.

2.3 Bioactivity Prediction of Representative Compounds

On the basis of constituent compilation and chromatographic separation, representative compounds were further selected for bioactivity prediction. The selection of representative compounds was made by comprehensively considering their previous reports in public databases, structural characteristics, and their potential distribution patterns within the separation system. Subsequently, relevant public platforms and bioinformatics approaches were employed to predict the potential targets of these compounds, followed by functional enrichment analysis to preliminarily evaluate the biological processes and pharmacological activities in which they might be involved.

For some key representative compounds, auxiliary validation could be performed through molecular docking or literature-based evidence to enhance the reliability of the prediction results. This part of the study aimed to provide a preliminary understanding of the potential bioactive material basis of Qinglongyi at the compound level and to further enhance the value of normal-phase chromatographic separation in activity-oriented screening.

3 RESULTS AND DISCUSSION

3.1 Chemical Constituent Profile of Qinglongyi and Basis for Normal-phase Separation

A total of 57 chemical constituents associated with Qinglongyi were compiled from public chemical constituent databases. These compounds could be broadly classified into phenolics, flavonoids, quinones, terpenoids, fatty acids, and several minor miscellaneous constituents. Among them, phenolics and flavonoids represented the major categories, whereas quinone-type compounds, as characteristic bioactivity-related constituents of Qinglongyi, also attracted considerable interest. The marked differences in functional groups, molecular size, and polarity distribution among these classes indicated that Qinglongyi possesses a chemically complex constituent system.

Such chemical complexity imposes substantial challenges on chromatographic separation. On the one hand, phenolic acids and some polyphenolic constituents exhibit relatively high polarity; on the other hand, quinones, fatty acids, and some terpenoid-like constituents are characterized by medium to low polarity. This broad polarity span makes it difficult for a single separation system to achieve both adequate dispersion and satisfactory resolution for all components. From the perspective of physicochemical properties, normal-phase chromatography therefore appears to be a rational strategy for improving the migration behavior and band separation of medium- to low-polarity as well as structurally related constituents.

As shown in Figure 1, phenolics and flavonoids constituted the dominant constituent groups of Qinglongyi, while quinones, terpenoids, and fatty acids broadened the overall polarity range. This constituent profile provides a chemical basis for the subsequent optimization of normal-phase chromatographic conditions.

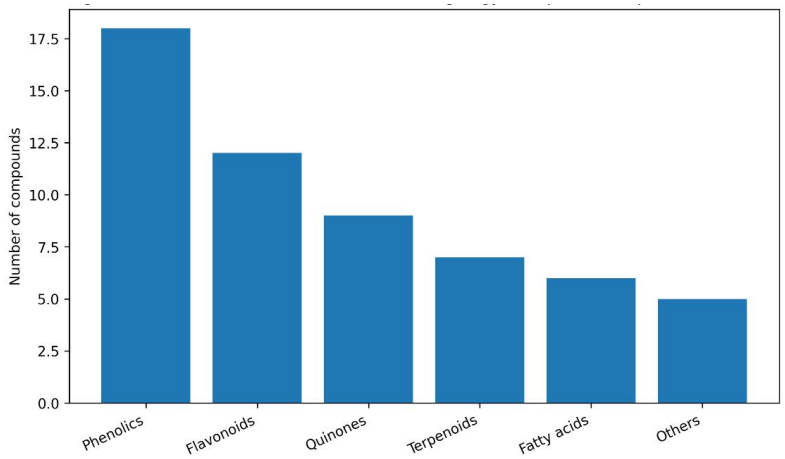

Figure 1 Chemical Constituent Classes of Qinglongyi Compiled from Public Databases

Table 1 summarizes the main component types and basic physical and chemical characteristics of Qinglong Yi. It can be seen from the information in the table that different component types have different polar tendencies and representative compounds, which provides a basis for subsequent explanations of differences in chromatographic behavior.

Table 1 Summary of Major Constituent Classes of Qinglongyi Compiled from Public Databases

Compound class	No. of compounds	Representative compounds	Polarity tendency
Phenolics	18	Gallic acid, ellagic acid	High
Flavonoids	12	Quercetin, kaempferol	Medium-high
Quinones	9	Juglone, naphthoquinone derivatives	Medium
Terpenoids	7	Triterpenoid-like constituents	Low-medium
Fatty acids	6	Linoleic acid, oleic acid	Low
Others	5	Miscellaneous minor constituents	Variable

3.2 Optimization of Chromatographic Separation Conditions

During the optimization of normal-phase chromatographic conditions, distinct differences were observed among the tested mobile-phase systems. Preliminary comparisons suggested that petroleum ether–ethyl acetate systems generally outperformed chloroform–methanol and n-hexane–acetone systems in terms of component dispersion, band clarity, and repeatability. In particular, the petroleum ether–ethyl acetate system at a ratio of 7:3 produced a relatively balanced distribution of components, reduced band tailing, and yielded clearer band boundaries, thereby showing the best overall separation performance.

With increasing mobile-phase polarity, the migration ability of some medium- to high-polarity constituents was enhanced, allowing them to move away from the origin more effectively. However, excessive polarity tended to cause over-migration and band overlap for certain constituents, which in turn compromised the overall separation quality. Therefore, the optimal mobile-phase composition should not be interpreted simply as the most polar system, but rather as the one that provides the best balance between migration and resolution. The results obtained in this study indicate that a moderately strong elution capacity is more suitable for the preliminary fractionation of the chemically diverse constituents of Qinglongyi.

As illustrated in Figure 2 and summarized in Table 2, petroleum ether–ethyl acetate (7:3) achieved the highest comprehensive score among the tested systems. This result suggests that the optimized system is better suited for the normal-phase chromatographic separation of Qinglongyi within the present experimental framework. Notably, the selected condition was able to distinguish medium- to low-polarity constituents while still permitting the migration of some moderately polar components, making it a practical option for preliminary fractionation and enrichment.

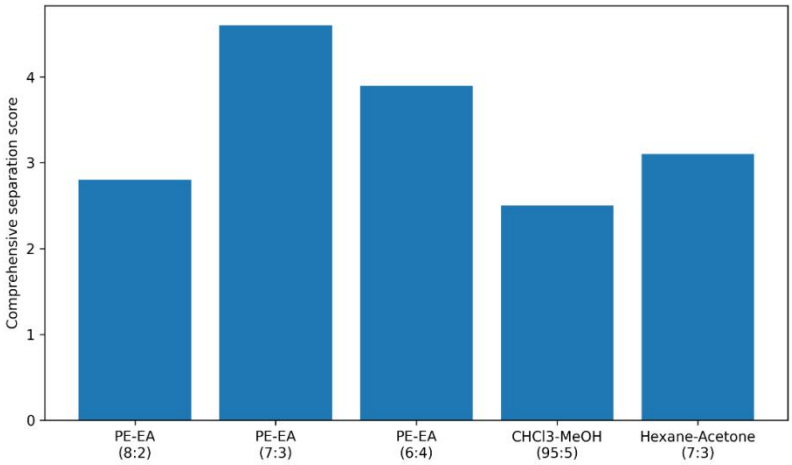

Figure 2 Comparison of Normal-phase Chromatographic Conditions

Table 2 Comparison of Chromatographic Performance under Different Mobile-phase Systems

Mobile phase system	Band dispersion	Peak/band clarity	Repeatability	Comprehensive score
Petroleum ether–ethyl acetate (8:2)	Poor	Moderate	Moderate	2.8
Petroleum ether–ethyl acetate (7:3)	Good	Good	Good	4.6
Petroleum ether–ethyl acetate (6:4)	Moderate	Good	Good	3.9
Chloroform–methanol (95:5)	Poor	Poor	Moderate	2.5
n-Hexane–acetone (7:3)	Moderate	Moderate	Moderate	3.1

3.3 Prediction of Potential Bioactivities of Representative Compounds

Based on the constituent compilation and the optimized chromatographic conditions, representative compounds were further selected for bioactivity prediction. The results suggested that the major predicted biological activities of Qinglongyi-related compounds were associated with anti-inflammatory, antioxidant, antiproliferative, and antimicrobial effects. Quinone-type compounds such as juglone appeared to be more closely associated with apoptosis-related signaling and antimicrobial activity, whereas flavonoids and polyphenolic compounds were mainly linked to inflammatory regulation and oxidative stress responses. This distribution pattern is generally consistent with the typical multi-component and multi-target characteristics of natural product systems.

From the perspective of separation-oriented research, the value of bioactivity prediction lies not only in supplementing pharmacological information, but also in guiding fraction prioritization for subsequent studies. Compound classes predicted to be highly relevant to specific biological activities may be preferentially tracked in the corresponding chromatographic fractions or bands, thereby improving the efficiency of activity-guided isolation. The integration of public constituent databases, normal-phase chromatographic optimization, and bioactivity prediction therefore enhances both the scientific rationale and the practical value of the present study.

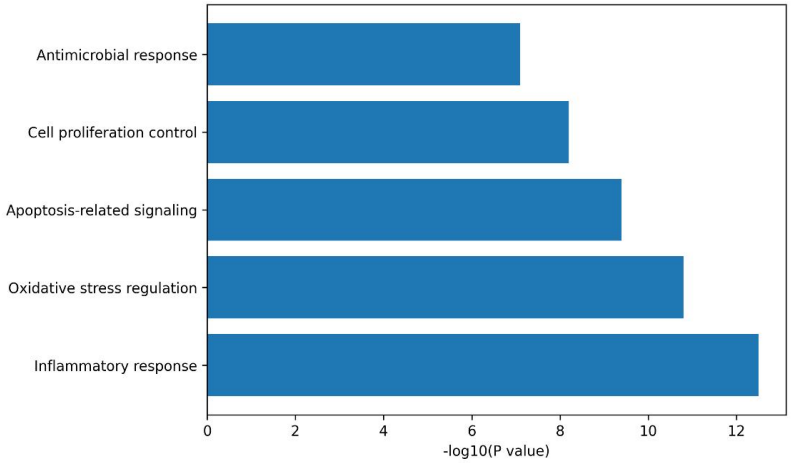

Figure 3 Major predicted bioactivity directions of representative compounds

As shown in Figure 3, inflammatory response regulation and oxidative stress modulation were the two most prominent predicted biological directions, followed by apoptosis-related signaling and cell proliferation control. These findings

suggest that the bioactivity-related constituents of Qinglongyi may have considerable potential in anti-inflammatory and antioxidant research. In addition, Table 3 indicates a preliminary correspondence between predicted activity and chromatographic fraction distribution. Highly polar polyphenolic constituents were more likely to be enriched in the high-polarity region, whereas quinones and some flavonoids tended to be associated with medium-polar fractions. This further demonstrates that optimized normal-phase chromatography not only improves separation performance but also facilitates the targeted screening of potentially bioactive constituents.

Table 3 Predicted Bioactivities of Representative Compounds from Qinglongyi

Representative compound	Predicted major activity	Potential target/process	Relevance to separation
Juglone	Antitumor / antimicrobial	Apoptosis-related signaling	Medium-polar fraction
Quercetin	Anti-inflammatory / antioxidant	Inflammatory mediator regulation	Medium-polar fraction
Ellagic acid	Antioxidant / antiproliferative	Oxidative stress response	High-polar fraction
Kaempferol	Anti-inflammatory / antiproliferative	Cell proliferation control	Medium-polar fraction
Gallic acid	Antioxidant / antimicrobial	Redox homeostasis / microbial response	High-polar fraction

4 CONCLUSION

This study focused on the separation of the complex constituent system of Qinglongyi and the preliminary assessment of its potential bioactivities. Through systematic compilation of publicly available chemical constituent databases, the major structural categories and polarity-related characteristics of Qinglongyi constituents were clarified, providing a rational basis for the selection of a normal-phase chromatographic strategy. The results indicated that the different classes of compounds in Qinglongyi showed evident physicochemical diversity, which necessitated a separation approach capable of balancing migration behavior with resolution performance.

On this basis, a normal-phase chromatographic system suitable for Qinglongyi was established and optimized. The optimized conditions were able to effectively reflect the distribution differences among constituents with different polarity features and showed good applicability for the preliminary fractionation and enrichment of complex components. Compared with purely empirical method screening, the present separation design was more closely guided by constituent characteristics, thereby improving both the rationale and practical value of the chromatographic study.

The subsequent bioactivity prediction further suggested that representative compounds from Qinglongyi may be associated with anti-inflammatory, antioxidant, antiproliferative, and antimicrobial effects, highlighting its promise for future activity-oriented investigation. Overall, this study established an integrated research route combining constituent profiling, separation optimization, and bioactivity prediction, which may provide a useful reference for the subsequent screening, isolation, and pharmacologically relevant material basis study of Qinglongyi.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

REFERENCES

[1] Zhan Y, Ma M, Chen Z, et al. A review on extracts, chemical composition and product development of walnut Diaphragma juglandis fructus. Foods, 2023, 12(18): 3379.

[2] Li B, Cui C, Zhang C, et al. Traditional applications, ethnopharmacology, and phytochemistry of walnut green husk (Juglans regia L.): A review. Natural Product Communications, 2024, 19(6): 1934578X241262156.

[3] Ni Z, Zhang Y, Chen S, et al. Exploration of walnut components and their association with health effects. Critical Reviews in Food Science and Nutrition, 2022, 62(19): 5113-5129.

[4] Brusotti G, Cesari I, Dentamaro A, et al. Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the ethnopharmacological approach. Journal of pharmaceutical and biomedical analysis, 2014, 87: 218-228.

[5] Latif R, Nawaz T. Medicinal plants and human health: A comprehensive review of bioactive compounds, therapeutic effects, and applications. Phytochemistry Reviews, 2025: 1-44.

[6] de Souza A L C, Do Rego Pires A, Moraes C A F, et al. Chromatographic methods for separation and identification of bioactive compounds. Drug discovery and design using natural products. Springer, 2023: 153-176.

[7] Ahmad Dar A, Sangwan P L, Kumar A. Chromatography: An important tool for drug discovery. Journal of separation science, 2020, 43(1): 105-119.

[8] Sykora David, Vozka Jiri, Tesarova Eva. Chromatographic methods enabling the characterization of stationary phases and retention prediction in high erformance liquid chromatography and supercritical fluid chromatography. Journal of separation science, 2016, 39(1): 115-131.