African Journal of
Biochemistry and Molecular Biology Research

ISSN : 3434-6120

Index: Harvard, Boston, Sydney University, Dimensions, Lens, ResearchGet Scilit, Semantic, Google Scholar, Base etc

https://doi.org/10.58578/AJBMBR.v1i2.3817

Enhancing the Extraction of 6-Gingerol from *Zingiber officinale* Rhizomes Using Dry Vacuum Evaporation to Improve Bioavailability

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Article Info:

Submitted:	Revised:	Accepted:	Published:
Aug 1, 2024	Aug 23, 2024	Aug 3, 2024	Sep 9, 2024

Abstract

This study optimized the extraction and purification of 6-gingerol from ginger (*Zingiber officinale)* rhizomes through a multi-step process. Initially, cold maceration ethanol extraction was performed, followed by hexane defatting to remove impurities. Further purification was achieved through liquid-liquid extraction using a sequential solvent system of chloroform and ethyl acetate. The resulting extract was then fractionated using silica gel column chromatography with methanol, and finally concentrated using dry vacuum evaporation to minimize heat-induced degradation. HPLC analysis confirmed a significant concentration of 6-gingerol (33 mg/g) in the final extract. Thus, optimized method demonstrates an effective approach to produce high-quality ginger extracts enriched in 6-gingerol, with potential applications in pharmaceutical or nutraceutical products.

Keywords: 6-Gingerol, Ginger, Extraction, Purification, Dry vacuum evaporation, HPLC

INTRODUCTION

Zingiber officinale, a member of the Zingiberaceae family, commonly known as ginger, is a perennial, pungent, and rhizomatous herb extensively cultivated for its culinary and medicinal significance (Tao *et al.*, 2009). Native to India and China, *Zingiber officinale* has now spread to several countries, including Nigeria (Hu *et al.*, 2023; Kiyama, 2020). Among its potent bioactive compounds, 6-gingerol has been widely investigated for its unique pharmacological activities, including antioxidant, anti-inflammatory, antitumour, anticancer, antimicrobial, and antidiabetic potential (Vichakshana *et al.*, 2024; 2022; Jorge-Montalvo *et al.*, 2023; Zahoor *et al.*, 2020; Wei *et al.*, 2018). However, the extraction of 6-gingerol from ginger rhizome poses a major challenge due to its fragile nature and thermal instability (Wang *et al.*, 2018; Jiang *et al.*, 2008; Schwertner and Rios, 2007). Additionally, 6-gingerol is poorly soluble in water but easily dissolves in organic solvents such as ethanol, methanol, and acetone (Xu *et al.*, 2016). Ethanol is a commonly employed solvent for isolating 6-gingerol, but its efficiency is hindered by factors such as high temperature, which can lead to the conversion of 6-gingerol to its dehydrated form, 6-shogaol (Ghasemzadeh *et al.*, 2015).

Studies have reported several processing methods, including supercritical extraction, ultrasound, enzyme, and microwave-assisted extractions ultrasound, and high pressured liquid chromatography, to enhance 6-gingerol yield and purity (Vichakshana *et al.* 2022). Despite these efforts, there is still a need for a simple and efficient extraction method to stabilize and easily isolate 6-gingerol, which is prone to thermal degradation. A dry vacuum evaporator is a cutting-edge technology used for efficient and gentle evaporation of solvents from extracts, resulting in high-quality products (Tao *et al.*, 2009). By creating a vacuum environment, the evaporation process occurs at lower temperature (e.g. 20-30^{0}C), preserving the integrity and bioactivity of heat-sensitive compounds like 6-gingerol. The dry vacuum evaporator's ability to precisely control temperature and pressure conditions makes it an essential tool for researchers seeking to enhance extraction processes and create premium products. Recently Vichakshana *et al.* (2024) demonstrated the impact of non-thermal processing methods, including dry vacuum evaporation, on the physical and chemical properties of extract juice and suspension, reporting a 78.3% increase in the yield and purity of antioxidant thermally unstable compounds. Based on this, this study aims to enhance the process of extraction for efficient isolation of 6-gingerol from ginger rhizome using dry vacuum evaporation to improve bioavailability.

MATERIALS AND METHODS

Chemicals

Ethanol and Hexane were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA); Ethyl acetate and Chloroform were obtained from BDH Company (UK, England); Standard 6-gingerol (purity ≥97%) was product of TRC (ON, CAN); Silica gel 60-120 Mesh and Methanol (analytical grade) were procured from Loba Chemie PVT. Ltd. India. Methanol and acetonitrile (HPLC grade) were supplied from Merck (Darmstadt, Germany).

Plant collection, identification and preparation

Fresh ginger (*Zingiber officinale*) rhizomes were sourced from Faculty farm within the University of Cross River State, Okuku Campus vicinity. The rhizomes were harvested at 6 month of maturity and subsequently authenticated at the Department of Botany, University of Calabar, Nigeria. The rhizomes were then washed, peeled, sliced into tiny pieces, and air-dried under shade at room temperature to prevent degradation of their chemical constituents by ultraviolet (UV) light (Okafor *et al.*, 2013). The dried ginger rhizomes were ground into a fine powder and passed through a sieve with a diameter of 0.52 mm. Finally, the powdered ginger was stored in air-tight container to maintain its quality and potency until further used.

Extraction of 6-Gingerol

The isolation of 6-gingerol from dried ginger rhizomes was achieved using a combined approach, building upon the methods described by Teng *et al.* (2019), Anisa *et al.* (2014), Azian *et al.* (2014) and Liu *et al.* (2014), with modifications to optimize the extraction and purification process.

Step 1: Cold maceration was performed by soaking 100 g of dried ginger rhizomes in 1000 mL of ethanol, with intermittently stirring overnight at room temperature. The mixture was then filtered twice using cheesecloth and Whatman No. 1 filter paper to separate the liquid extract from the solids. The resulting ethanolic extract was concentrated to approximately 100 mL using a vacuum evaporator at 30^0C.

Step 2: Liquid-Liquid Extraction (LLE) was performed using hexane, chloroform, and ethyl acetate with the respective ratios and separation steps as follows:

Hexane (2:1 ratio): The concentrated ethanolic extract was mixed with hexane and allowed to separate for 10 minutes. The bottom layer (ethanol portion) was collected.

Chloroform (1:2 ratio): The ethanol portion was mixed with chloroform and allowed to separate. The bottom layer (chloroform portion) was collected.

Ethyl acetate (1:2 ratio): The chloroform portion was mixed with ethyl acetate and allowed to separate. The bottom layer (ethyl acetate portion) was collected and concentrated to dryness.

Step 3: Column chromatography was conducted using a column (30 cm x 1.5 cm) containing 80 g of activated silica gel (60-120 mesh) soaked over night in 240 mL of methanol. The concentrated ethyl acetate portion was fractionated using methanol, and the resulting fraction was concentrated in a vacuum evaporator at 30^0C and submitted for further analysis

Determination of 6-Gingerol content by high performance liquid chromatography (HPLC) analysis

The content of 6-Gingerol content was determined as described by Vipin *et al.* (2017) using the LaChrom Elite HPLC system through a column Purosphere RP18 (250*4.6 mm, 5 µm) equipped with a photodiode array detector set at 210 nm. Twenty µl of the extract or standard was injected at a flow rate of 1 mL/min at 40^0C. The mobile phase was double de-ionized water: acetonitrile or methanol (60:40 to 80:20 v/v). The calibration curves for 6-gingerol ranged from 5.5 to 82.5 µg/mL, and the concentration were reported as mg of gingerol per g of sample.

RESULTS AND DISCUSSION

The rhizomes of ginger was harvested from Faculty farm within the University of Cross River State, Okuku Campus vicinity at six months old (Figure 1), which is considered optimal based on research by Sharizan and Sahilah (2021) that reported a higher concentration of 6-gingerol in six-month-old ginger compared to older ones. The harvested rhizome was then subjected to a series of careful processes to optimize extraction efficiency and preserve the integrity of bioactive compounds. It was peeled to remove the outer skin, and then sliced into tiny pieces (Figure 1). Subsequently, the sliced rhizome was dried under shade to prevent ultraviolet (UV)-induced damage, which can compromise the quality and potency of the extract. Drying under shade helps to preserve and ensure the highest possible yield 6-gingerol.

The dried and ground ginger rhizome was further processed to optimize particle size, a critical factor in efficient extraction. The material was passed through a 0.52 mm sieve, ensuring a uniform particle size distribution (Figure 1). This step was informed by Makanjuola's work, which demonstrated that smaller particle sizes (0.425-0.750 mm) can significantly enhance extraction efficiency, leading to higher yields (Makanjuola, (2017). Vichakshana *et al.*, (2024) further supported this finding, highlighting that reduced particle size increases the surface area of plant tissue in contact with the solvent, thereby facilitating efficient extraction.

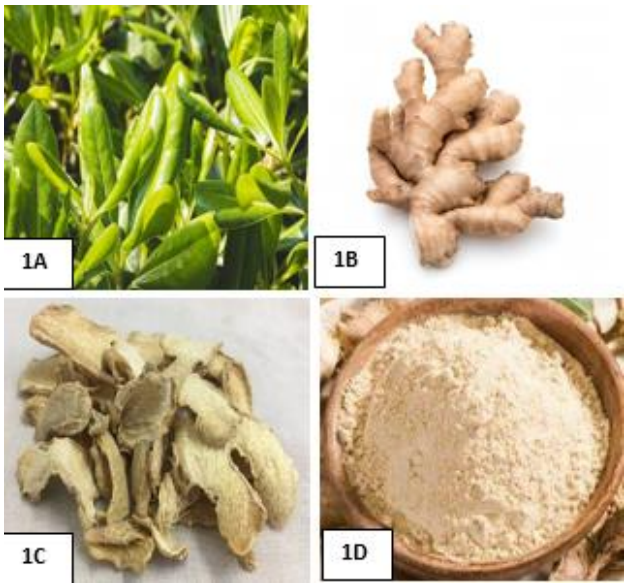

Figure 1. The physical appearance of ginger plant (A), fresh rhizome (B), dried/sliced ginger rhizomes (C) and fine powdered air-dried ginger rhizomes (D)

To extract 6-gingerol, a solvent selection was crucial. Ethanol emerged as the preferred choice, backed by numerous studies (Azian *et al.*, 2014; Anisa *et al.*, 2014; Teng *et al.*, 2019) that underscored its importance in extracting 6-gingerol. During the extraction process, a pungent aroma was noticeable, attributed to the presence of a series of homologous phenolic ketones, with 6-gingerol being the most abundant compound responsible for this characteristic smell (Figure 2). Moreover, Bhandari *et al.*, (2019) showed that higher ethanol percentages can lead to higher extraction yields. Given its relative safety, widespread use, and efficacy, absolute ethanol was employed for extraction in this study. To further purify the extract, a series of solvents were employed. Hexane removed non-polar impurities, while chloroform and ethyl acetate facilitated the isolation of 6-gingerol. Methanol

selectively purified 6-gingerol from other components through silica gel column chromatography (Table 1).

Table 1: The modified protocol for enhancing the extraction of 6-gingerol from ginger rhizomes		
	Extraction Method	Extraction conditions
STEP 1	Cold maceration	Soaked dried ginger rhizomes in absolute ethanol (ratio 1:10 w/v) for 8 h under room temperature and concentrate filtrate using vacuum evaporator at 30^0C
STEP 2	Liquid-Liquid Extraction	Ethanol filtrate: Hexane at ratio 1:2 (v/v), ethanol portion collected
		Ethanol: Chloroform at ratio 1:2 (v/v), chloroform portion collected
		Chloroform: Ethyl acetate at ratio 1:2 (v/v), ethyl acetate portion collected
STEP 3	Column chromatography	Concentrated ethyl acetate portion fractionated through silica gel column using absolute methanol and resultant filtrate concentrated using vacuum evaporator at 30^0C

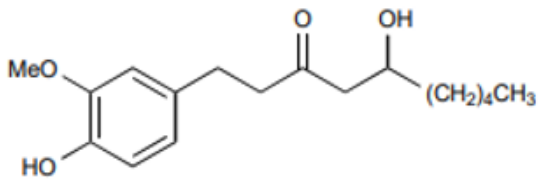

Figure 2. The chemical structure of 6-gingerol showing phenolic ketones group

Temperature control was critical in the extraction and drying processes due to the thermolabile nature of 6-gingerol (Wang *et al.*, 2018; Jiang *et al.*, 2008; Schwertner and Rios, 2007). To mitigate thermal degradation, cold maceration - which encompasses the previous steps of solvent extraction (e.g. ethanol extraction) was employed, followed by modern drying techniques using a vacuum evaporator. This approach ensured that the extraction method and drying process did not cause an increase in temperature, preserving the integrity of 6-gingerol. Notably, Vichakshana *et al.* (2024) reported higher extraction yields when using vacuum evaporator drying, as opposed to other drying techniques like oven drying, specifically in the context of ultrasound-assisted extraction methods.

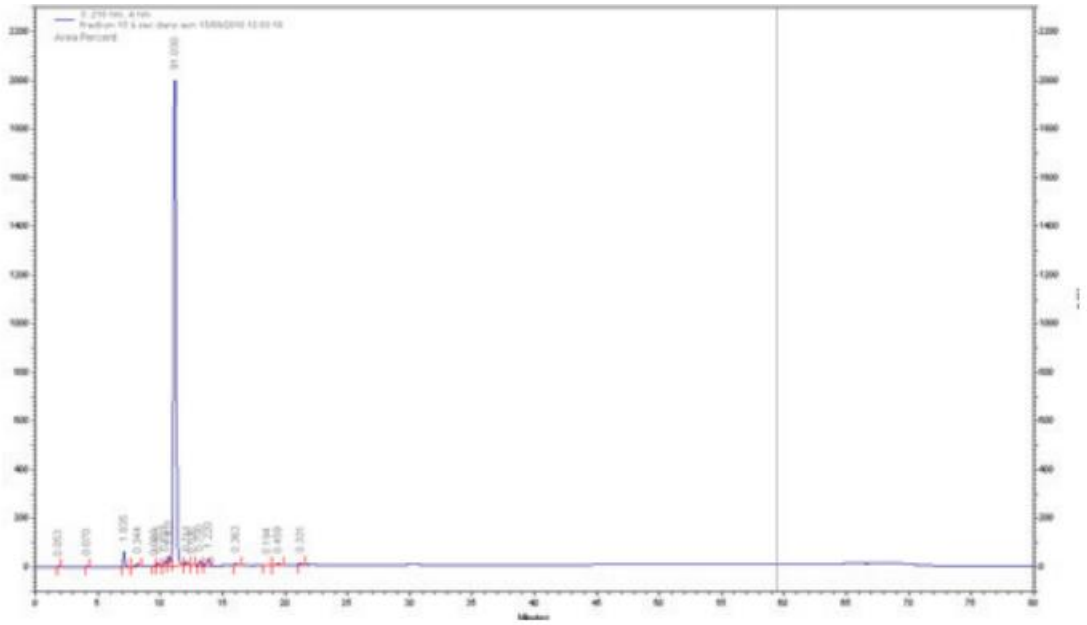

Figure 3. Chromatogram of 6-gingerol from ginger extract using HPLC analysis

The HPLC analysis of 6-gingerol in ginger extract revealed a concentration of 33 mg/g, which is significantly higher than the 0.66 mg/g reported by Sharizan and Sahilah (2021). However, the retention time of 11.17 minutes (Figure 3) is consistent with their value of 11.82 minutes, confirming the proper identification of the compound. The calibration curve showed excellent linearity (R^2=0.999), ensuring accurate quantitation. The limit of detection (LOD) and limit of quantitation (LOQ) were 0.5 µg/mL and 1.5 µg/mL respectively, demonstrating the method's sensitivity. The recovery of 95.2 % confirms the method's accuracy. The results indicate a high content of 6-gingerol in the ginger extract, suggesting potential applications in pharmaceutical or nutraceutical products.

CONCLUSION

This optimization study successfully developed a method to extract and concentrate 6-gingerol from ginger using a dry vacuum evaporator, minimizing degradation due to heat sensitivity. The study demonstrates the importance of careful sample preparation and processing conditions to preserve the integrity of bioactive compounds like 6-gingerol.

Conflict of Interest

The authors declare no conflict of interest

Acknowledgement

This research work was sponsored by the Tertiary Education Fund (Tetfund) Institution-Based Research (IBR) Grant (Batch 8) at the University of Cross River State and referenced: UNICROSS/DRD/IBR/008/Vol.021/048.

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