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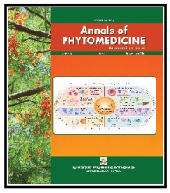

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Sinigrin: A next-generation bioactive compound for pharmaceutical and industrial applications

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Abstract

Sinigrin, a prominent aliphatic glucosinolate predominantly found in Brassicaceae species, has gained increasing attention due to its diverse biological activities and industrial relevance. This review provides a comprehensive overview of sinigrin, covering its natural sources, chemical structure, physicochemical properties, biosynthesis, metabolism, pharmacological activities and industrial applications. Sinigrin is widely distributed in plants such as *Brassica nigra*, *Brassica juncea*, *Brassica oleracea* and *Armoracia rusticana*, with its content influenced by genetic, environmental and processing factors. Structurally, sinigrin is characterized by a thioglucoside linkage and a sulfate group, which contribute to its high polarity and reactivity. The biological activity of sinigrin is primarily attributed to its enzymatic hydrolysis product, allyl isothiocyanate, which plays a key role in mediating its pharmacological effects. These include anticancer, antimicrobial, anti-inflammatory, antioxidant, antidiabetic, cardioprotective and neuroprotective properties, largely through modulation of cellular signaling pathways such as Nrf2, NF-κB and MAPK. The review also highlights the importance of sinigrin metabolism and bioavailability, emphasizing the role of myrosinase and gut microbiota in its activation. In addition to its health benefits, sinigrin has significant industrial applications. It is widely used as a natural flavoring agent, antimicrobial preservative in food systems, biofumigant in agriculture and functional ingredient in pharmaceutical and cosmetic formulations. However, challenges such as instability, low bioavailability, variability in natural sources and difficulties in standardization limit its full exploitation. Emerging approaches, including nanotechnology-based delivery systems and metabolic engineering, offer promising solutions to enhance its stability and efficacy.

1. Introduction

Plants synthesize an extraordinary array of chemical compounds that extend beyond those directly required for growth and development. These compounds, collectively referred to as secondary metabolites, play a central role in plant survival, adaptation and ecological interactions. Unlike primary metabolites, secondary metabolites are often species-specific and are produced in response to environmental stimuli such as herbivory, pathogen attack and abiotic stress. These compounds function as chemical defenses, signaling molecules and mediators of plant-plant and plant-microbe interactions. In recent years, plant secondary metabolites have attracted considerable attention due to their wide range of biological activities and potential applications in pharmaceuticals, nutraceuticals and sustainable industrial systems (Nguyen *et al*., 2020; Bansal *et al*., 2024). Their chemical diversity and bioactivity make them valuable candidates for drug discovery and functional food development. Among these compounds, glucosinolates constitute a unique group of sulfur- and nitrogen-containing secondary metabolites predominantly found in the Brassicales order, particularly within the Brassicaceae family. Structurally, glucosinolates share a common core consisting of a thioglucose moiety, a sulfonated oxime group and a variable side chain derived from amino acids. Based on their precursor amino acids, glucosinolates are broadly classified into aliphatic, indolic and aromatic types, originating from methionine, tryptophan and phenylalanine or tyrosine, respectively. This structural diversity contributes to their wide range of biological functions and ecological roles. In intact plant tissues, glucosinolates remain biologically inactive; however, upon tissue disruption, they are hydrolyzed by the enzyme myrosinase to produce bioactive compounds such as isothiocyanates, nitriles and thiocyanates. This enzymatic system, often referred to as the "mustard oil bomb," serves as an effective plant defense mechanism against herbivores and pathogens (Blaževiæ *et al*., 2020; Nguyen *et al*., 2020).

Within the diverse glucosinolate family, sinigrin has emerged as one of the most prominent and extensively studied aliphatic glucosino-

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lates. Sinigrin, also known as allyl glucosinolate, is widely distributed in economically important Brassicaceae crops such as mustard, cabbage and horseradish. It is particularly abundant in seeds and roots, where it contributes significantly to plant defense. Upon enzymatic hydrolysis, sinigrin produces allyl isothiocyanate, a volatile compound responsible for the pungent aroma and characteristic flavor of mustard-based products. Beyond its sensory attributes, sinigrin-derived compounds exhibit a wide spectrum of biological activities, including antimicrobial, antioxidant, anti-inflammatory and anticancer effects (Ferara *et al*., 2025; Du *et al*., 2025). These properties have positioned sinigrin as a key bioactive phytochemical with significant relevance in both human health and industrial applications. The scientific investigation of sinigrin dates back to early studies on mustard and related plants, where its pungency and biological effects were initially observed. Over time, advancements in analytical chemistry facilitated the isolation and structural elucidation of glucosinolates, including sinigrin. Modern analytical techniques such as high-performance liquid chromatography and spectrophotometric methods have further enabled precise quantification and characterization of glucosinolates in plant tissues (Galasso *et al*., 2025; Redha *et al*., 2024). These developments have significantly contributed to understanding the distribution, variability and functional roles of sinigrin across different plant species and environmental conditions.

In recent years, sinigrin has gained renewed interest due to its potential role in addressing global health challenges. Increasing evidence suggests that sinigrin-derived isothiocyanates can modulate key molecular pathways involved in inflammation, oxidative stress and carcinogenesis. For instance, sinigrin has been reported to influence signaling pathways such as NF-κB and MAPK, thereby reducing inflammatory responses and oxidative damage (Du *et al*., 2025). Additionally, studies have indicated its potential in improving metabolic functions and reducing the risk of chronic diseases, further supporting its role as a functional food component (Ferara *et al*., 2025). The growing body of research highlights the importance of sinigrin in preventive healthcare and nutraceutical development. Apart from its pharmacological significance, sinigrin also exhibits considerable potential in industrial and agricultural applications. In the food industry, sinigrin and its hydrolysis products are increasingly being explored as natural preservatives due to their antimicrobial properties (Figure 1). The shift toward clean-label and chemical-free food systems has further accelerated research into glucosinolate-based preservation strategies. In agriculture, sinigrin-rich plant materials are utilized in biofumigation practices, where the release of isothiocyanates suppresses soil-borne pathogens and pests. Recent studies have also demonstrated the allelopathic effects of sinigrin, suggesting its potential in weed management and sustainable crop production systems (Ko *et al*., 2024). Furthermore, the diversity of glucosinolate profiles in *Brassica* germplasm provides opportunities for crop improvement and functional trait enhancement (Iwar *et al*., 2024).

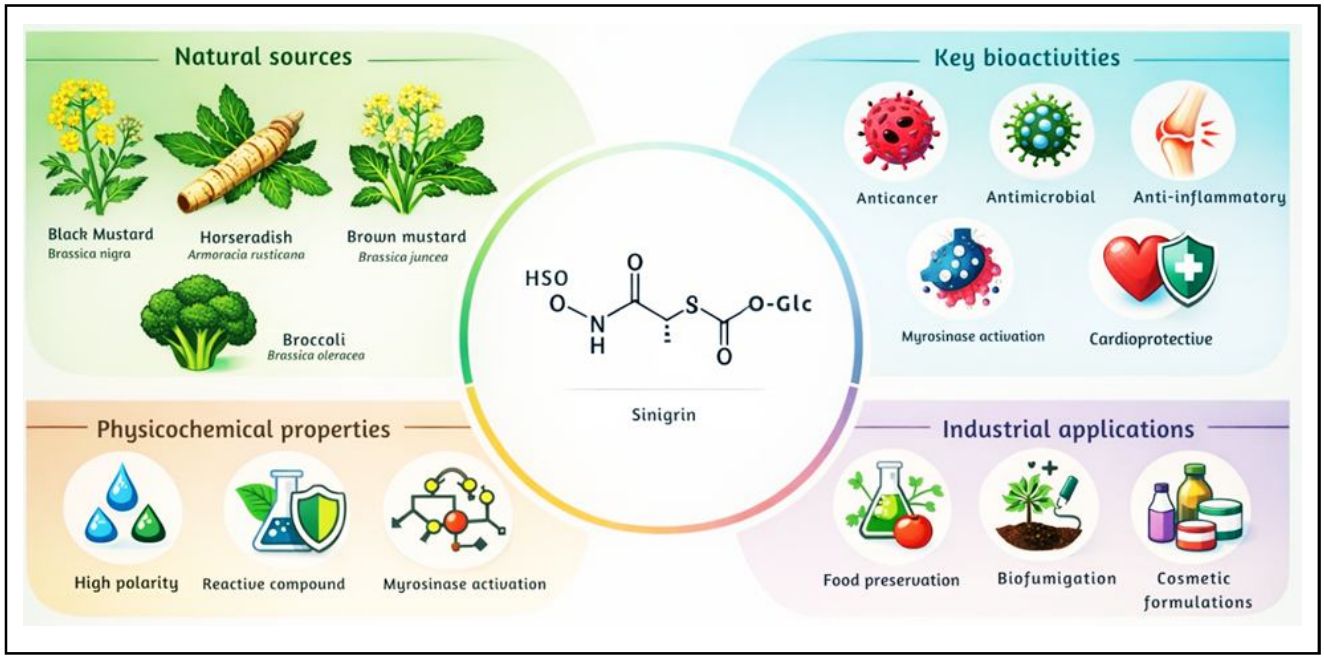

Figure 1: Natural sources, chemical structure, physicochemical properties, bioactivities and applications of sinigrin.

Another critical aspect of sinigrin research is its biosynthesis and genetic regulation. Sinigrin is synthesized through a complex metabolic pathway involving amino acid chain elongation, core structure formation and secondary modifications. Advances in genomics and metabolomics have enabled the identification of key genes and enzymes involved in glucosinolate biosynthesis, opening new avenues for metabolic engineering and crop improvement (Blaževiæ *et al*., 2020; Bansal *et al*., 2024). These approaches have significant implications for enhancing the nutritional quality and functional properties of Brassicaceae crops. Additionally, emerging technologies such as nanotechnology and encapsulation strategies are being explored to improve the stability, bioavailability and controlled delivery of sinigrin in food and pharmaceutical systems. Despite these promising developments, several challenges remain in fully exploiting the potential of sinigrin. Variability in sinigrin content due to genetic, environmental and agronomic factors poses challenges for

standardization and large-scale utilization. Moreover, the bioavailability and metabolic fate of sinigrin in humans are influenced by multiple factors, including gut microbiota composition and dietary interactions. Concerns regarding the safety and potential toxicity of certain hydrolysis products also necessitate careful evaluation. Addressing these challenges requires a multidisciplinary approach integrating plant science, analytical chemistry, food technology and clinical research (Singh *et al*., 2025).

In this context, sinigrin represents a promising bridge between plant-derived natural products and modern therapeutic and industrial applications. Its well-defined biosynthetic pathway, diverse biological activities and wide distribution in commonly consumed crops make it an ideal candidate for functional and translational research. Therefore, the present review aims to provide a comprehensive overview of sinigrin, focusing on its sources, biosynthesis, pharmacological properties and industrial applications. By integrating recent research findings, this review seeks to highlight the significance of sinigrin as a next-generation bioactive compound and to identify future directions for its scientific and industrial utilization.

2. Natural sources and distribution of sinigrin

2.1 Botanical sources

Sinigrin (allyl glucosinolate) is one of the most predominant aliphatic glucosinolates occurring in plants of the Brassicaceae family, a group widely recognized for its nutritional and phytochemical richness. These sulfur-containing metabolites are characteristic of cruciferous vegetables and play essential roles in plant defense and ecological interactions. Glucosinolates, including sinigrin, are biosynthesized from amino acids and are particularly abundant in Brassica crops such as mustard, cabbage and radish (Abdel-Massih *et al*., 2023; Baldelli *et al*., 2025). The occurrence of sinigrin is closely linked to plant evolutionary adaptation, where it functions as a chemical defense compound against herbivores and pathogens through its hydrolysis products. Among the Brassicaceae members, *B. nigra* (black mustard) and *B. juncea* (Indian mustard) are recognized as major reservoirs of sinigrin. Mustard seeds, in particular, are rich in glucosinolates, with sinigrin being the dominant compound responsible for their pungency and biological activity (Das *et al*., 2022; Zhang *et al*., 2022). In *B. juncea*, sinigrin accumulation contributes significantly to its phytochemical profile, supporting its use in food, medicine and industrial applications (Hu *et al*., 2025). Similarly, *B. oleracea* species, including cabbage, broccoli and cauliflower, contain sinigrin along with other glucosinolates, although its concentration varies depending on cultivar and plant organ (Table 1).

Another important botanical source of sinigrin is *A. rusticana* (horseradish), where it is highly concentrated in roots. The hydrolysis of sinigrin in horseradish produces allyl isothiocyanate, contributing to its sharp flavor and antimicrobial properties. In addition to these major crops, sinigrin is also found in *Raphanus sativus* and other cruciferous vegetables, albeit in varying amounts. The diversity of glucosinolate composition across Brassicaceae species reflects both genetic and metabolic specialization, as well as adaptation to environmental pressures (Zhu *et al*., 2023). The distribution of sinigrin within plant tissues is not uniform and varies significantly among seeds, roots, leaves and reproductive organs. Seeds are generally the richest source of sinigrin, as they require strong chemical protection against pests and microbial attack during dormancy. Studies have shown that sinigrin is the predominant glucosinolate in mustard seeds, while its hydrolysis product, allyl isothiocyanate, plays a key role in defense and flavor (Zhang *et al*., 2022). In vegetative tissues, sinigrin is commonly present in leaves and stems, although its concentration is typically lower than in seeds. However, in certain mustard varieties, sinigrin has been reported as a major glucosinolate in aerial plant parts, indicating its broader distribution across plant organs (ResearchGate study, 2025).

Table 1: Natural sources and distribution of sinigrin in Brassicaceae plants

Plant species	Common name	Plant part	Sinigrin content	Key influencing factors	References
B. nigra	Black mustard	Seeds	Very high	Genotype, sulfur availability	Bondonno *et al*., 2022
B. juncea	Indian mustard	Seeds, leaves	High	Climate, soil nutrients	Ali *et al*., 2025
B. oleracea	Cabbage, broccoli	Leaves, florets	Moderate	Growth stage, storage	Costa-Pérez *et al*., 2023
A. rusticana	Horseradish	Roots	High	Processing conditions	Hanschen *et al*., 2020
Brassica rapa	Turnip	Roots, leaves	Moderate	Environmental stress	Ko *et al*., 2024
Sinapis alba	White mustard	Seeds	Low	Genetic variation	Blaževiæ *et al*., 2020
R. sativus	Radish	Roots	Moderate	Harvest maturity	Traka and Mithen, 2022

Roots, particularly in horseradish and root mustard, also accumulate significant amounts of sinigrin, contributing to below-ground defense against soil pathogens. The distribution pattern is influenced by plant developmental stage, with young tissues generally exhibiting higher glucosinolate levels due to increased susceptibility to herbivory. Such spatial and temporal variation highlights the functional importance of sinigrin in plant defense and ecological adaptation.

2.2 Geographical and environmental influence

The accumulation and distribution of sinigrin are strongly influenced by environmental conditions, including climate, soil properties and agricultural practices. Temperature is a critical factor regulating glucosinolate biosynthesis, as enzymatic activities involved in the pathway are sensitive to thermal conditions. Moderate temperatures generally promote glucosinolate accumulation, whereas extreme heat or cold can disrupt metabolic processes and reduce sinigrin content. Environmental stress conditions, such as drought and high exposure, may also induce glucosinolate production as part of the plant's adaptive response (Zhu *et al*., 2023). Soil composition, particularly sulfur and nitrogen availability, plays a crucial role in sinigrin biosynthesis. Since glucosinolates are sulfur-containing compounds, adequate sulfur nutrition is essential for their production. Studies

have demonstrated that sulfur fertilization significantly enhances glucosinolate content in Brassica crops, whereas deficiency leads to reduced accumulation. Nitrogen availability also influences amino acid metabolism, which serves as the precursor for glucosinolate biosynthesis. The balance between sulfur and nitrogen nutrition is therefore critical for optimizing sinigrin production (Baldelli *et al*., 2025).

Geographical factors such as altitude, photoperiod and regional climatic conditions contribute to variations in sinigrin content across different growing regions. Plants grown at higher altitudes often experience increased UV radiation and temperature fluctuations, which can stimulate secondary metabolite production. Additionally, field studies have revealed substantial variation in glucosinolate profiles among populations and species collected from different geographical locations, indicating the influence of environmental heterogeneity (Recent Brassicaceae diversity study, 2025). Agronomic practices, including irrigation, fertilization and cultivation systems, further affect sinigrin accumulation. Water stress, for example, can lead to increased production of secondary metabolites as part of the plant's defense mechanism, although excessive stress may negatively impact overall plant growth. Organic farming systems have been reported to enhance glucosinolate content due to reduced chemical inputs and increased reliance on natural soil amendments. Furthermore, genotype × environment interactions play a significant role in determining sinigrin levels, as different cultivars respond differently to environmental conditions (Singh *et al*., 2025).

2.3 Factors affecting sinigrin content

The concentration of sinigrin in plant tissues is influenced by multiple factors, including plant developmental stage, post-harvest handling and processing methods. Growth stage is one of the most important determinants of glucosinolate content. Young seedlings and actively growing tissues generally exhibit higher levels of sinigrin due to their increased vulnerability to herbivores and environmental stress. Studies have shown that glucosinolate content peaks during specific growth stages, such as bolting or early reproductive phases and subsequently declines as the plant matures (Research Gate study, 2025). Post-harvest handling and storage conditions significantly affect the stability of sinigrin. After harvest, enzymatic activity and environmental factors such as temperature and humidity can lead to degradation or hydrolysis of glucosinolates. Improper handling may result in the premature conversion of sinigrin into allyl isothiocyanate, reducing its availability in raw plant material. Cold storage is generally effective in preserving glucosinolate content, whereas prolonged storage at ambient conditions can lead to substantial losses (Redha *et al*., 2024).

Processing methods also play a crucial role in determining sinigrin content and its bioavailability. Thermal treatments such as boiling and steaming can lead to the degradation of glucosinolates or inactivation of myrosinase, thereby affecting the formation of bioactive compounds. Boiling, in particular, may cause significant losses due to leaching of water-soluble glucosinolates into cooking water. In contrast, milder processing methods such as steaming or microwave cooking are more effective in preserving glucosinolate content. Fermentation processes can further modify glucosinolate profiles through microbial activity, leading to the formation of new metabolites and influencing flavor and nutritional properties (Li *et al*., 2025; Wang *et al*., 2025). Recent advances in food processing and formulation technologies have focused on improving the stability and bioavailability of sinigrin. Techniques such as encapsulation and controlled-release systems are being explored to protect sinigrin from degradation during processing and digestion. Additionally, the dynamics of the glucosinolate-myrosinase system during food processing, particularly fermentation, have been extensively studied, highlighting the importance of microbial interactions in modulating sinigrin content (Yu *et al*., 2025).

3. Chemical structure and properties of sinigrin

3.1 Chemical structure

Sinigrin (allyl glucosinolate) is a structurally well-defined aliphatic glucosinolate belonging to sulfur-rich plant secondary metabolites. It follows the characteristic glucosinolate backbone composed of a β-thioglucose moiety, a sulfonated oxime group and an amino acid-derived side chain. The molecular formula of sinigrin is $C_{10}H_{16}KNO_9S_2$ and it typically exists as a potassium salt within plant tissues. Its defining structural feature is the allyl side chain, derived from methionine, which distinguishes it from other glucosinolates and governs its biological activity. The molecule contains several important functional groups, including hydroxyl groups, a sulfate ester and a thioglucosidic linkage. These groups collectively contribute to its polarity, water solubility and chemical stability. The thioglucosidic bond is particularly important, as it remains intact in undamaged tissues but is rapidly cleaved by the enzyme myrosinase upon tissue disruption. This cleavage leads to the formation of reactive intermediates that rearrange into biologically active compounds. The sulfate group enhances ionic character and facilitates intracellular storage, while the allyl group contributes to the formation of volatile hydrolysis products such as isothiocyanates (Baldelli *et al*., 2025). Recent structural studies have highlighted that glucosinolates, including sinigrin, exhibit remarkable chemical diversity due to variations in side chain structure, which directly influence their biological roles and metabolic fate. The presence of sulfur atoms in both the glucosinolate core and side chain plays a critical role in determining their reactivity and ecological function (Ullah *et al*., 2025).

3.2 Physicochemical properties

Sinigrin is a highly polar and water-soluble compound due to its multiple hydroxyl groups and sulfate moiety. This hydrophilic nature facilitates its accumulation in plant vacuoles and allows efficient extraction using aqueous solvents. However, it shows limited solubility in non-polar organic solvents. Its physicochemical behavior is strongly influenced by environmental factors such as pH, temperature and enzymatic activity. In contrast, milder processing methods such as steaming better preserve glucosinolate content. Recent studies emphasize that processing conditions critically determine the retention and bioavailability of sinigrin and its derivatives (Baldelli *et al*., 2025). The most important chemical transformation of sinigrin is its enzymatic hydrolysis catalyzed by myrosinase. This reaction results in the formation of allyl isothiocyanate (AITC), a volatile compound responsible for the pungent flavor of mustard and horseradish. AITC exhibits strong antimicrobial, antioxidant and anticancer properties. The formation of hydrolysis products depends on several factors, including pH, presence of specifier proteins and reaction conditions. Under certain conditions, alternative products such as nitriles and thiocyanates

may also form, influencing biological activity (Baldelli *et al*., 2025; Ullah *et al*., 2025). Recent advances have also highlighted the importance of controlling hydrolysis conditions to maximize beneficial compounds. For example, food processing and gut microbiota interactions significantly influence the conversion of sinigrin into bioactive metabolites, thereby affecting its health-promoting potential (Baldelli *et al*., 2025).

3.3 Analytical techniques for identification and quantification

The accurate identification and quantification of sinigrin are essential for evaluating its distribution, biological activity and industrial applications. Modern analytical techniques have significantly improved the sensitivity and precision of glucosinolate detection. High-performance liquid chromatography (HPLC) remains one of the most widely used methods for sinigrin analysis. It enables efficient separation and quantification of glucosinolates, often following desulfation to improve detection. HPLC is widely applied in food chemistry and plant metabolite studies due to its reliability and reproducibility. Liquid chromatography - tandem mass spectrometry (LC-MS/MS) offers enhanced sensitivity and specificity, allowing precise identification based on molecular mass and fragmentation patterns. This technique is particularly valuable in metabolomics studies and for detecting low concentrations of sinigrin in complex matrices (Ullah *et al*., 2025).

Gas chromatography-mass spectrometry (GC-MS) is primarily used to analyze volatile hydrolysis products such as allyl isothiocyanate. Since sinigrin itself is non-volatile, it must first undergo enzymatic hydrolysis before GC-MS analysis. This method is especially useful for studying functional and sensory properties. Spectroscopic techniques such as nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy provide detailed structural information. NMR is widely used for elucidating molecular structure, while IR spectroscopy helps identify functional groups. Recent analytical advancements, including high-throughput metabolomics and spectroscopic profiling, have further enhanced the detection and characterization of glucosinolates (Ullah *et al*., 2025).

4. Biosynthesis of sinigrin

4.1 Overview of glucosinolate biosynthesis

The biosynthesis of sinigrin is part of the glucosinolate metabolic pathway derived primarily from the amino acid methionine, which serves as the precursor for aliphatic glucosinolates. This pathway is highly conserved among Brassicaceae species and consists of three major stages: chain elongation of the amino acid, formation of the glucosinolate core structure and secondary modifications that yield specific compounds such as sinigrin. These processes are tightly regulated at both biochemical and genetic levels and are closely associated with plant defense mechanisms. Glucosinolate biosynthesis is influenced by developmental signals and environmental factors, enabling plants to modulate metabolite production in response to biotic and abiotic stresses (Cheng *et al*., 2024; Ali *et al*., 2025).

4.2 Chain elongation process

The chain elongation stage plays a crucial role in determining the diversity and structural variation of aliphatic glucosinolates. This process involves the extension of the methionine side chain through a series of enzymatic reactions. The key enzymes involved include methylthioalkylmalate synthase (MAM), isopropylmalate isomerase (IPMI) and isopropylmalate dehydrogenase (IPMDH). These enzymes catalyze sequential condensation, isomerization and oxidative decarboxylation reactions, leading to the formation of elongated amino acid derivatives. The number of elongation cycles determines the final side chain length, which contributes significantly to the structural diversity of glucosinolates. The expression and activity of these enzymes are influenced by genetic and environmental factors, highlighting their importance in shaping glucosinolate composition in plants (Cheng *et al*., 2024).

4.3 Core structure formation

Following chain elongation, the glucosinolate core structure is formed through a series of reactions catalyzed by cytochrome $P4_{50}$ enzymes and various transferases. The enzyme CYP79 converts the elongated amino acid derivatives into aldoximes, which are subsequently processed by CYP83 into reactive intermediates. These intermediates are conjugated with glutathione through the action of glutathione S-transferase (GST), followed by further transformation and glucosylation mediated by UDP-glucosyltransferase (UGT). The final step involves sulfation by sulfotransferase (SOT), resulting in the formation of complete glucosinolate molecules. The CYP79 and CYP83 enzyme families are particularly important in controlling metabolic flux and ensuring efficient biosynthesis of glucosinolates such as sinigrin (Tang *et al*., 2025; Ullah *et al*., 2025).

4.4 Secondary modifications

Secondary modifications are responsible for refining the glucosinolate structure and generating specific compounds such as sinigrin. These modifications include oxidation, desaturation and structural rearrangements that lead to the formation of the characteristic allyl side chain of sinigrin. These reactions are highly specific and enzyme-dependent, ensuring the production of structurally distinct glucosinolates. The regulation of these modifications contributes to the diversity of glucosinolate profiles observed across different plant species and plays a significant role in determining their biological activity and functional properties (Cheng *et al*., 2024).

4.5 Genetic regulation

The biosynthesis of sinigrin is under strict genetic control, primarily regulated by MYB transcription factors, including MYB28, MYB29 and MYB76, which are key regulators of aliphatic glucosinolate biosynthesis. These transcription factors control the expression of genes encoding enzymes such as CYP79 and MAM, thereby coordinating the entire biosynthetic pathway. MYB28 is considered a major regulator, while MYB29 and MYB76 contribute to fine-tuning the process. Environmental factors, particularly sulfur availability and stress conditions, significantly influence gene expression and metabolic activity. For instance, sulfur deficiency can suppress glucosinolate biosynthesis through transcriptional regulation, demonstrating the integration of nutrient signaling with secondary metabolism (Ali *et al*., 2025). Additionally, recent studies have highlighted the role of epigenetic mechanisms, including DNA methylation and histone modifications, in regulating glucosinolate biosynthesis. These mechanisms enable plants to adapt dynamically to environmental changes. Advances in metabolic engineering and genome editing technologies have further enabled the manipulation of key genes and regulatory networks, offering new opportunities to enhance sinigrin production for applications in food, pharmaceuticals and agriculture (Ben Ammar *et al*., 2025).

5. Extraction and isolation techniques of sinigrin

5.1 Conventional extraction methods

The extraction of sinigrin from plant matrices, particularly from Brassicaceae species such as mustard seeds, cabbage and horseradish, has traditionally relied on conventional solvent-based techniques. These methods are widely used due to their simplicity, cost-effectiveness and scalability. Sinigrin, being a highly polar and water-soluble glucosinolate, is typically extracted using aqueous solvents or hydroalcoholic mixtures such as methanol-water or ethanol-water systems. The efficiency of solvent extraction depends on several factors, including solvent polarity, temperature, extraction time and particle size of the plant material. Polar solvents facilitate the disruption of plant cell walls and enhance the solubilization of glucosinolates, thereby improving extraction yield. However, one of the challenges associated with conventional solvent extraction is the potential co-extraction of unwanted compounds such as sugars, proteins and phenolics, which may interfere with subsequent purification steps (Herrero *et al*., 2021).

Another widely used conventional technique is Soxhlet extraction, which involves continuous extraction of compounds using a refluxing solvent system. In this method, the solvent repeatedly passes through the plant material, ensuring thorough extraction of target compounds such as sinigrin. Soxhlet extraction is particularly advantageous for exhaustive extraction, as it allows prolonged contact between the solvent and plant matrix. However, this method is associated with several limitations, including high solvent consumption, long extraction times and potential thermal degradation of heat-sensitive compounds. Since sinigrin can undergo degradation under prolonged heating conditions, careful optimization of Soxhlet parameters is required to minimize losses. Despite these limitations, Soxhlet extraction remains a standard method for laboratory-scale studies and comparative analyses due to its reproducibility and efficiency (Azmir *et al*., 2022). Conventional extraction techniques often require subsequent steps such as filtration, centrifugation and solvent evaporation to concentrate the extract and remove impurities. These additional steps can increase processing time and cost, highlighting the need for more efficient and environmentally friendly extraction approaches. Nevertheless, conventional methods continue to be widely used as baseline techniques for evaluating the efficiency of newer extraction technologies.

5.2 Green extraction techniques

In recent years, there has been a growing emphasis on the development of green extraction techniques that are environmentally sustainable, energy-efficient and capable of preserving the integrity of bioactive compounds such as sinigrin. These techniques aim to reduce solvent usage, extraction time and energy consumption while enhancing extraction efficiency and selectivity. Ultrasound-assisted extraction (UAE) is one of the most widely adopted green extraction methods. This phenomenon disrupts plant cell walls, enhances mass transfer and facilitates the release of intracellular compounds. UAE has been shown to significantly improve the extraction yield of glucosinolates, including sinigrin, while reducing extraction time and solvent consumption (Chemat *et al*., 2021).

Microwave-assisted extraction (MAE) is another advanced technique that utilizes microwave energy to heat the solvent and plant matrix simultaneously. The rapid heating effect leads to cell rupture and enhanced diffusion of target compounds into the solvent. MAE offers several advantages, including reduced extraction time, lower solvent usage and improved extraction efficiency compared to conventional methods. For sinigrin extraction, MAE has been reported to yield higher concentrations with minimal degradation when optimized conditions are applied. The technique also allows precise control over extraction parameters, making it suitable for both laboratory and industrial applications (Zhang *et al*., 2021). Supercritical fluid extraction (SFE) represents a highly innovative and eco-friendly approach for extracting bioactive compounds. This technique utilizes supercritical fluids, most commonly carbon dioxide (CO_2), which exhibit unique properties of both liquids and gases. Supercritical CO_2 has low viscosity and high diffusivity, allowing it to penetrate plant matrices effectively. Although, sinigrin is polar and less soluble in pure CO_2 the addition of co-solvents such as ethanol enhances its extraction efficiency. SFE offers several advantages, including solvent-free extracts, high selectivity and minimal thermal degradation. This method is increasingly being explored for the extraction of glucosinolates due to its sustainability and scalability (Herrero *et al*., 2021). These green extraction techniques not only improve the efficiency of sinigrin recovery but also align with the principles of green chemistry, which emphasize the reduction of hazardous substances and environmental impact. The adoption of these technologies is expected to play a significant role in the industrial processing of glucosinolate-rich plant materials.

5.3 Purification and standardization

Following extraction, purification and standardization are essential steps to obtain sinigrin in a form suitable for analytical, pharmaceutical and industrial applications. Chromatographic techniques are widely employed for the purification of sinigrin due to their high resolution and specificity. Column chromatography, particularly ion-exchange chromatography, is commonly used to separate glucosinolates based on their charge properties. Sinigrin, being an anionic compound due to its sulfate group, can be effectively isolated using anion-exchange resins. High-performance liquid chromatography (HPLC) is extensively used for both purification and quantification of sinigrin. Preparative HPLC allows the isolation of high-purity sinigrin fractions, while analytical HPLC is used for quality control and standardization. The use of reverse-phase columns and appropriate mobile phases enables efficient separation of sinigrin from other glucosinolates and interfering compounds. Advances in chromatographic techniques, including ultra-high-performance liquid chromatography (UHPLC), have further improved the speed and sensitivity of analysis (Rivera-Pérez *et al*., 2022).

In addition to chromatographic methods, solid-phase extraction (SPE) is often employed as a preliminary purification step to concentrate and clean up sinigrin extracts. SPE cartridges containing specific sorbents can selectively retain glucosinolates, allowing impurities to be washed away before elution. This technique is particularly useful in sample preparation for analytical studies. Standardization of sinigrin involves the establishment of quality control parameters to ensure consistency, purity and reproducibility. These parameters include determination of purity, concentration and identification of degradation products. Analytical techniques such as HPLC, LC-MS/MS and spectrophotometry are commonly used for this purpose. Quality control also involves monitoring factors such as extraction efficiency, stability and storage conditions. The development of

standardized protocols is essential for ensuring the reliability of sinigrin in research and industrial applications (Rivera-Pérez *et al*., 2022). Furthermore, regulatory frameworks and quality standards play a crucial role in the commercialization of sinigrin-containing products. Ensuring compliance with safety and quality guidelines is essential for their acceptance in food, pharmaceutical and nutraceutical industries. Advances in analytical chemistry and process optimization continue to improve the efficiency of purification and standardization, thereby enhancing the overall quality of sinigrin products.

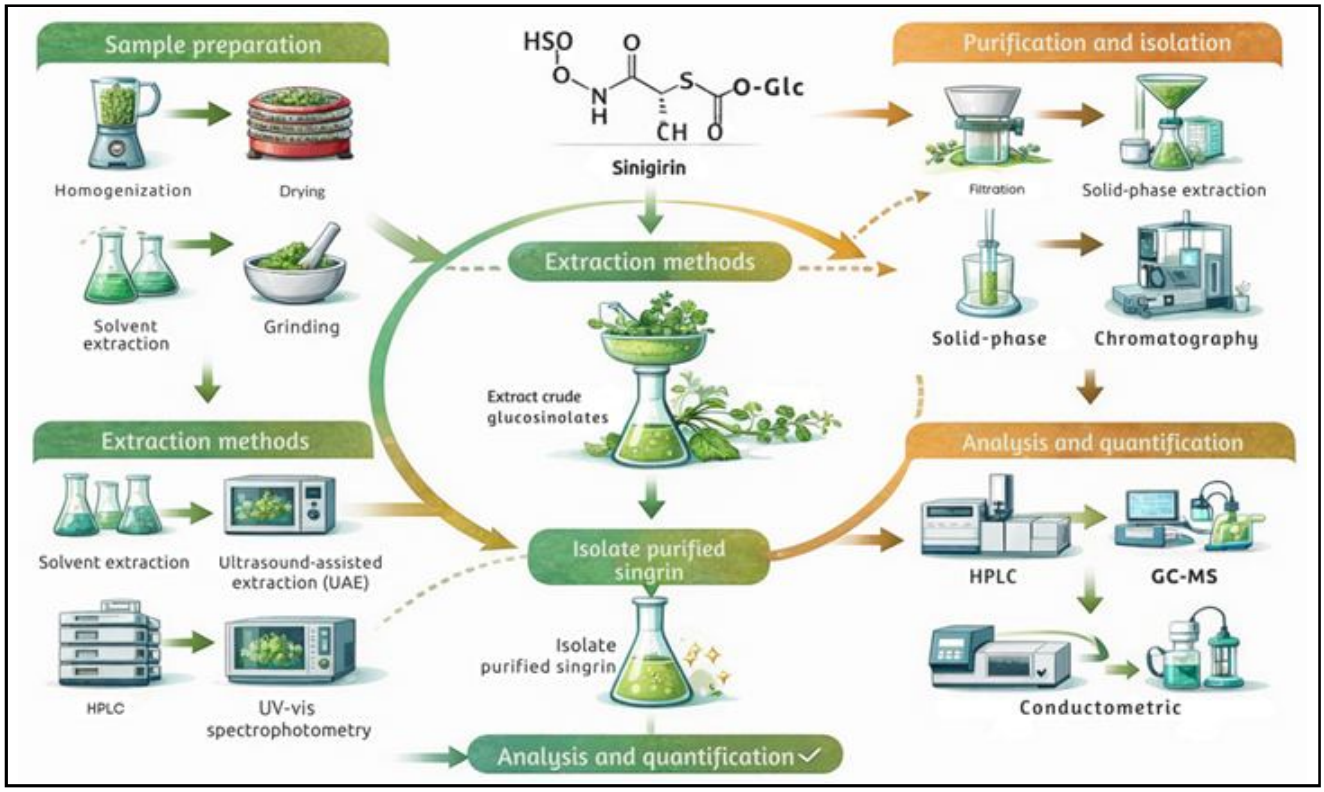

Figure 2: Extraction and isolation techniques of sinigrin: From sample preparation to analytical quantification.

Table 2: Extraction, biosynthesis and analytical techniques of sinigrin

Category	Technique/process	Key features	Advantages	Limitations	References
Conventional extraction	Solvent extraction	Uses water/ methanol	Simple, cost-effective	Low selectivity	Redha *et al*., 2024
Conventional extraction	Soxhlet extraction	Continuous extraction	High yield	Thermal degradation	Azmir *et al*., 2022
Green extraction	Ultrasound-assisted extraction (UAE)	Cavitation -based	Fast, efficient	Equipment cost	Chemat *et al*., 2021
Green extraction	Microwave-assisted extraction (MAE)	Rapid heating	High efficiency	Possible degradation	Zhang *et al*., 2021
Green extraction	Supercritical fluid extraction (SFE)	CO_2 -based	Eco-friendly	Expensive setup	Herrero *et al*., 2021
Biosynthesis	Methionine pathway	Amino acid precursor	Natural synthesis	Complex regulation	Cheng *et al*., 2024
Biosynthesis	Enzymes (MAM, CYP79, CYP83)	Catalyze steps	High specificity	Sensitive to environment	Tang *et al*., 2025
Analysis	HPLC	Quantification	Accurate, reliable	Time-consuming	Rivera-Pérez *et al*., 2022
Analysis	LC-MS/MS	High sensitivity	Precise identification	High cost	Ullah *et al*., 2025
Analysis	GC-MS	Volatile analysis	Detects AITC	Requires hydrolysis	Hanschen *et al*., 2020

6. Metabolism and bioavailability of sinigrin

6.1 Enzymatic hydrolysis

The biological activity of sinigrin is largely dependent on its enzymatic conversion into reactive metabolites, a process primarily mediated by the enzyme myrosinase (β-thioglucoside glucohydrolase) (Table 2). In intact plant tissues, sinigrin and myrosinase are compartmentalized in separate cellular structures, preventing premature hydrolysis. However, upon mechanical damage such as chewing, cutting, or processing, these components come into contact, initiating the hydrolytic reaction. Myrosinase catalyzes the cleavage of the thioglucosidic bond in sinigrin, releasing glucose and forming an unstable aglycone intermediate, which subsequently rearranges into various biologically active compounds (Hanschen *et al*., 2020).

The activity of myrosinase is influenced by several factors, including pH, temperature and the presence of cofactors or specifier proteins. Under neutral to slightly acidic conditions, the hydrolysis of sinigrin predominantly yields isothiocyanates, whereas alternative products such as nitriles and thiocyanates may form under different environmental conditions. Epithiospecifier proteins (ESPs) and nitrile-specifier proteins (NSPs) play a crucial role in directing the formation of specific hydrolysis products, thereby influencing the biological activity of sinigrin (Björkman *et al*., 2021). Additionally, thermal processing can inactivate plant-derived myrosinase, reducing the formation of bioactive metabolites; however, microbial myrosinase-like activity in the human gut can partially compensate for this loss. Recent studies have also highlighted the importance of gut microbiota in the hydrolysis of sinigrin. Certain intestinal bacteria possess myrosinase-like enzymes capable of converting glucosinolates into isothiocyanates, thereby contributing to the overall metabolic fate of sinigrin in the human body. This microbial conversion is particularly relevant when plant myrosinase is inactivated during cooking, emphasizing the role of gut microbiota in modulating glucosinolate bioactivity (Liou *et al*., 2020).

6.2 Formation of bioactive metabolites

The enzymatic hydrolysis of sinigrin results in the formation of a variety of bioactive compounds, among which allyl isothiocyanate (AITC) is the most prominent. AITC is a volatile sulfur-containing compound responsible for the pungent aroma and flavor of mustard and horseradish. More importantly, it exhibits a wide range of biological activities, including antimicrobial, antioxidant, anti-inflammatory and anticancer effects. The formation of AITC involves the spontaneous rearrangement of the unstable aglycone intermediate produced during myrosinase-mediated hydrolysis (Hanschen *et al*., 2020). The profile of hydrolysis products is highly dependent on environmental conditions such as pH, temperature and the presence of specifier proteins. For instance, in the presence of epithiospecifier proteins, sinigrin may yield epithionitriles instead of isothiocyanates, while nitrile-specifier proteins favor the formation of simple nitriles. These alternative products generally exhibit lower biological activity compared to isothiocyanates, highlighting the importance of reaction conditions in determining the functional properties of sinigrin (Björkman *et al*., 2021).

Allyl isothiocyanate has been extensively studied for its pharmacological potential. It is known to induce phase II detoxification enzymes, modulate oxidative stress pathways and inhibit the proliferation of cancer cells. Additionally, AITC exhibits strong antimicrobial activity against a broad spectrum of bacteria and fungi, making it a valuable compound in food preservation and safety applications. Other minor hydrolysis products, including thiocyanates and nitriles, may also contribute to the overall biological activity, although their effects are generally less pronounced. Recent research has also focused on the interaction between sinigrin-derived metabolites and cellular signaling pathways. AITC has been shown to influence key molecular targets such as nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor kappa B (NF-κB), which are involved in antioxidant defense and inflammation. These findings underscore the significance of sinigrin as a precursor to bioactive compounds with therapeutic potential (Dinkova-Kostova and Kostov, 2022).

6.3 Absorption, distribution, metabolism and excretion

The bioavailability of sinigrin and its metabolites is a complex process influenced by factors such as food matrix, enzymatic activity and individual physiological differences. In humans and animals, intact sinigrin is generally not readily absorbed in the upper gastrointestinal tract due to its hydrophilic nature and molecular size. Instead, it undergoes hydrolysis either by plant-derived myrosinase during food consumption or by gut microbiota in the colon, resulting in the formation of absorbable metabolites such as isothiocyanates (Angelino and Jeffery, 2019). Once formed, allyl isothiocyanate is rapidly absorbed across the intestinal epithelium and enters systemic circulation. Due to its lipophilic nature, AITC can easily diffuse across biological membranes and distribute to various tissues. After absorption, AITC undergoes conjugation with glutathione through the mercapturic acid pathway, forming glutathione conjugates that are further metabolized into cysteine and N-acetylcysteine derivatives. These metabolites are eventually excreted in urine, making urinary mercapturic acids reliable biomarkers for glucosinolate intake and metabolism (Angelino and Jeffery, 2019).

The efficiency of sinigrin bioavailability is influenced by several factors, including the presence of active myrosinase, food processing methods and individual gut microbiota composition. Cooking methods that inactivate myrosinase can significantly reduce the formation of isothiocyanates, thereby lowering bioavailability. However, the gut microbiota can partially compensate for this by converting residual glucosinolates into bioactive metabolites, although the efficiency of this process varies among individuals (Liou *et al*., 2020). In animal studies, sinigrin and its metabolites have been shown to distribute to various organs, including the liver, kidneys and lungs, where they exert biological effects. The rapid metabolism and excretion of isothiocyanates suggest that their biological activity is transient, requiring continuous dietary intake to maintain therapeutic levels. Additionally, inter-individual variability in metabolism highlights the importance of personalized nutrition approaches in optimizing the health benefits of sinigrin.

7. Pharmacological activities of sinigrin

Sinigrin (allyl glucosinolate) has attracted considerable attention due to its broad spectrum of pharmacological properties, primarily mediated through its enzymatic hydrolysis product, allyl isothiocyanate (AITC). These bioactivities encompass anticancer, antimicrobial, anti-inflammatory, antioxidant, antidiabetic, cardioprotective and neuroprotective effects. The biological efficacy of sinigrin is closely linked to its ability to modulate cellular signaling pathways, regulate gene expression and influence redox homeostasis. Recent

advances in molecular pharmacology, nutraceutical science and metabolomics have provided deeper insights into the mechanisms underlying these effects, positioning sinigrin as a promising bioactive compound for therapeutic and preventive applications (Table 3).

Table 3: Pharmacological activities of sinigrin and mechanisms of action

Pharmacological activity	Mechanism of action	Target pathways	Biological effects	References
Anticancer	Induces apoptosis, cell cycle arrest	Nrf2, p53, caspases	Tumor inhibition, anti-metastasis	Tarar *et al*., 2022
Antimicrobial	Disrupts microbial membranes	Protein/enzyme inhibition	Bacterial and fungal inhibition	Luciano and Holley, 2021
Anti-inflammatory	Suppresses cytokine production	NF-κB, MAPK	Reduced inflammation	Kim *et al*., 2021
Antioxidant	Enhances detox enzymes	Nrf2 pathway	ROS scavenging	Dinkova-Kostova and Kostov, 2022
Antidiabetic	Improves glucose metabolism	AMPK activation	Reduced blood glucose	Kumari *et al*., 2023
Cardioprotective	Regulates lipid metabolism	Oxidative stress pathways	Reduced LDL, improved HDL	Traka and Mithen, 2022
Neuroprotective	Reduces neuroinflammation	Nrf2, mitochondrial pathways	Protects neurons	Zhang *et al*., 2021

7.1 Anticancer activity

The anticancer potential of sinigrin and its derivatives has been extensively studied across various in vitro and in vivo models. One of the primary mechanisms involves the induction of apoptosis, a programmed cell death process essential for eliminating damaged or malignant cells. Sinigrin-derived AITC has been shown to activate intrinsic apoptotic pathways by increasing mitochondrial membrane permeability, leading to the release of cytochrome c and subsequent activation of caspases such as caspase-3 and caspase-9. This cascade ultimately results in DNA fragmentation and cell death in cancer cells (Zhang *et al*., 2021; Dinkova-Kostova and Kostov, 2022). In addition to apoptosis, sinigrin plays a significant role in cell cycle regulation. Studies have demonstrated that AITC can arrest cancer cell proliferation by interfering with key regulators of the cell cycle, particularly at the G2/M phase. This effect is mediated through the downregulation of cyclins and cyclin-dependent kinases (CDKs), as well as the upregulation of tumor suppressor proteins such as p53 and p21. By inhibiting cell cycle progression, sinigrin effectively suppresses tumor growth and proliferation.

Another important mechanism underlying the anticancer activity of sinigrin is its ability to modulate detoxification enzymes. AITC is known to induce phase II detoxifying enzymes such as glutathione S-transferases (GSTs), NAD(P)H quinone oxidoreductase 1 (NQO1) and UDP-glucuronosyltransferases (UGTs). These enzymes enhance the detoxification and elimination of carcinogens, thereby reducing the risk of cancer initiation and progression. This process is largely mediated through activation of the Nrf2 (nuclear factor erythroid 2-related factor 2) signaling pathway, which regulates antioxidant and detoxification responses (Dinkova-Kostova and Kostov, 2022). Furthermore, sinigrin has been shown to inhibit angiogenesis and metastasis, two critical processes involved in cancer progression. AITC suppresses the expression of vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), thereby reducing tumor vascularization and invasion. These combined effects highlight the multifaceted anticancer potential of sinigrin and support its use as a chemopreventive and therapeutic agent.

7.2 Antimicrobial activity

Sinigrin and its hydrolysis products exhibit strong antimicrobial activity against a wide range of microorganisms, including bacteria, fungi and pathogenic microbes. The antimicrobial effect is primarily attributed to AITC, which disrupts microbial cell membranes and interferes with essential metabolic processes. AITC is highly reactive and can penetrate microbial cells, where it interacts with proteins, enzymes and nucleic acids, leading to cellular damage and cell death. Studies have demonstrated that sinigrin-derived AITC is effective against both Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* species. The mechanism of action involves disruption of membrane integrity, inhibition of enzyme activity and interference with energy metabolism. Additionally, AITC has been shown to inhibit biofilm formation, which is a critical factor in microbial resistance and persistence (Luciano and Holley, 2021).

In addition to antibacterial activity, sinigrin also exhibits antifungal properties. AITC has been reported to inhibit the growth of fungal pathogens such as *Candida albicans* and *Aspergillus* species by disrupting cell wall integrity and inhibiting spore germination. This makes sinigrin a potential candidate for applications in food preservation and agricultural disease management. Recent studies have also highlighted the antiviral potential of isothiocyanates, suggesting that sinigrin may have broader antimicrobial applications. The ability of sinigrin to act against a wide spectrum of pathogens, combined with its natural origin, makes it an attractive alternative to synthetic antimicrobial agents in both food and pharmaceutical industries.

7.3 Anti-inflammatory activity

The anti-inflammatory effects of sinigrin are primarily mediated through its ability to modulate key inflammatory signaling pathways. Chronic inflammation is a major contributor to various diseases, including cancer, cardiovascular disorders and neurodegenerative conditions. Sinigrin-derived AITC has been shown to suppress inflammatory responses by inhibiting the activation of nuclear factor kappa B (NF-κB), a key transcription factor involved in the regulation

of pro-inflammatory genes. AITC reduces the expression of inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukins (IL-1β, IL-6) and cyclooxygenase-2 (COX-2). This is achieved through the inhibition of upstream signaling pathways, including mitogen-activated protein kinases (MAPKs) and IκB kinase (IKK). By suppressing these pathways, sinigrin effectively reduces inflammation at the molecular level (Kim *et al*., 2021). Additionally, sinigrin has been shown to enhance the activity of antioxidant enzymes, thereby reducing oxidative stress, which is closely linked to inflammation. The dual action of sinigrin as both an anti-inflammatory and antioxidant agent further enhances its therapeutic potential.

7.4 Antioxidant properties

The antioxidant activity of sinigrin is closely associated with its ability to modulate redox homeostasis and enhance cellular defense mechanisms against oxidative stress. Although, sinigrin itself is not a strong direct free radical scavenger, its hydrolysis product AITC plays a significant role in activating endogenous antioxidant systems. AITC activates the Nrf2 signaling pathway, which leads to the upregulation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes neutralize reactive oxygen species (ROS) and prevent oxidative damage to cellular components such as lipids, proteins and DNA (Dinkova-Kostova and Kostov, 2022). Furthermore, sinigrin has been shown to reduce lipid peroxidation and enhance glutathione levels, thereby maintaining cellular redox balance. The antioxidant properties of sinigrin contribute to its protective effects against various diseases, including cancer, diabetes and cardiovascular disorders.

7.5 Antidiabetic and cardioprotective effects

Sinigrin has shown promising potential in the management of metabolic disorders, particularly diabetes and cardiovascular diseases. Its antidiabetic effects are primarily attributed to its ability to regulate glucose metabolism and improve insulin sensitivity. Studies have demonstrated that sinigrin can enhance glucose uptake in peripheral tissues and reduce blood glucose levels by modulating key metabolic enzymes. One of the key mechanisms involves the activation of AMP-activated protein kinase (AMPK), a central regulator of energy metabolism. Activation of AMPK leads to increased glucose uptake, enhanced fatty acid oxidation and improved insulin sensitivity. Additionally, sinigrin has been shown to inhibit α-glucosidase and β-amylase enzymes, thereby reducing carbohydrate digestion and postprandial glucose levels (Zhang *et al*., 2021).

In terms of cardioprotective effects, sinigrin contributes to the regulation of lipid metabolism and prevention of atherosclerosis. It has been reported to reduce levels of low-density lipoprotein (LDL) cholesterol and triglycerides while increasing high-density lipoprotein (HDL) cholesterol. These effects help maintain cardiovascular health and reduce the risk of heart disease. Moreover, the antioxidant and anti-inflammatory properties of sinigrin play a crucial role in protecting cardiovascular tissues from oxidative damage and inflammation. By modulating lipid profiles and reducing oxidative stress, sinigrin provides comprehensive protection against cardiovascular disorders.

7.6 Neuroprotective effects

The neuroprotective potential of sinigrin has gained increasing attention in recent years, particularly in the context of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Oxidative stress, inflammation and mitochondrial dysfunction are key factors contributing to neuronal damage and sinigrin has been shown to target these pathways effectively. AITC exhibits neuroprotective effects by reducing oxidative stress and enhancing antioxidant defense mechanisms in neuronal cells. It activates the Nrf2 pathway, leading to increased expression of antioxidant enzymes and protection of neurons from oxidative damage. Additionally, sinigrin has been shown to inhibit neuroinflammation by suppressing the activation of microglia and reducing the production of pro-inflammatory cytokines (Kim *et al*., 2021).

Another important aspect of sinigrin's neuroprotective activity is its ability to modulate neurotransmitter systems and prevent neuronal apoptosis. Studies have demonstrated that AITC can protect neurons from apoptosis by regulating mitochondrial function and inhibiting caspase activation. Furthermore, sinigrin has been reported to improve cognitive function and memory in experimental models, suggesting its potential role in preventing or slowing the progression of neurodegenerative diseases.

8. Mechanisms of action

The biological activity of sinigrin is primarily mediated through its hydrolysis product, allyl isothiocyanate (AITC), which interacts with multiple molecular targets and signaling pathways. One of the key mechanisms involves activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which regulates antioxidant and detoxification responses. Activation of Nrf2 leads to increased expression of cytoprotective enzymes such as glutathione S-transferase, heme oxygenase-1 and NAD(P)H quinone oxidoreductase, thereby enhancing cellular resistance to oxidative stress (Tarar *et al*., 2022). Simultaneously, sinigrin-derived metabolites inhibit the nuclear factor kappa B (NF-κB) pathway, which is responsible for the regulation of pro-inflammatory cytokines and mediators. This dual modulation of oxidative and inflammatory pathways contributes significantly to its therapeutic effects (Costa-Pérez *et al*., 2023). In addition to Nrf2 and NF-κB signaling, sinigrin influences other important cellular pathways such as mitogen-activated protein kinases (MAPKs) and the PI3K/Akt pathway. These pathways are involved in regulating cell proliferation, survival and apoptosis. AITC has been reported to induce apoptosis by modulating mitochondrial pathways and activating caspase enzymes, while also inhibiting survival pathways that are often upregulated in cancer cells (Yadav *et al*., 2022). Furthermore, the modulation of these signaling cascades helps regulate cellular stress responses and maintain homeostasis under adverse conditions.

Sinigrin also plays a crucial role in gene expression modulation. Through its interaction with transcription factors such as Nrf2, NF-κB and activator protein-1 (AP-1), AITC can regulate the expression of genes involved in detoxification, inflammation and cell cycle control. These changes in gene expression allow cells to adapt to oxidative and inflammatory stress. Recent studies have also suggested that isothiocyanates may influence epigenetic mechanisms, including histone modification and DNA methylation, thereby contributing to long-term regulation of gene activity (Tarar *et al*., 2022). Another

important mechanism involves enzyme inhibition and activation. Sinigrin-derived compounds inhibit phase I enzymes, particularly cytochrome P450 enzymes involved in the activation of procarcinogens, while inducing phase II detoxification enzymes that facilitate the elimination of harmful substances. Additionally, AITC has been shown to inhibit enzymes associated with inflammation, such as cyclooxygenase (COX) and lipoxygenase (LOX), thereby reducing the synthesis of inflammatory mediators (Costa-Pérez *et al*., 2023). The biological activity of sinigrin is largely attributed to the electrophilic nature of isothiocyanates, which enables them to react with nucleophilic groups in proteins and other biomolecules. This interaction results in the modification of protein function and signaling pathways, particularly through binding to cysteine residues. Such modifications play a critical role in activating protective cellular responses and inhibiting pathogenic processes. Overall, the mechanisms of action of sinigrin involve a coordinated network of molecular interactions that contribute to its wide range of biological activities.

9. Industrial applications

The diverse biological properties of sinigrin and its hydrolysis products have led to their wide application in various industrial sectors, including food, pharmaceuticals, agriculture and cosmetics. Its natural origin, safety profile and multifunctional characteristics make it a valuable alternative to synthetic compounds, particularly in industries focusing on sustainability and health.

9.1 Food industry

In the food industry, sinigrin is widely used as a natural flavoring agent due to its ability to produce allyl isothiocyanate, which imparts the characteristic pungent flavor of mustard and horseradish. This compound is commonly utilized in condiments, sauces and processed foods to enhance sensory quality. Its natural origin makes it highly desirable in clean-label food products, where consumers prefer natural additives over synthetic ones (Yadav *et al*., 2022). In addition to its flavoring properties, sinigrin has significant potential in food preservation. Allyl isothiocyanate exhibits strong antimicrobial activity against foodborne pathogens, making it suitable for use in active food packaging systems. These systems incorporate antimicrobial compounds into packaging materials to extend shelf life and improve food safety. Recent studies have demonstrated that isothiocyanate-based packaging can effectively inhibit microbial growth and reduce spoilage in perishable food products (Ko *et al*., 2024).

9.2 Pharmaceutical industry

In the pharmaceutical industry, sinigrin is being explored for its potential in drug development and nutraceutical applications. Its ability to modulate key signaling pathways and regulate gene expression makes it a promising candidate for therapeutic interventions, particularly in cancer, inflammation and metabolic disorders. Sinigrin-derived compounds have been shown to enhance detoxification processes and reduce oxidative stress, which are critical factors in disease prevention (Tarar *et al*., 2022). Sinigrin is also incorporated into functional foods and dietary supplements aimed at improving overall health and preventing chronic diseases. Advances in formulation technologies, including encapsulation and nano-delivery systems, have improved the stability and bioavailability of sinigrin, making it more effective in pharmaceutical applications. These developments have opened new avenues for the use of sinigrin in preventive healthcare and personalized nutrition (Costa-Pérez *et al*., 2023).

9.3 Agriculture

In agriculture, sinigrin plays a crucial role in sustainable crop protection and soil management. One of its most important applications is in biofumigation, where glucosinolate-rich plant materials are incorporated into soil. Upon hydrolysis, sinigrin releases isothiocyanates that act as natural fumigants, suppressing soil-borne pathogens, nematodes and weeds. This method provides an environmentally friendly alternative to synthetic pesticides and contributes to sustainable agricultural practices (Ko *et al*., 2024). Sinigrin also contributes to natural plant defense mechanisms. The release of isothiocyanates upon tissue damage acts as a deterrent to herbivores and insects, reducing pest infestation. This natural defense system can be utilized in integrated pest management strategies to minimize the use of chemical pesticides. Additionally, sinigrin-containing crops are used in crop rotation systems to improve soil health and reduce disease incidence.

9.4 Cosmetic industry

The cosmetic industry has increasingly recognized the potential of sinigrin and its derivatives in skincare and personal care products. The antimicrobial and antioxidant properties of allyl isothiocyanate make it a valuable ingredient in formulations designed to protect the skin from microbial infections and oxidative damage. Sinigrin-derived compounds are used in creams, lotions and topical products to enhance skin health and hygiene. In addition to antimicrobial activity, sinigrin exhibits anti-inflammatory effects that help reduce skin irritation and redness. This makes it suitable for use in products targeting sensitive or acne-prone skin. Furthermore, its antioxidant properties contribute to antiageing formulations by protecting skin cells from oxidative stress and promoting cellular repair. The growing demand for natural and plant-based ingredients has further increased the interest in sinigrin for cosmetic applications (Yadav *et al*., 2022).

10. Toxicity and safety aspects

The safety evaluation of sinigrin and its hydrolysis product, allyl isothiocyanate (AITC), is critical due to their increasing application in food, pharmaceutical and industrial sectors. Sinigrin is commonly consumed through cruciferous vegetables and is generally regarded as safe at normal dietary levels. Epidemiological and nutritional studies indicate that moderate intake of glucosinolate-rich foods contributes positively to human health, particularly through antioxidant and chemopreventive effects (Bondonno *et al*., 2022). However, its biological activity is strongly dose-dependent and excessive exposure may lead to adverse physiological effects. At elevated concentrations, sinigrin-derived isothiocyanates exhibit high electrophilicity, allowing them to interact with cellular proteins and nucleic acids, which may result in cytotoxicity. Experimental studies have demonstrated that excessive intake of glucosinolates can interfere with thyroid function by inhibiting iodine uptake and altering thyroid hormone synthesis, particularly under iodine-deficient conditions (Mirmiran *et al*., 2023). Such effects highlight the importance of controlled consumption and careful formulation in functional foods and nutraceutical products. Potential toxicity is also associated with the irritant properties of AITC. Direct exposure to concentrated forms may cause irritation to the skin, eyes and respiratory tract. In food systems, regulatory

limits have been established to ensure safe consumption levels. For instance, allyl isothiocyanate is approved as a flavoring agent within defined limits to prevent adverse effects while maintaining its functional benefits (EFSA Panel on Food Additives, 2022). Additionally, recent toxicological evaluations have emphasized that chronic exposure to high concentrations of isothiocyanates may lead to mild gastrointestinal disturbances and cellular stress responses (Dolan *et al*., 2024).

The metabolic pathway of sinigrin also plays an important role in determining its safety. After ingestion, sinigrin is hydrolyzed into isothiocyanates, which are further metabolized via the mercapturic acid pathway and rapidly excreted. This efficient metabolism reduces the likelihood of bioaccumulation and long-term toxicity. However, inter-individual variability in gut microbiota composition can influence the rate of conversion and overall biological response (Traka and Mithen, 2022). From a regulatory perspective, sinigrin and its derivatives are considered safe when used within recommended limits. International regulatory agencies, including the European Food Safety Authority and other food safety bodies, have established guidelines for the use of isothiocyanates in food and consumer products. These guidelines ensure that exposure levels remain within safe thresholds while allowing the beneficial properties of sinigrin to be utilized. Nevertheless, further clinical studies are required to establish precise dosage recommendations and to evaluate long-term safety in different population groups.

11. Challenges and limitations

Despite its significant biological and industrial potential, the application of sinigrin faces several challenges related to stability, bioavailability, variability and standardization. One of the primary limitations is its chemical instability under certain environmental and processing conditions. Sinigrin is susceptible to enzymatic hydrolysis and degradation when exposed to heat, light and moisture. Food processing techniques such as boiling and high-pressure treatment can reduce glucosinolate content and alter myrosinase activity, thereby affecting the formation of bioactive isothiocyanates. These changes can lead to reduced efficacy and inconsistency in functional properties. Another major challenge is the low and variable bioavailability of sinigrin. As a hydrophilic compound, sinigrin is not readily absorbed in its intact form and requires enzymatic conversion into isothiocyanates for biological activity. The efficiency of this conversion depends on factors such as myrosinase activity, food matrix and gut microbiota composition. Variability in these factors among individuals results in differences in absorption and therapeutic outcomes, limiting the predictability of its effects.

Variability in natural sources further complicates the utilization of sinigrin. The concentration of sinigrin in plants varies significantly depending on species, genotype, environmental conditions and agronomic practices. Factors such as soil composition, climate and nutrient availability influence glucosinolate biosynthesis, leading to inconsistent levels across different plant materials. This variability poses challenges for quality control and industrial standardization. Standardization itself remains a critical limitation. Differences in extraction, purification and analytical techniques result in variations in sinigrin content and purity. Advanced analytical methods such as spectroscopic and chromatographic techniques have improved detection and quantification; however, consistent protocols are still lacking. Without standardized procedures, it is difficult to ensure reproducibility and reliability in both research and commercial applications.

12. Emerging trends and future perspectives

Recent advancements in science and technology have opened new avenues for enhancing the application and effectiveness of sinigrin. One of the most promising areas is the development of nanotechnology-based delivery systems. Encapsulation techniques using nanoparticles, liposomes and nanoemulsions have been shown to improve the stability, solubility and controlled release of bioactive compounds. These systems protect sinigrin from degradation and enhance its bioavailability, making it more effective in pharmaceutical and functional food applications. Another important trend is the application of metabolic engineering and synthetic biology to increase sinigrin production. Advances in genetic engineering have enabled the manipulation of biosynthetic pathways in plants and microorganisms, allowing for enhanced production of glucosinolates. These approaches offer sustainable and scalable alternatives to traditional extraction from plant sources. Additionally, understanding the genetic and environmental factors influencing glucosinolate biosynthesis can help in developing high-yield crop varieties. Clinical research and translational studies are also gaining momentum. While numerous preclinical studies have demonstrated the pharmacological potential of sinigrin, there is a growing need for human clinical trials to validate its efficacy and safety. Recent reviews emphasize the importance of integrating glucosinolate-rich foods into dietary strategies for disease prevention and health promotion. Such studies will provide valuable insights into dosage optimization, bioavailability and long-term effects.

The role of sinigrin in precision nutrition and functional foods represents another emerging area of interest. Personalized nutrition approaches consider individual genetic, metabolic and microbiome profiles to optimize dietary recommendations. Given the variability in glucosinolate metabolism, sinigrin has strong potential for inclusion in tailored dietary interventions. Functional foods enriched with sinigrin or its derivatives can provide targeted health benefits, including antioxidant and anti-inflammatory effects.

13. Conclusion

Sinigrin is a biologically significant glucosinolate with diverse applications in health, agriculture and industry. Its conversion into allyl isothiocyanate underpins its wide range of pharmacological activities, including anticancer, antimicrobial, anti-inflammatory, antioxidant and metabolic regulatory effects. These properties are mediated through complex molecular mechanisms involving modulation of signaling pathways, gene expression and enzymatic activity. In addition to its therapeutic potential, sinigrin plays an important role in industrial applications such as natural flavoring, food preservation, biofumigation and cosmetic formulations. Its natural origin and multifunctional characteristics make it a valuable alternative to synthetic compounds in sustainable and health-oriented products. Despite these advantages, challenges such as stability issues, low bioavailability and variability in natural sources remain significant barriers to its widespread utilization. Advances in nanotechnology, metabolic engineering and analytical techniques offer promising solutions to these limitations.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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