

Isolation of Secondary Metabolites of *Zingiberaceae* Rhizomes by Spectrofotometry and Chromatography: A Review

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Abstract

The Zingiberaceae family, known as the ginger family, is rich in secondary metabolites that contribute to its extensive use in traditional and modern medicine. Understanding effective methods for isolating these metabolites is crucial for advancing their pharmaceutical and nutraceutical applications. A literature review of 10 journal articles published between 2015 and 2025 was conducted. The focus was on spectrophotometric (UV-Vis, FTIR) and chromatographic (TLC, HPLC, LC-MS) techniques applied to the rhizomes of Zingiberaceae species. Data on compound identification, quantification, and method performance were analysed. Rhizomes of *Curcuma aeruginosa*, *Alpinia purpurata*, *Zingiber officinale*, and *Boesenbergia pandurata* were reported to contain flavonoids, alkaloids, phenolics, terpenoids, and curcuminoids. Spectrophotometric methods provided functional group identification and estimation of total metabolite concentrations. Chromatographic approaches enabled separation, purification, and structural elucidation with high specificity. Notably, flavenochromane C from *Zingiber cassumunar* and piperine from *Alpinia purpurata* were identified using LC-MS and NMR spectroscopy. In addition, TLC-densitometry for curcuminoids and xanthorrhizol in *Curcuma xanthorrhiza* demonstrated high accuracy and precision. Spectrophotometric techniques offer rapid and cost-effective screening, while chromatographic methods provide detailed identification and quantification of specific compounds. The complementary use of both approaches enhances the accuracy and reliability of isolating Zingiberaceae metabolites, supporting their further utilisation in medicinal research and product development.

Keywords: *Zingiberaceae*, spectrophotometry, chromatography, secondary metabolites, phytochemistry

Abstrak

Famili Zingiberaceae, dikenal sebagai famili jahe-jahean, kaya akan metabolit sekunder yang berkontribusi pada penggunaannya secara luas dalam pengobatan tradisional maupun modern. Pemahaman mengenai metode isolasi yang efektif sangat penting untuk mendukung pengembangan aplikasi farmasi dan nutraseutikal. Studi ini merupakan tinjauan pustaka terhadap 10 artikel jurnal yang dipublikasikan antara tahun 2015 hingga 2025. Fokus kajian adalah pada teknik spektrofotometri (UV-Vis, FTIR) dan kromatografi (TLC, HPLC, LC-MS) yang diaplikasikan pada rimpang spesies Zingiberaceae. Data mengenai identifikasi senyawa, kuantifikasi, serta kinerja metode dianalisis. Rimpang *Curcuma aeruginosa*, *Alpinia purpurata*, *Zingiber officinale*, dan *Boesenbergia pandurata* dilaporkan mengandung flavonoid, alkaloid, fenolik, terpenoid, dan kurkuminoid. Metode spektrofotometri digunakan untuk mengidentifikasi gugus fungsi dan mengukur konsentrasi total metabolit. Teknik kromatografi memungkinkan pemisahan, pemurnian, serta elucidasi struktur dengan spesifisitas tinggi. Sebagai contoh, flavenochromane C dari *Zingiber cassumunar* dan piperin dari *Alpinia purpurata* berhasil diidentifikasi menggunakan LC-MS dan spektroskopi NMR. Selain itu, analisis berbasis TLC-densitometri untuk kurkuminoid dan xanthorrhizol pada *Curcuma xanthorrhiza* menunjukkan akurasi dan presisi yang tinggi. Teknik spektrofotometri menawarkan pemeriksaan yang cepat dan ekonomis, sedangkan kromatografi memberikan identifikasi serta kuantifikasi yang lebih mendetail. Kombinasi kedua pendekatan ini meningkatkan akurasi dan keandalan dalam isolasi metabolit Zingiberaceae, sehingga mendukung pemanfaatannya lebih lanjut dalam riset medis dan pengembangan produk.

Kata kunci: *Zingiberaceae*, spektrofotometri, kromatografi, metabolit sekunder, fitokimia

Received: 30 July 2025

Revised: 28 August 2025

Accepted: 03 September 2025

Publish: 04 September 2025

INTRODUCTION

The *Zingiberaceae* family, commonly known as the ginger family, belongs to the

kingdom Plantae, division Angiospermae, and class Monocotyledon. Taxonomically, it is classified under the order Zingiberales.

This family comprises approximately 50 genera and over 1,600 species of perennial flowering plants distributed across tropical regions of Africa, Asia, and the Americas⁵. *Zingiberaceae* is recognized for its medicinal significance, with numerous species used in traditional medicine worldwide, making it a valuable source of natural therapeutic agents¹. Moreover, endophytic microorganisms associated with *Zingiberaceae* have been reported to produce diverse secondary metabolites with significant pharmacological activities⁷.

In tropical regions, including Indonesia, members of this family are pharmacologically and ethnomedicinally important. Rhizomes of species such as *Curcuma xanthorrhiza* (temulawak), *Zingiber officinale* var. *rubrum* (red ginger), *Curcuma zedoaria* (white turmeric), *Zingiber officinale* var. *officinatum* (white ginger), *Alpinia purpurata* (red galangal), *Zingiber cassumunar* (bangle), *Curcuma aeruginosa* (black ginger), *Etlingera elatior* (torch ginger), and *Boesenbergia pandurata* (fingerroot) contain bioactive secondary metabolites with antimicrobial, antioxidant, and antibacterial activities¹⁰. The major compounds frequently identified include flavonoids, alkaloids, saponins, steroids/terpenoids, tannins, phenolics, polyphenols, quinones, curcuminoids, and xanthorrhizol⁸.

Assessing the chemical content and pharmacological activities of *Zingiberaceae* rhizomes requires reliable isolation and characterization of these secondary metabolites. Two major analytical approaches are widely employed: spectrophotometry and chromatography. Spectrophotometric methods such as UV-

Vis and FTIR are useful for identifying functional groups, quantifying total flavonoids and polyphenols, and evaluating antioxidant potential¹⁰. Meanwhile, chromatographic methods—including column chromatography, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and liquid chromatography–mass spectrometry (LC-MS)—enable effective separation, purification, and identification of metabolites with high specificity^{2,3}.

Typically, the isolation process begins with extraction of rhizomes using polar solvents (e.g., methanol, acetone), followed by liquid–liquid partitioning and fractionation. Subsequent purification is carried out using column chromatography (silica gel as stationary phase), centrifugal, or preparative chromatography. Structural elucidation and quantification are then performed using LC-MS and complementary spectroscopic techniques. This workflow has enabled the identification of dominant metabolites, such as the flavonoid flavenochromane C in *Zingiber cassumunar*¹⁰. As emphasized by Rahman et al.¹⁰, proper extraction and fractionation are crucial to obtain bioactive compounds from *Zingiberaceae* rhizomes, while characterization using LC-MS provides accurate identification of key metabolites with potential therapeutic value¹².

Based on these considerations, this study aims to summarise and provide an overview of methods for isolating secondary metabolites from *Zingiberaceae* rhizomes using spectrophotometric and chromatographic techniques, as discussed in previous studies.

METHODOLOGY

Using a literature review approach, this study collected, analyzed, and synthesized data from various relevant sources, including books, journal articles, and other scientific publications related to the research topic. Journals published between 2015 and 2025, as well as digital books, were reviewed, focusing on the separation of secondary metabolites from *Zingiberaceae* rhizomes using spectrophotometric and chromatographic methods^{1-4, 7-12, 14, 15}. The literature sources were obtained from trusted online databases and repositories, including Google Scholar, PubMed, official journal websites, and applications such as iPusnas.

This review highlights analytical methods applied in previous studies, particularly UV-Vis spectrophotometry, FTIR, TLC, HPLC, and LC-MS, as well as their application in the identification, quantification, and characterization of bioactive compounds in *Zingiberaceae* rhizomes^{2, 3, 8-10}. The purpose of this approach is to provide a comprehensive overview of the techniques used in secondary metabolite isolation and to summarize the results achieved in prior research for better understanding and further application in the field of pharmaceutical and phytochemical sciences.

RESULT AND DISCUSSION

A literature review was conducted to identify, analyze, and compare studies related to the isolation and characterization of secondary metabolites from *Zingiberaceae* rhizomes. Various analytical methods,

particularly spectrophotometric and chromatographic techniques, have been employed across different species to reveal the diversity of bioactive compounds and their pharmacological potential.

The summary of these findings is presented in Table 1, which outlines the title of each study, the analytical methods applied, the main results obtained, and the corresponding references. As shown in Table 1, thin-layer chromatography (TLC) combined with UV-Vis spectrophotometry has frequently been applied to detect phenolics, alkaloids, and terpenoids, while advanced techniques such as LC-MS and NMR spectroscopy have enabled precise structural elucidation of compounds such as flavenochromane C and bis-(2-ethylhexyl) phthalate.

By presenting the results in Table 1, this review provides a concise overview of the experimental approaches, analytical methods, and main findings from previous research. This not only demonstrates the chemical richness of *Zingiberaceae* rhizomes but also underscores the importance of applying appropriate analytical techniques to achieve accurate metabolite identification. Furthermore, these findings highlight the contribution of *Zingiberaceae* plants as a valuable source of bioactive compounds with significant pharmacological potential. In addition, the comparative analysis of different methods provides useful insights for selecting the most effective approaches in future research and drug discovery efforts.

Table 1. Summary of studies on the isolation and characterization of secondary metabolites from Zingiberaceae rhizomes using spectrophotometric and chromatographic methods

No	Title/Topic	Method	Results	Reference
1	Isolation and identification of secondary metabolite compounds from black turmeric rhizome (<i>Curcuma aeruginosa</i> Roxb.)	Thin Layer Chromatography (TLC) and UV Spectrophotometry	A yellow oil weighing 0.0216 g was obtained. A single blue spot was observed under UV light (254 nm and 366 nm), with vanillin sulfate spot formation on the TLC plate, indicating a terpenoid compound. The UV spectrum in methanol showed absorption at 206.14 nm with an absorbance of 0.216.	8
2	Isolation and identification of alkaloid compounds from red galangal rhizome (<i>Alpinia purpurata</i>)	Thin Layer Chromatography (TLC) and UV-Vis Spectrophotometry	TLC produced R _f values: 0.46 (yellow), 0.56 (purple), 0.73 (orange), 0.79 (bluish green), 0.96 (blue). UV-Vis confirmed alkaloids with absorptions at 2361.68, 2337.71, 1632.91, 1511.29, 1050.85, 2923.03, and 2812.34 cm ⁻¹ .	14
3	Isolation and antibacterial activity test of phenolic compounds from ethyl acetate extract of red ginger rhizome (<i>Zingiber officinale</i> Roscoe var. <i>sunti</i>)	Thin Layer Chromatography (TLC) and UV-Vis Spectrophotometry	FeCl ₃ addition to fraction 7 identified phenols, appearing as yellow spots under UV 254 nm. UV-Vis spectrum of compound 1 showed peaks at 280 nm (band I) and 233 nm (band II).	3
4	Isolation of secondary metabolites and antibacterial activity test on methanol extract of temu kunci (<i>Boesenbergia pandurata</i>)	NMR Spectroscopy	1D NMR (¹ H-NMR, ¹³ C-NMR) indicated the isolate was bis-(2-ethylhexyl) phthalate.	15
5	Isolation of secondary metabolite compounds from ethyl acetate fraction of bangle rhizome (<i>Zingiber cassumunar</i> Roxb.) and activity test against <i>Candida albicans</i>	Thin Layer Chromatography (TLC) and LC-MS	LC-MS identified flavenochromane C (C ₂₁ H ₂₀ O ₆ , MW 369.13). Separation yielded 16 fractions; FK1 (78 mg), FK2 (67 mg), FK3 (278 mg).	4

6	Validation of the analysis method of curcuminoids and xanthorrhizole in Javanese ginger rhizome (<i>Curcuma xanthorrhiza</i>) using TLC-densitometry	Densitometric Thin Layer Chromatography	Three curcuminoids identified: bisdemethoxycurcumin (Rf 0.04), demethoxycurcumin (Rf 0.13), and curcumin (Rf 0.26). Xanthorrhizol Rf values: 0.90, 0.87, 0.69.	9
7	Torch ginger rhizome (<i>Etlingera elatior</i> Jack.) as a source of phenolics	Densitometric Thin Layer Chromatography	TLC-densitometry of methanol extract gave Rf values: 0.76 (sample I), 0.75 (sample II), 0.80 (sample III) at 254 nm.	13
8	Secondary metabolite compounds and antioxidant activity test of white ginger leaf extract (<i>Zingiber officinale</i> Rosc. var. officinarum)	Thin Layer Chromatography (TLC)	TLC-densitometry with linear equation $y = 25.728x - 103.37$ quantified phenolic content of methanol extract of <i>Etlingera elatior</i> rhizome with average 0.14443 µg/ml (3 replications).	6
9	Pharmacognostic testing and compound identification in several fractions of ethanol extract of galangal leaves (<i>Alpinia galanga</i>)	Thin Layer Chromatography (TLC)	n-Hexane fraction: tannins, steroids, alkaloids. Methanol fraction: organic compounds, alkaloids, flavonoids, phenolics, saponins, terpenoids. Ethyl acetate fraction: phenolics, tannins, steroids.	11
10	Comparison of flavonoid content of instant white turmeric powder (<i>Curcuma zedoaria</i> Rosc.) circulating in the market using UV-Vis spectrophotometry	UV-Vis Spectrophotometry	Brand V showed highest flavonoid (5.366% batch A); brand II 5.423% (batch B). Brand IV showed lowest flavonoid (3.024% batch A, 2.851% batch B).	12

Based on the reviewed studies, several bioactive compounds have been identified from different *Zingiberaceae* plant extracts, including terpenoids, alkaloids, phenolics, flavonoids, and curcuminoids. The yellow oil isolate from *Curcuma aeruginosa* exhibited terpenoid characteristics through TLC and UV spectra with maximum absorption at 206.14 nm⁸. TLC analysis of total alkaloids from *Alpinia purpurata* revealed several spots with distinct R_f values, supported by UV-Vis absorptions at 2361.68 cm⁻¹ and 1632.91 cm⁻¹, confirming the presence of alkaloid functional groups¹⁴.

Phenolic identification from *Zingiber officinale* var. *sunti* using FeCl₃ revealed a yellow spot under UV 254 nm, with UV-Vis absorptions at 280 nm and 233 nm [3]. NMR analysis confirmed bis-(2-ethylhexyl) phthalate as the isolated compound from *Boesenbergia pandurata*¹⁵. LC-MS analysis of the ethyl acetate fraction of *Zingiber cassumunar* identified flavenochromane C (C₂₁H₂₀O₆) as the dominant flavonoid⁴.

TLC-densitometry of *Etlingera elatior* extract demonstrated an average phenolic content of 0.14443 µg/ml⁷, while phytochemical screening of *Alpinia galanga* leaves revealed different compounds depending on the solvent used: tannins and steroids in the n-hexane fraction, phenolics in the ethyl acetate fraction, and flavonoids and saponins in the methanol fraction¹¹. In instant white turmeric powder (*Curcuma zedoaria*), the highest flavonoid content was recorded in

brand II (5.423%) and the lowest in brand IV (2.851%)¹².

Overall, TLC, spectroscopic methods (UV-Vis, NMR, LC-MS), and phytochemical screening successfully identified various bioactive compounds, underscoring their potential applications in pharmaceuticals and nutraceuticals.

CONCLUSION

This literature review concludes that the isolation and characterization of secondary metabolites from *Zingiberaceae* rhizomes using spectrophotometric and chromatographic methods have successfully identified diverse bioactive compounds, including flavonoids, alkaloids, terpenoids, and phenolics, which exhibit significant pharmacological potential. These findings address the research problem of isolation and identification techniques for active compounds and achieve the objective of providing an overview of effective analytical methods.

However, the results also highlight the need for further exploration regarding methodological optimization and clinical trials to ensure the practical application of these compounds in drug discovery and nutraceutical development.

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