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Review Article

Extraction and Quantification of Allicin: A Bioactive Component of *Allium sativum*

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ABSTRACT

Allicin, a bioactive compound found in garlic, exhibits remarkable pharmacological properties such as antimicrobial, antioxidant, and anticancer effects. Its therapeutic potential has garnered substantial attention, leading to efforts to refine extraction and quantification methods for precise analysis in garlic-based products. This review examines advanced techniques for allicin extraction from garlic, including Ultrasonic-Assisted Extraction (UAE), Supercritical Water Extraction (SCWE), and Microwave-Assisted Extraction (MAE). UAE employs ultrasonic waves to improve mass transfer and solvent penetration, optimizing allicin yield while reducing extraction time. SCWE utilizes the unique properties of water above its critical point to extract allicin efficiently and selectively, providing an eco-friendly alternative. MAE uses microwave energy to rapidly heat solvents, enhancing allicin extraction through improved penetration and dissolution. Alongside these extraction methods, this review highlights analytical techniques for allicin quantification, including High-Performance Liquid Chromatography (HPLC), High-Performance Thin-Layer Chromatography (HPTLC), and Ultraviolet (UV) spectroscopy. HPLC offers exceptional sensitivity and specificity for splitting and measuring allicin in complex matrices, making it invaluable for accurate pharmacokinetic studies and quality control of pharmaceutical formulations. HPTLC offers quick screening with minimal sample preparation, ideal for routine investigation and batch testing of garlic extracts. UV spectroscopy, a cost-effective technique, detects allicin through its characteristic absorption spectra, enabling quick evaluations of garlic product quality and stability. This review consolidates recent advancements in allicin extraction and quantification techniques, emphasizing their applications in the pharmaceutical, nutraceutical, and food sectors. Future research aims to optimize extraction parameters to maximize allicin yield and develop robust analytical methods to address the growing demand for quality assurance and therapeutic efficacy in garlic-based products.

Keywords: Garlic, Allicin, Extraction, Quantification.

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INTRODUCTION

In addition to being a common food and spice, garlic (*Allium sativum* Linn.) is also used as a common medicine all throughout the world.[1] Garlic has been shown in several studies to have potential benefits in preventing age-related, cardiovascular, and carcinogenic disorders.[2] In particular, it has been firmly proposed that certain organosulfur compounds are responsible for its therapeutic and advantageous qualities.[3] For thousands of years, ancient civilizations including the Egyptians, Greeks, and Chinese have utilized garlic for its therapeutic benefits. Its health benefits are primarily attributed to a sulfur containing compound known as allicin. Allicin is accountable for garlic's characteristic odor and a significant portion of its therapeutic properties.[4]

Garlic has been widely consumed and used as a conventional medication in China for centuries. In current times, extensive research has revealed its impressive biological functions, comprising antibacterial, anticancer, antioxidant, cardio shielding, hypoglycaemic, anti-inflammatory, immunomodulatory, anti-fattening effects. Research has increasingly centered on black garlic, a treated form of garlic that contains higher levels of polyphenols and flavonoids, along with enhanced reducing actions in comparison to fresh garlic.[5] Sulfoxides are known to exhibit therapeutic effects; for instance, alliin has demonstrated anticancer actions,[6] while both alliin and cycloalliin are associated with lipid-lowering benefits. Similarly, γ-glutamyl peptides have been stated to reduce blood pressure and contribute to cholesterol-reducing effects.[7,8] When garlic is

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crumpled, its components are converted into other derivatives, like allicin, diallyl polysulfides, ajoene, and dithiins.[8,9] Therefore, careful control of sample preparation is essential to lessen errors. The biosynthetic pathway of organosulfur compounds in garlic is believed to involve the conversion of γ-glutamyl peptides into their respective sulfoxides, facilitated by the enzymes γ-glutamyl transpeptidase and oxidase.[10-12] Additionally, studies have shown that the levels of organosulfur compounds fluctuate during cultivation and storage.[12-15]

Garlic is distinguished for its therapeutic benefits, primarily due to allicin, a sulfur-containing compound derived from alliin (Figure 1). Effective extraction and precise quantification of allicin are crucial for assessing its therapeutic potential and maintaining the quality of garlic-derived products. Numerous techniques have been developed for allicin extraction and quantification, each offering unique benefits and challenges.

Allicin has a rich history embedded in the medicinal practices of ancient civilizations, with its discovery representing a significant breakthrough in understanding the therapeutic properties of garlic. Spanning from traditional remedies to contemporary scientific research, allicin remains a focal point of research for its potential health benefits. Exploring its historical background highlights the enduring value of garlic as a medicinal resource throughout human history.[17]

The chemical properties and stability of allicin

Semmler's ground-breaking research identified diallyl disulfide and diallyl trisulfide as key contributors to the flavor of garlic distillates. Cavallito and Bailey (1944) discovered the main physiologically active ingredient in garlic, allicin (diallyl thiosulfinate) who also first reported its strong antimicrobial properties.[17,18] Allicin is absent in raw garlic but is quickly generated when alliin is converted by the enzyme CS-lyase (allinase), which becomes active upon crushing or cutting garlic cloves.[18-20] Allicin accounts for approximately 70% of the total thiosulfinates formed in garlic cloves during automated crushing. Mechanistic and pharmacokinetic research on allicin and its derivatives emphasizes the importance of using a labeled compound for study.[20-23] However, labeling this volatile and unsteady solvent demands careful treatment. Miron *et al.*, outlined a straightforward methodology for preparing tritium-labeled (^{3}H) allicin.[24]

The primary sulfur compound in both raw garlic and garlic powder is alliin, with garlic cloves typically containing around 8 g/kg of alliin. In an ideal dehydration procedure without ingredient loss, the resulting powder would contain 20-25 mg/g of alliin. However, garlic powders generally contain a maximum of 10 g/kg alliin, indicating significant loss during the dehydration process. Crumpled raw garlic is rich in allicin, with approximately 37 mg/g present. Allicin has been identified as a major component in garlic obtained by solvent extraction. Allicin goes through dehydration, resulting in the creation of two isomeric disulfides through a reorganisation process. After a day, the primary products of this reaction include sulfur dioxide, along with diallyl mono, di, and trisulfides.[25] In commercial garlic oils, allyl and methyl sulfides are the chief constituents. Allicin degrades into ajoenes, diallyl disulfide, and vinyldithiins at varying rates, reliant on factors such as concentration, temperature, and pH.[26] Upon exposure to heated water-based solutions, allicin converts into a fat-soluble oligosulfide namely diallyl disulfide.[27] Allyl mercaptan, a scented component, is primarily responsible for garlic smell after consuming garlic cloves. It is quantitatively produced from allicin or diallyl disulfide through their reaction with cysteine, forming the intermediary S-allylmercaptocysteine when exposed to whole blood. Allyl mercaptan, or one of its auxiliary metabolites, could serve an essential role in the pharmacological actions (excluding topical and enteral actions) of allicin or diallyl disulfide.[28] Figure 2 depicts the chemical constructions of the constituents found in garlic.

EXTRACTION

Allicin can be extracted from garlic through various methods, depending on the equipment available, the feasibility of the process, and the desired product type. Typically, garlic is peeled, then chopped or crushed using tools such as a mortar and pestle or a garlic press. Grinding the garlic is a crucial step to activate the enzyme alliinase, allowing it to interact with alliin and facilitate the production of allicin. Grinding can be performed using any equipment capable of delivering maximum crushing strength to optimize enzymatic yield. The ground garlic is then placed into a beaker containing cold water, sealed, and shaken vigorously for a few seconds. This process is repeated with the addition of cold water. During shaking, the beaker is held at the top to minimize heat transfer. Finally, the mixture is passed through a 0.45 μM glass filter.

Garlic and its extracts are widely utilized in the food industry. Beyond their culinary applications, the phytochemicals in garlic are also employed for the management and prevention of numerous ailments. The biologically active components of fresh garlic extract, primarily thiosulfinates- many of the health advantages of garlic are linked to the most common class of organosulfur compounds found in freshly sliced or crumpled garlic. Allicin is the predominant thiosulfinate in garden fresh garlic, making up about 70% (w/w) of the entire thiosulfinates, which represent around 0.4% of the fresh mass. As a crystalline solid, allicin has a half-life of 16 hr at ambient temperature, whereas in crushed garlic, its half-life extends to around 2.5 days.[29]

Ultrasonic Assisted Extraction (UAE)

UAE methods for natural product extraction have gained popularity in both labs and industries because of their

rapid energy transfer, absence of heating requirements, and environmentally friendly nature. The use of ultrasound interrupts cell wall structures and enhances diffusion across membranes, leading to cell lysis and the efficient discharge of intracellular substances. UAE has demonstrated to be a highly efficient technique for extracting compounds from garlic. Loghmanifar *et al.*, explored the optimization of ultrasonic extraction parameters to maximize allicin yield from garlic.[30] Extracting allicin, a highly bioactive compound in garlic, poses challenges due to its instability and intricate interactions with the extraction matrix. Their study examines the effectiveness of UAE in improving both the yield and stability of allicin from garlic cloves. Several factors, like ultrasonic power, extraction duration, solvent type, and temperature, were optimized to identify the best conditions for maximizing allicin extraction. The findings revealed that UAE significantly enhanced allicin yield compared to traditional extraction techniques. The optimal conditions included an ultrasonic power of 100 W, a 30-min extraction time, ethanol as the solvent, and 25°C as temperature. In this context, the allicin yield was 45% higher than that achieved with conventional solvent extraction methods.

The study also examined the effect of ultrasonic frequency on allicin stability, finding that a frequency of 40 kHz effectively minimized allicin degradation during extraction. These results highlight UAE as a promising method for the efficient and stable extraction of allicin, with potential applications in both the food and pharmaceutical industries. Additionally, researchers such as Dhwani *et al.*, have investigated the efficiency of ultrasonic-assisted extraction in comparison to other extraction methods for allicin.[31] Extracting allicin, a biologically active compound in garlic, is particularly challenging due to its thermal instability and rapid degradation. This research investigates the use of UAE to improve allicin yield while maintaining its stability. The research systematically evaluates the influence of ultrasonic power, extraction duration, solvent concentration, and temperature on allicin extraction efficiency.

Through a series of experiments, the optimal conditions for allicin extraction were determined to be an ultrasonic power of 150 W, an extraction time of 20 min, 70 % ethanol as the solvent, and a temperature of 20°C, which yielded the highest amount of allicin. Compared to conventional extraction techniques, UAE enhanced allicin yield by 55%, highlighting its efficiency. Furthermore, the study evaluated allicin stability across various ultrasonic frequencies and found that a frequency of 35 kHz most effectively preserved its bioactivity. These results underscore the potential of UAE as a highly effective method for allicin extraction, with significant implications for its use in the nutraceutical and pharmaceutical industries. UAE employs ultrasound energy combined with diluents for extraction of desired components from plant matrices. Ultrasound refers to mechanical waves that have frequencies above 20 kHz, which are higher than the human hearing range of 20 Hz to 20 kHz.

The molecules separate and create cavitation bubbles when the negative pressure during rarefaction is greater than the molecular forces holding them together at high sound wave intensities. Hot spots and extreme local conditions are produced when these bubbles increase through coalescence and collapse during the compression phase. In the United Arab Emirates, bioactive chemicals are typically extracted from fruit and vegetable by-products using frequencies between 20 and 120 kHz.

Garlic cloves were obtained from a local market and stored in the lab under dark conditions at 25°C. To prepare the sample, 10 g of garlic cloves were combined with 100 mL of ion free water. UAE was carried out in an ultrasound cleaning bath (Sonorex, DT1028/H, with interior dimensions of (500 mm×300 mm×200 mm) using indirect sonication. The process was conducted at a stationary frequency of 35 kHz, with the working liquid being used for the extraction. The pre-treated sample mixture was placed into a 100 mL sample flask and positioned in the ultrasonic void for the extraction procedure. The factors optimized included extraction period (30, 60, 90, 120, and 150 min), temperature of extraction (25°C, 30°C, and 35°C), and particle size (combined and cut garlic bulbs). Following sonication, the solution was purified by centrifugation at 3,000 RPM for 2 min. It was then filtered to eliminate any undissolved garlic and stored at 4°C.[32-34]

Supercritical Water Extraction

Supercritical Fluid Extraction (SFE) using Supercritical Carbon Dioxide (SCCO2) is considered the utmost effective approach for isolating individual components from the garlic plant matrix, which comprises a complex blend of unstable components containing sulfur.[35] Rybak *et al.*, (2004) authors of a detailed text on supercritical fluid extraction, provide extensive information on the use of supercritical water in this process.[36] Allicin, a bioactive compound present in garlic, is widely recognized for its antimicrobial, antioxidant, and anticancer benefits. However, traditional extraction methods often result in the degradation of allicin due to its unstable and reactive nature. This research investigates Supercritical Water Extraction (SCWE) as an innovative approach to efficiently extract allicin while preserving its structural stability. Supercritical water, characterized by its exceptional solvent properties at temperatures and pressures beyond its critical point, provides a sustainable and effective alternative to conventional extraction techniques.

Through a series of experiments, the researchers optimized key SCWE parameters such as temperature, pressure, and extraction duration. The findings reveal that SCWE achieves high yield and purity in allicin extraction. When compared to traditional solvent-based methods, SCWE demonstrated notable benefits, including reduced solvent consumption and improved extraction efficiency. The study also delves into the mechanistic details of

allicin extraction using supercritical water, offering valuable insights into the molecular-level interactions between allicin and the supercritical water environment.

The findings indicate that SCWE is a highly promising method for allicin extraction, with the potential to be scaled up for industrial applications. The study contributes to the advancement of more sustainable and efficient extraction methods for bioactive compounds, offering significant benefits for the pharmaceutical, nutraceutical, and food industries. Additionally, Saka and Ueno (1999) conducted extensive studies on the use of supercritical water for environmental remediation and waste management.[37] Allicin, a vital bioactive compound in garlic, is renowned for its therapeutic benefits, including antimicrobial, anticancer, and antioxidant properties. Traditional extraction methods frequently compromise its stability and yield, highlighting the need for more effective and gentle approaches. This study explores the use of SCWE as a method to improve extraction efficiency while maintaining the structural integrity of allicin.

Supercritical water, known for its high diffusivity, low viscosity, and adjustable solvent properties, provides an ideal medium for extracting delicate compounds like allicin. The reported study aims to optimize SCWE conditions-such as temperature, pressure, and extraction time-to maximize allicin yield. Through systematic experiments, the authors determined the optimal parameters that ensure high extraction efficiency while minimizing degradation.

Comparative analyses with conventional extraction methods highlight the benefits of SCWE, including lower solvent consumption, shorter extraction durations, and greater purity of the extracted allicin. Furthermore, the study explores the thermodynamic and kinetic factors involved in allicin extraction using supercritical water, offering a thorough understanding of the underlying process mechanisms.[38]

The findings demonstrate that SCWE is an effective and advanced method for allicin extraction, with promising applications in the pharmaceutical and nutraceutical sectors. This research underscores the potential of supercritical water as a sustainable and efficient solvent for isolating high-value bioactive compounds, setting the stage for future innovations in natural product extraction technologies. SCWE holds significant potential for extracting allicin, as the enzymatic conversion of alliin to allicin requires water for activation. SCWE presents numerous advantages as an entirely green extraction method, utilizing water as the solvent. It also offers economic efficiency in operational costs while providing high selectivity for extracting various classes of compounds.

Carbon dioxide has a crucial role in warranting the complete extraction and separation of samples, offering the advantage of high selectivity. It is particularly effective for extracting oxidation-sensitive substances, as the process avoids exposure to high temperatures and oxygen. Optimal efficiency and garlic yield are achieved at temperatures between 35-50°C and pressures of 300-400 bar, using ethanol as the diluent. In contrast, a study by Rafe and Nadjafi (2014) employed a dynamic mode with a CO_2 flow rate of 20 g/min at 50°C and a pressure of 100 bar.[39]

A sample was prepared by fermenting a 25 g portion of finely chopped garlic in air for 10 min, which is sufficient time to fully convert alliin into allicin, before extraction. Extraction of the garlic constituents were then performed using supercritical carbon

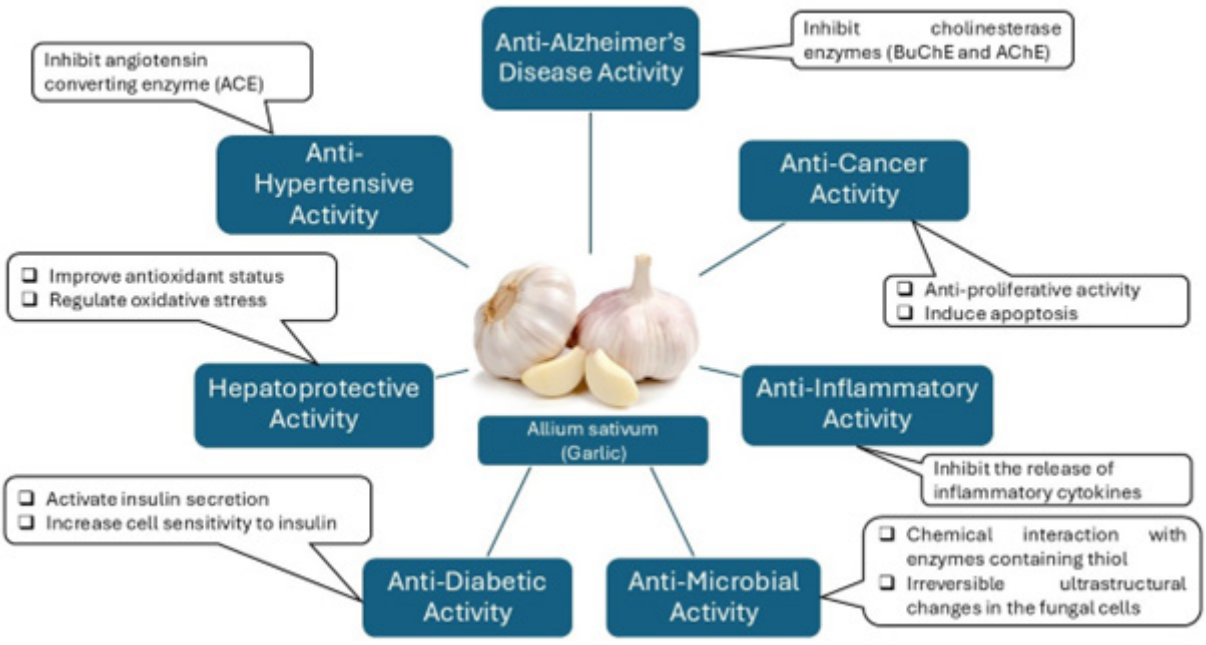

Figure 1: A schematic overview illustrating the diverse pharmacological activities of garlic (*Allium sativum*) and their underlying mechanisms.[16]

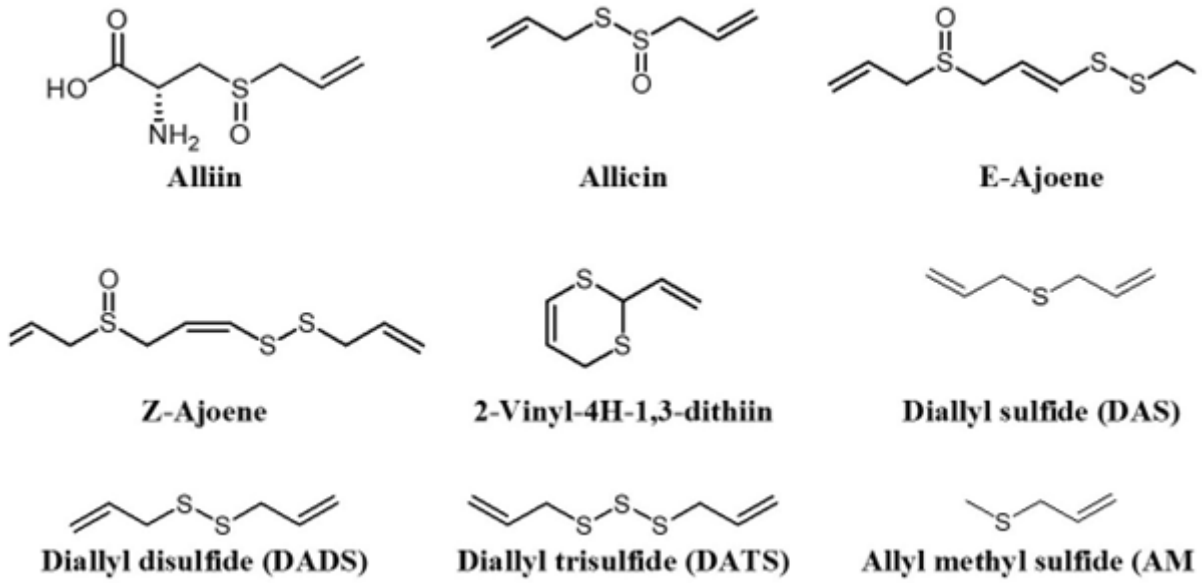

Figure 2: Chemical constructions of garlic components.

dioxide in an arrangement designed for this purpose. The entire setup was constructed from stainless steel, with gaskets made of either Teflon or Graflex, a high thermoresistant graphite-based substance. The apparatus was charged with liquid carbon dioxide to a pressure of approximately 300 bar using a Knauer pump, and the extraction of the samples was carried out using a 50 mL Keystone reactor. Extraction was accomplished for 2 hrs at 250 bar and 40°C (regime 1) or 90°C (regime 2), with a flow rate of 2 mL/min of SCCO2. The supercritical water extraction process yielded allicin concentrations varying from 0.31 to 1.76 μg/g.[35]

Microwave Extraction Method

The extraction of essential oil from garlic is a key area of research due to its significant potential in both the pharmaceutical and food industries. MAE is an emerging methodology for extracting garlic's essential oil. MAE provides several benefits, such as shorter extraction times, reduced solvent usage, and greater efficiency compared to conventional distillation techniques.

MAE of allicin from garlic has been widely studied for its efficiency and effectiveness in comparison to traditional extraction methods. Several researchers have made significant contributions to this area by conducting studies that compare various extraction techniques to optimize the MAE process and analyze the yields and concentrations of alliin and allicin in garlic.[40]

MAE operates by using electromagnetic waves to heat both the solvent and plant tissues, leading to changes in the cell structure that facilitate the release of bioactive compounds like allicin. In order to facilitate the transfer of analytes from the sample matrix into the solvent, it uses microwave energy to heat solvents that come in contact with a sample. The capacity of MAE to quickly heat the sample solvent mixture is its main benefit. The extraction process can be carried out at higher temperatures by employing closed vessels, which speeds up the mass transfer of the target chemicals from the sample matrix. A typical extraction process lasts between 15 and 30 min and utilizes little portions of solvent, typically ranging from 10 to 30 mL. Ethanol is commonly used as the solvent, with fixed extraction parameters such as temperature, time, and microwave power. Domestic microwaves are employed for the extraction, which can be done either with or without diluent. Solvent-assisted microwave extraction is more effective, as the solvent helps break down the cell structure. The principles of MAE differ from those of conventional methodologies (such as solid-liquid extraction) because the extraction process is driven by changes in the cell structure induced by electromagnetic waves. Prolonged contact with microwave radiation can result in the allicin decomposition.[41]

The samples were meticulously sorted and dried in air in an open platter till a steady weight was reached before the MAE extraction trial. Once dried, the samples were powdered to a fine size (0.1 mm) with the help of a laboratory crusher and stored in an airtight container.

MAE of garlic powder was carried out by means of a reformed domestic microwave oven (HMT72M450, Bosch, Gerlingen), (275 mm×460 mm×380 mm) and operates at a frequency of 2450 kHz. The equipment was fitted with a digital control system to regulate the microwave power and irradiation time according to different experimental design parameters. A powdered garlic sample was placed into a 250 mL round-bottom flask, along with 100 mL of distilled water as extraction solvent, to achieve the desired solid-liquid ratio for the extraction mixture. The extract was obtained at various extraction times and cooled to room temperature. It was then filtered through a funnel with Whatman No. 1 filter paper, and the filtrate was accumulated in a laboratory volumetric flask. Lastly, the extract was stored at 40°C for the

analysis of Total Phenolic Content (TPC).[42] Overview of various techniques employed for extracting active compounds from garlic is given in Table 1.

QUANTIFICATION METHODS

After extraction, the quantification of allicin is the next step to determine its concentration. Chromatographic methods are preferred due to their reliability, selectivity, and accuracy in minimizing interference from other sulfur-containing compounds. High performance liquid chromatography is commonly used for this intention. These techniques rely on external standards to compare and analyze the samples. HPLC is commonly preferred for allicin quantification because of its simplicity, accessibility, and precision. This process aids in isolating the desired amount of allicin and provides an accurate measurement of its quantity. Quantifying allicin is crucial as it supports further research and analysis of garlic's therapeutic and medicinal properties.

High Performance Liquid Chromatography (HPLC)

HPLC is typically utilized for the detection of analytes. Unlike many other phytoconstituents, allicin is rarely detected using the simpler HPTLC technique due to challenges posed by its unique structure, despite the method's simplicity and its high demand for analytical monitoring of Allium species. Pure commercial allicin was used as a reference, and the sample was made by dissolving fresh garlic cloves in deionized water. The methodology was effectively utilized to real Allium species samples, yielding results that closely aligned with HPLC data. A solvent mixture, typically composed of aqueous and organic solvents like methanol or acetonitrile in a 60:40 (v/v) ratio, was utilized as the elution solvent. Phosphate buffers, such as potassium dihydrogen phosphate, were often employed to adjust the solution's pH, usually within the range of 2 to 4. The flow rate was generally fixed at 1.0 mL/min. HPLC produced distinct peaks, as shown in Figures 3 and 4.[52]

Chromatogram of allicin extracted from garlic, analyzed using HPLC and UV Spectroscopy. Lawson and Hughes are recognized for their contributions to the stability and analysis of allicin in garlic using HPLC.[53] Allicin, a powerful bioactive compound found in garlic, is extensively studied for its antimicrobial, anti-inflammatory, and anticancer effects. Precise quantification and characterization of allicin are essential for research and industrial purposes. HPLC provides a reliable analytical method for detecting and quantifying allicin, ensuring accuracy and consistency.

This investigation details the development and optimization of an HPLC methodology specifically designed for allicin determination. Critical factors like eluent solvent composition, flow rate, detection wavelength, and column selection were systematically assessed to improve sensitivity and resolution. The optimized HPLC conditions were authenticated for Limit of Detection (LOD), Limit of Quantification (LOQ), linearity, accuracy and precision.

The results indicate that the optimized HPLC method offers a reliable and efficient solution for allicin analysis, exhibiting high sensitivity and specificity. This methodology was utilized for different garlic extracts to evaluate allicin content, uncovering

Table 1: Overview of techniques employed for extracting active compounds from garlic (*Allium sativum* L.).

Type of extract	Extraction Methodology	Analyses Performed	References
Aqueous	Distillation of the garlic extract using lowered pressure	Evaluation of the antiproliferative effects of copper-rich garlic extract.	[43]
	Pressing extraction	Detection of allicin with antitumor properties.	[44]
Methanol	Maceration	Examination of cell viability and apoptosis in leukemia cells.	[45]
Ethanol	Solvent extraction	Assessment of motor synchronisation and Purkinje cell count in rats.	[46]
	Solvent extraction	Evaluation of antibacterial activity against Staphylococcus aureus.	[47]
Chloroform	Solvent extraction using lowered pressure	Evaluation of the anti-inflammatory effects of aged black garlic.	[48]
Fresh material	Combined with water	Examination of Nitric Oxide (NO) and Interferon-(IFN-) concentration in plasma.	[49]
Freeze dried material	NDA*	Storage of broken up meat.	[50]
Oil	Steam distillation	Storage of minced meat.	[50]
	NDA*	Examination of the cytotoxic mechanism of DATS in leukemia cells.	[51]

*NDA: No data available.

notable variations in allicin concentration based on the extraction technique and garlic variety.

This study also examines allicin's stability under various storage conditions and its degradation kinetics, offering important insights into the handling and processing of garlic-based products. The HPLC method developed has applications beyond research settings, presenting potential advantages for quality control in the nutraceutical and pharmaceutical industries.

In conclusion, the optimized HPLC methodology established in this research serves as an effective tool for the accurate quantification and analysis of allicin, supporting further research and industrial utilization of this bioactive compound. Iberl *et al.*, (1990) carried out studies focused on optimizing HPLC conditions for allicin quantification.[54] Allicin, an active biocompound in garlic, possesses diverse therapeutic properties, including antimicrobial, antioxidant, and anticancer effects. Precise quantification and analysis of allicin are critical for clinical research and the development of garlic-based health products. HPLC is widely regarded as a dependable and accurate technique for detecting and quantifying allicin across various matrices.

This research outlines the development and validation of a reliable HPLC method for allicin analysis. Key parameters, including selecting stationary phase, elution solvent constitution, flow rate, and detection wavelength, were optimized to ensure high sensitivity and specificity. The methodology was validated in conformity with ICH guidelines, evaluating factors like repeatability, linearity, accuracy, precision, Limit of Detection (LOD), and Limit of Quantification (LOQ).

The optimized HPLC method exhibited outstanding linearity across a broad concentration series, along with high precision and accuracy. It was utilized to quantify allicin in various garlic extracts and formulations, revealing differences in allicin content among products.

Their findings suggest that the developed HPLC method is highly efficient for routine allicin analysis, supporting quality control and standardization in the production of garlic-based supplements and pharmaceuticals. This study highlights the significance of HPLC in the analytical profiling of bioactive compounds and its contribution to advancing nutraceutical and pharmaceutical research.

In summary, the HPLC method established in this study offers an accurate and dependable approach for quantifying allicin, enabling further research and application of this powerful bioactive compound in various health-related fields.

High Performance Thin Layer Chromatography (HPTLC)

Allicin is a bioactive compound present in garlic (*Allium sativum*) and other Allium species, recognized for its strong antimicrobial, antioxidant, and potential therapeutic effects. Accurate quantification of allicin is crucial for assessing the quality of garlic products and investigating its health benefits. HPTLC provides a reliable technique for the separation, identification, and quantification of allicin, owing to its high sensitivity and specificity. Pure commercial allicin is used as a reference compound, while the sample is prepared from freshly harvested garlic cloves, extracted with solvents like ethanol, methanol, or acetone. An example of a developing solvent used in thin-layer chromatography is a mixture of ethyl acetate and methanol (75:20:5 v/v/v). Anisaldehyde-sulfuric acid is used as visualizing agent in the method. Silica gel 60 F254 plates were utilized as stationary phase and were kept in oven to remove impurities before use. Ensure proper spotting technique to achieve well-defined spots with minimal overlap. The spotted plate was placed in pre-saturated developing chamber. Allicin spots were observed under UV light at a wavelength of 254 nm. Wagner and Bladt are pioneers in the field of HPTLC, making significant contributions to the development of methods for analyzing plant-derived compounds, including allicin.[55] Allicin,

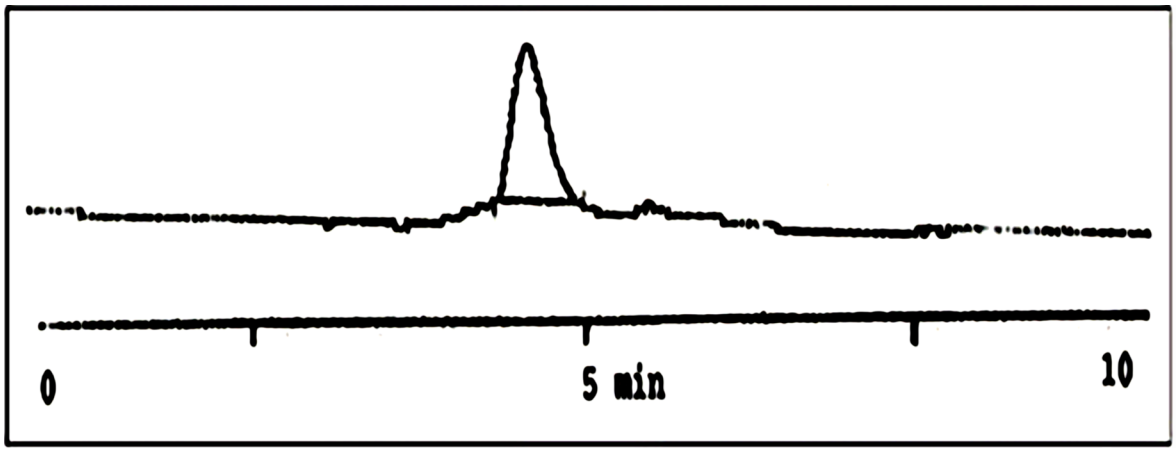

Figure 3: Chromatogram of a standard allicin solution (10 µg/mL) obtained using high-performance liquid chromatography and a UV analyzer.

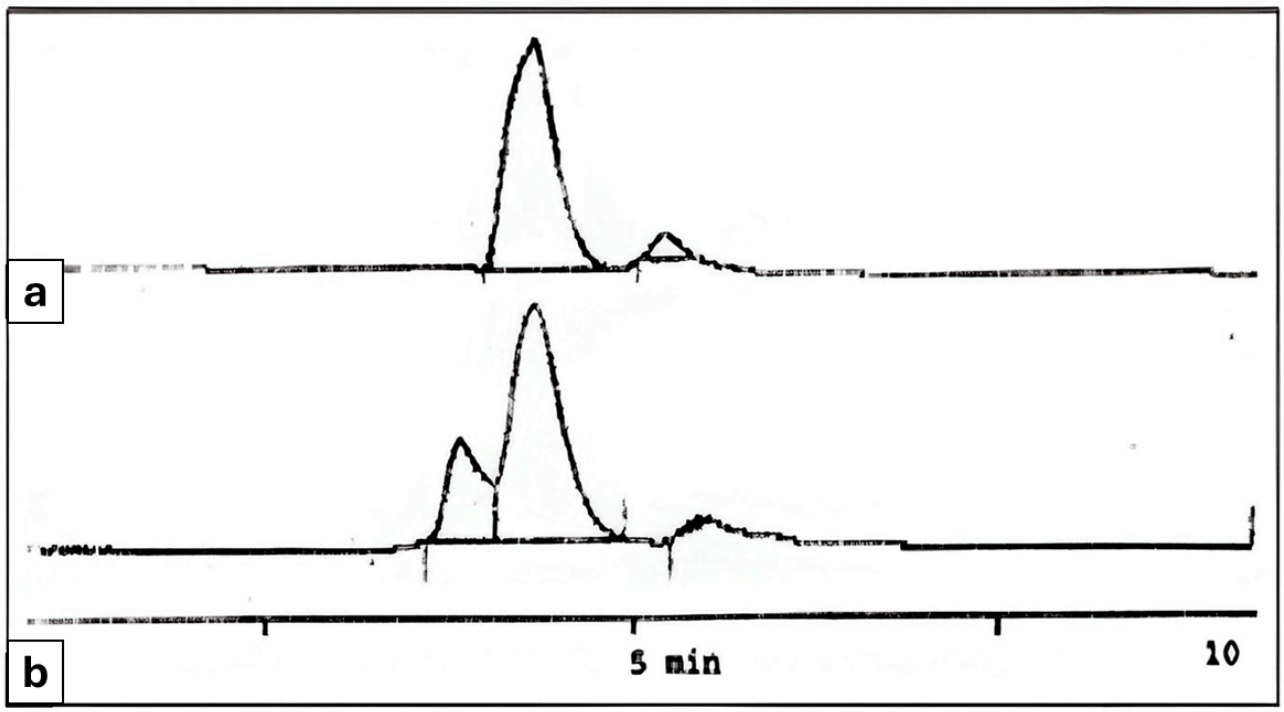

Figure 4: Chromatogram of purified garlic extracts (10 µg/mL) achieved through HPLC with ultraviolet detection, showcasing the application of (a) bath sonication and (b) probe sonication techniques.

a sulfur-containing compound extracted from garlic, is renowned for its broad pharmacological actions, such as antimicrobial, antioxidant, and anticancer effects. Accurate quantification and analysis of allicin are essential for scientific research and quality control in the nutraceutical and pharmaceutical industries. HPTLC provides a fast, cost-effective, and efficient method for analyzing allicin in a variety of garlic-based products.

This research outlines the development and optimization of an HPTLC methodology for both qualitative and quantitative analysis of allicin. Key factors like the choice of stationary phase, elution solvent composition, application technique, and detection method were systematically optimized to improve resolution and sensitivity. The methodology was validated for parameters including specificity, Limit of Detection (LOD), and Limit of Quantification (LOQ), linearity, precision, accuracy.

The optimized HPTLC methodology showed excellent separation and quantification of allicin, with high reproducibility and accuracy. It was effectively utilized to analyze allicin content in various garlic extracts and commercial formulations, highlighting notable differences in allicin concentration. The study also examined the stability of allicin during sample preparation and storage, offering valuable insights for the proper handling of garlic-based products.

The results suggest that HPTLC is a reliable and effective analytical technique for the quick screening and quantification of allicin. This method provides notable benefits as per speed, cost, and simplicity in comparison with conventional chromatographic methodology, making it ideal for routine quality control and large-scale screening applications.

In conclusion, the HPTLC methodology developed in this research work offers an effective and precise approach for analyzing allicin, aiding both research activities and quality control in the production of garlic-based health products. Blania and Spangenberg (1991) contributed to the determination of allicin and ajoene in dried garlic and garlic preparations.[56] Allicin, the main bioactive compound in garlic, is well-known for its broad range of therapeutic benefits, including antimicrobial, antioxidant, and anticancer properties. Accurate analysis and quantification of allicin are crucial for assessing the effectiveness and quality of garlic-based products. HPTLC offers a flexible and efficient technique for the rapid screening and quantification of allicin.

This study aims to develop and validate an HPTLC methodology specifically designed for the accurate investigation of allicin and ajoene. Several experimental factors, including the choice of stationary phase, optimization of the eluent solvent composition, sample application techniques, and detection methods, were carefully assessed to achieve the best resolution and sensitivity. The method validation factors, like Limit of Detection (LOD), and Limit of Quantification (LOQ), precision, specificity, linearity, and accuracy were thoroughly evaluated in accordance with ICH strategies.

The optimized HPTLC method showed excellent separation of allicin, with high reproducibility and accuracy. When applied to

various garlic extracts and commercial formulations, the method revealed significant differences in allicin content, highlighting the importance of standardized analytical techniques for quality control. Furthermore, the study examined the stability of allicin under different sample preparation and storage conditions, offering important insights into the proper handling and preservation of garlic products.

The results demonstrate that HPTLC is a strong, cost-efficient, and dependable analytical methodologies for both qualitative and quantifiable analysis of allicin. This approach provides notable benefits with regard to speed, ease, and cost in comparison conventional chromatographic methods, making it highly suitable for regular quality control and large-scale screening in the nutraceutical and pharmaceutical industries.

In conclusion, the HPTLC method established and validated in this research offers a robust and effective answer for the precise quantification of allicin, facilitating ongoing research and ensuring quality control in the production of garlic-based health products.

UV Spectroscopy

UV-visible spectrophotometry offers a simple and dependable approach for quantitatively analyzing allicin, thanks to its distinct absorption in the UV spectrum. While methods described in literature can be complex and require costly equipment, more affordable and efficient techniques are desirable for rapid quantification of allicin in garlic extracts, particularly in industrial settings. Thus, the purpose of this research was to develop a quick and economical UV spectrophotometric method for determining allicin in aqueous garlic extracts. 1 g of garlic cloves is collected and crushed in a mortar and pestle with 10 mL of water. After 24 hr, the garlic solution is filtered through Whatmann filter paper no. 42, and the filtrate is stored in a refrigerator. To analyze the allicin content of the garlic extract, it is passed through an SPE cartridge and eluted using solvents of different polarities. Water, being highly polar solvent, effectively elutes allicin within 4 mL, whereas methanol and ethanol fail to elute it. Elution of garlic fraction was done with water showed an absorbance ratio of A240 nm/A254 nm ranging from 1.4 to 1.5, which is characteristic of allicin. Phosphate buffer (pH 7.4) was made use to maintain the stability of the allicin solutions. Previous research has reported that the absorbance ratio of allicin, measured at wavelengths of 240 nm and 254 nm using water as blank, typically falls within the range of 1.4 to 1.5. This method can be employed for quality control of garlic bulbs, monitoring batch consistency in formulations based on garlic, and usually for the standardization of products based on garlic.

Wanyika *et al.*, (2010) carried out pioneering research on the quantitative determination of allicin in aqueous garlic extracts.[57] Allicin, a major sulfur-containing compound in garlic, is recognized for its strong biological actions, like antimicrobial, reducing, and antitumor activities. Precise quantification and analysis of allicin are essential for research and the development of garlic-based therapeutic products. UV spectroscopy offers a fast, straightforward, and affordable method for analyzing allicin in different garlic extracts and formulations.

This research explores the developing and optimizing a UV spectroscopic methodology for the quantitative analysis of allicin. The absorption properties of allicin were analyzed to determine the ideal wavelength for detection. Important factors such as solvent choice, sample preparation, and the creation of a calibration curve were carefully optimized to improve the method's sensitivity and accuracy. The methodology was validated by assessing factors like Limit of Detection (LOD), and Limit of Quantification (LOQ), linearity, precision, accuracy.

The outcomes reveal that the UV spectroscopic methodology offers a reliable and effective way to quantify allicin, exhibiting excellent linearity and reproducibility. This methodology was efficaciously employed to different garlic extracts, enabling quick evaluation of allicin content. Comparative studies with well-established chromatographic techniques emphasized the benefits of UV spectroscopy, such as its simplicity, speed, and cost-effectiveness, making it especially suitable for day to day investigation and quality control.

Furthermore, the study examined the stability of allicin under various environmental conditions, providing essential insights into the best practices for storing and handling garlic products. The UV spectroscopic method proved to be highly effective for the fast screening and quality evaluation of allicin in both research and industrial applications.

In conclusion, the UV spectroscopy methodology established in this research offers a robust, efficient, and accessible approach for quantifying allicin, supporting ongoing research and ensuring quality control in the production of garlic-based health products.

Chaudhari *et al.*, (2024) simultaneously estimated allicin and *Azadirachta indica* extracts in niosomal formulation.[58] Allicin, the primary bioactive compound in garlic, is well-accepted for its therapeutic benefits, like antimicrobial, antioxidant, and anticancer properties. Accurate quantification and analysis of allicin are crucial for scientific research and the creation of garlic-based health products. UV spectroscopy provides a fast, straightforward, and affordable approach for detecting and measuring allicin.

This study centers on the development and validation of a UV spectroscopic methodology for quantifying allicin. The absorption spectrum of allicin was examined to identify the ideal wavelength for its detection. Key factors, including the selection of solvent, sample preparation methods, and the construction of the calibration curve, were carefully optimized to improve the

method's sensitivity and accuracy. The methodology was then validated as per ICH recommendations

The optimized UV spectroscopic methodology showed outstanding sensitivity and specificity for allicin, with a clear linear correlation across a wide concentration range. This method was effectively used to quantify allicin in different garlic extracts and commercial garlic products, revealing notable differences in allicin content. A comparative analysis with conventional chromatographic methods demonstrated that UV spectroscopy offers a quicker and more cost-effective option for routine analysis.

In addition, the stability of allicin under various storage and preparation conditions was examined, offering crucial insights for the proper handling and preservation of garlic products. The UV spectroscopic method was found to be a reliable and efficient tool for quick screening and quality control of allicin.

In conclusion, the UV spectroscopic methodology established in this research offers an effective and efficient means for the precise quantification of allicin, contributing to ongoing research and quality assurance in the nutraceutical and pharmaceutical sectors. Cavallito and Bailey (1944) were early pioneers in identifying and characterizing allicin using UV absorption techniques.[59] Allicin, a bioactive compound present in garlic, demonstrates strong antioxidant, antimicrobial, and anticancer effects. Precisely quantifying allicin is essential for assessing its therapeutic value and maintaining the quality of garlic-based products. UV spectroscopy offers a simple and economical approach for the quantitative analysis of allicin.

This study centers on the creation and validation of a UV spectroscopic methodology specifically designed for the detection and quantification of allicin. Key parameters, including wavelength selection, solvent composition, and sample preparation techniques, were optimized to improve the sensitivity and accuracy of the method. Further, the method underwent thorough validation in line with established guidelines.

The optimized UV spectroscopic methodology exhibited exceptional sensitivity and specificity for allicin, showing a linear response over a broad concentration range. When applied to different garlic extracts and formulations, the method revealed notable differences in allicin content, highlighting the necessity of standardized analytical techniques for quality control.

Comparative studies with traditional chromatographic methods highlighted the benefits of UV spectroscopy, including its simplicity, speed, and cost-effectiveness, making it particularly suitable for day-to-day investigation and large-scale screening in the pharmaceutical and food industries.

Additionally, the study examined allicin's stability under various environmental conditions, offering critical insights into its degradation kinetics and optimal storage requirements. The UV spectroscopic method demonstrated robustness and reliability for the rapid evaluation of allicin content across a range of samples. In conclusion, the UV spectroscopic methodology established in this research offers a practical and effective solution for the precise quantification of allicin, facilitating progress in research and ensuring quality control in the production of garlic-based products.

CONCLUSION

Garlic has been traditionally utilized in many regions for its numerous biological benefits and therapeutic effects. It exhibits an extensive variety of biological actions, including antidiabetic, anti-inflammatory, and antimicrobial properties. Garlic is particularly effective in managing cardiovascular diseases and has shown promising results as a hepatoprotective agent. Allicin, a sulfur-containing compound responsible for garlic's characteristic pungent smell, is known for its potent anticancer properties. Several extraction methodologies have been applied to separate allicin, followed by its quantification to determine its concentration. Techniques such as HPLC, HPTLC, and UV spectroscopy are highly sensitive and specific, making them valuable tools for accurately quantifying allicin.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UAE: Ultrasonic-Assisted Extraction; **SCWE:** Supercritical Water Extraction; **MAE:** Microwave-Assisted Extraction; **HPLC:** High-Performance Liquid Chromatography; **HPTLC:** High-Performance Thin-Layer Chromatography; **UV:** Ultraviolet Spectroscopy; **LOD:** Limit of Detection; **LOQ:** Limit of Quantification; **ICH:** International Conference on Harmonization; **SFE:** Supercritical Fluid Extraction; **$SCCO_2$:** Supercritical Carbon Dioxide; **TPC:** Total Phenolic Content.

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