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Research Article

Identification and Network Analyses of Salicylic Acid-Induced Overexpression of *P*-Glycoprotein Inhibitors in *Sesamum indicum* and *Coriandrum sativum*

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Abstract

Background: Plants are major sources of bioactive metabolites, contributing to over one-third of modern pharmaceuticals. These metabolites arise from primary and secondary metabolic pathways essential for plant growth and defense. Salicylic acid, a phytohormone, acts as an elicitor that enhances secondary metabolite biosynthesis. Such metabolites may help overcome multidrug resistance, particularly through inhibition of the efflux transporter P-glycoprotein. **Objectives:** Building on previous identification of P-glycoprotein inhibitors via molecular docking, this study investigated their biosynthetic pathways and evaluated the impact of salicylic acid elicitation on the phytochemical profiles of selected medicinal plants. **Methods:** Biosynthetic pathways of identified inhibitor compounds were retrieved from the Plant Metabolic Network database and analyzed to identify common enzymes. Protein–protein interaction analysis was conducted using the STRING database. For elicitation studies, *Sesamum indicum* and *Coriandrum sativum* were treated with salicylic acid on alternate days. Harvested seeds were subjected to GC-MS profiling to assess phytochemical changes. **Results and Conclusion:** Twelve of 33 inhibitors were mapped to phytosterol, tocopherol, sesamin, and cis-abienol biosynthesis pathways, involving 14 common enzymes. Among these, 3β-hydroxysteroid-4α-carboxylate 3-dehydrogenase (decarboxylating) showed the highest connectivity, highlighting its role in sterol biosynthesis. GC-MS analysis revealed that elicited *Sesamum indicum* showed an increase in phytochemicals from 22 to 50, with potential P-glycoprotein inhibitor candidates increasing from 4 to 19. In *Coriandrum sativum*, compounds increased from 42 to 44. Salicylic acid elicitation enhanced phytochemical diversity and increased the occurrence of compounds previously reported as potential P-glycoprotein inhibitors, particularly in *Sesamum indicum*, highlighting its utility as an elicitor for modulating secondary metabolite production.

Keywords: *GC-MS; P-Glycoprotein Inhibitors; S`esamum Indicum; Salicylic Acid*

Introduction

Plants are a rich source of diverse bioactive phytochemicals that have been utilized for centuries in traditional medicine and continue to play a pivotal role in modern therapeutic strategies. These plant-derived natural compounds have significantly contributed to the advancement of medical treatments and the improvement of human health by offering a wide array of pharmacologically active molecules (Yruela, 2015). Metabolic pathways, classified as anabolic or catabolic, regulate the synthesis and transformation of biomolecules at the cellular level. In plants, these pathways support the synthesis of primary and secondary metabolites, essential for basic cellular function and defense (Srivastava, 2024). Notably, phytohormones such as salicylic acid (SA) play a significant role in modulating

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secondary metabolism. SA acts as an elicitor that enhances the biosynthesis of defense-related phytochemicals by activating stress-responsive signaling pathways (Khan *et al*., 2013).

Phytochemicals have many biomedical applications, and one increasingly important area is their potential to combat multidrug resistance (MDR), a significant barrier to effective chemotherapy that is often caused by the overexpression of efflux transporters such as P-glycoprotein (Tian *et al*., 2023). P-glycoprotein reduces the intracellular concentration of drugs and xenobiotics by actively transporting them out of cells, thereby diminishing their efficacy. Phytochemicals, particularly flavonoids, alkaloids, and terpenoids, have demonstrated promising inhibitory activity against P-glycoprotein, which is attributed to their ability to modulate transporter function and expression (Abdallah *et al*., 2015). The elicitation of these compounds through SA treatment offers a strategic approach to enhance their accumulation and potentially increase their therapeutic efficacy.

This work extends the previous report on putative P-glycoprotein inhibitors identified through a molecular docking study (Mukhopadhyay *et al*., 2024) by using a modified pathway enrichment analysis. It also explores the impact of SA application on the selected plant species and examines the enhancement of the phytochemical repertoire of P-glycoprotein inhibitor compounds.

Materials and Methods

A total of 33 compounds from 25 spices have been identified in the literature as potential or putative P-glycoprotein inhibitors (Mukhopadhyay *et al*., 2024). Therefore, the current study identifies the biosynthetic pathways responsible for the production of these inhibitor compounds, followed by network analysis to locate their interacting partners and GC-MS analysis for phytochemical profiling of the selected spices.

Pathway Identification of the Phytochemicals:

Text mining was employed to identify the biosynthetic pathways responsible for the production of phytocompounds recognized as inhibitors. The Plant Metabolic Network (PMN) database was used to identify pathways, which were then visualized using the built-in tools of the database. Additionally, the common enzymes that make up the core modules of these pathways were noted (Guo *et al*., 2024).

Network Identification and Protein-Protein Interaction Enrichment Analysis of the Enzymes:

The amino acid sequences of the query proteins, obtained from the UniProt database, were entered into the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database to identify interacting partners and potential protein interactions of the query proteins, which were common enzymes across the identified pathways. The STRING database helps explain the functional context of these enzymes by revealing their potential interacting partners and the pathways in which they may be involved (Szklarczyk *et al*., 2021).

Elicitation of the Phytochemicals by SA:

Two sets of *Sesamum indicum* and *Coriandrum sativum* plants were cultivated in a rooftop garden in Halisahar, West Bengal, India, from March to June 2024 and from October 2024 to January 2025. For each species, five pots were maintained as controls (non-elicited) and five pots were assigned to the SA treatment group. Plants were grown in garden soil under natural environmental conditions. SA was used as the elicitor at concentrations of 0.5 mM and 1 mM, selected based on previous reports demonstrating their effectiveness in stimulating secondary metabolite production while avoiding phytotoxic effects, making them suitable concentrations for elicitation studies in medicinal and aromatic plants (Singh, 2023; Karalija *et al*., 2025). Plants were initially allowed to establish under normal growth conditions during March 2024 without SA treatment. Subsequently, 0.5 mM SA was applied during April 2024, followed by 1 mM SA during May and June 2024 (Figure 1). The solutions were applied directly to both the soil and foliage of the plants twice daily throughout the treatment period (Singh, 2023; Karalija *et al*., 2025). Once the plants matured and proper seed formation occurred, fresh seeds were harvested from both the control and elicited groups. The seeds were

dehusked, separated from the seed coat, and air-dried for 10 days. After drying, the seeds were surface-sterilized using distilled water and 70% ethanol for 5 minutes, then rinsed with double-distilled water and air-dried for an additional 10 to 20 minutes (Qadir *et al*., 2018).

The sterilized seeds, including both the seed kernel and seed coat, were then ground into a fine powder using a high-speed grinder. Seeds harvested from the five pots within each treatment group were pooled to obtain a representative composite sample. The resulting powder was stored in airtight containers until further analysis. Five grams of seed powder from each pooled sample were placed into separate airtight containers and subjected to GC-MS analysis.GC-MS analysis was performed at the Sophisticated Analytical Instrument Facility (SAIF), IIT Madras. Samples were analyzed in scan mode using electron impact (EI) ionization with an injection volume of 1.0 µL. Mass spectra were processed using the NIST17 mass spectral library, and compound identification was based on spectral matching, retention time information, match scores, and probability values provided by the library search.

Figure 1: *Stages of Growth and Development of Sesamum Indicum (left) and Coriandrum Sativum (Right) Seed. [DAP- Day After Planting].*

Results

Pathway Identification of the Phytochemicals

Plant metabolic systems are highly complex and generate a wide array of compounds, including secondary metabolites such as alkaloids, flavonoids, and terpenoids. The biosynthetic pathways involve sequential enzymatic reactions that convert precursor molecules into bioactive end products. Specific enzymes catalyze each step and are tightly regulated by genetic and environmental factors. In the PMN database, only 12 out of the 33 P-glycoprotein inhibitors were identified. Table 1 summarizes the phytochemical-specific pathways along with the corresponding enzymes involved in their biosynthesis, revealing 14 common enzymes across all pathways involved in the synthesis of the 12 inhibitor compounds.

Network Identification and Protein-Protein Interaction Enrichment Analysis of the Enzymes

Protein-protein interaction networks, functional associations, and network analyses of all 14 common enzymes involved in these pathways were examined using the STRING database. The network details are listed in Table 2. The 3D images of the networks involving these enzymes are illustrated in Figure 2, and the results indicate that 3-beta-hydroxysteroid-4-alpha-carboxylate 3-dehydrogenase possesses an extremely low P-value of 2.14E-14 (Figure 2), followed by homogentisate phytyl transferase with a P-value of 1.00E-10 (Figure 2). Among the 14 enzymes analyzed, two STRING

networks are presented to highlight the most functionally significant and highly interactive protein associations relevant to the study objectives; additional network visualizations are provided in Supplementary File 2.

Table 1: *Identified Phytochemicals and Their Regulatory Pathways*

Phytochemicals	Pathways	Enzymes
Campesterol, Stigmasterol, Beta-Sitosterol, Delta 5 arenasterol, γ-sitosterol	Phytosterol biosynthesis (plants)	3-beta-hydroxysteroid-4-alpha-carboxylate 3-Dehydrogenase (decarboxylating)
		Cycloeucalenol isomerase
		Obtusifoliol-14α-demethylase
		4α-methyl-5α-ergosta-8,24-dien-3β-ol isomerase
		Sterol 24C methyltransferase
		Delta(7)-sterol 5(6)-desaturase
		Epi-sterol-desaturase
α-Tocopherol, γ-Tocopherol	Vitamin E biosynthesis	4-hydroxyphenylpyruvate dioxygenase
		Homogentisate phytyl transferase
		2-methyl-6-phytyl-1,4-benzoquinone methyltransferase
		γ-tocotrienol cyclase
		γ-tocotrienol methyltransferase
Piperitol, Sesamin, Sesamolin, Sesamolinol	Sesamin biosynthesis	Sesamin synthase
Sclareol	Cis-abienol biosynthesis	Sclareol synthase

The results of Table 2 depict that the majority of the proteins have 11 nodes, indicating a consistent size. The number of edges, which represents the interactions between the proteins, varies significantly. *3-beta-hydroxysteroid-4-alpha-carboxylate 3-dehydrogenase* has the highest number of edges (48), suggesting a highly interconnected module. The average local clustering coefficient, which measures the tendency of proteins to form clusters or modules, ranges from 0.588 to 1. This enzyme also has the highest average node degree (8.73), implying that the proteins in this component are highly interconnected. Being highly connected, this protein may play a central role and interact with other proteins, acting as a hub for multiple biological pathways that coordinate multiple biological processes, crucial for network stability, functional robustness, signal transduction and metabolic regulation. Disruption of this node could lead to significant downstream effects, indicating its importance in the studied biological system.

Table 2: *Protein Network Details of the Enzymes*

Enzymes	No. of nodes	No. of edges	Average node degree	Average local clustering coefficient	*p*-value
3-beta-hydroxysteroid-4-alpha-carboxylate 3-dehydrogenase (decarboxylating)	11	48	8.73	0.882	2.14E-14
Cycloeucalenol isomerase	11	10	1.82	0.909	0.627
Obtusifoliol-14α-demethylase	11	31	5.64	0.72	1.00E+00
4α-methyl-5α-ergosta-8,24-dien-3β-ol isomerase	11	35	6.36	0.876	5.92e-08
Sterol 24C methyltransferase	11	38	6.91	0.946	3.82E-10
Delta(7)-sterol 5(6)-desaturase	11	39	7.09	0.588	5.27E-10
Epi-sterol-desaturase	11	39	7.09	0.588	5.27E-10
4-hydroxyphenylpyruvate dioxygenase	11	34	6.18	0.801	5.56E-07
Homogentisate phytyl transferase	11	38	6.91	0.846	1.00E-10
2-methyl-6-phytyl-1,4-benzoquinone methyltransferase	11	30	5.45	0.738	3.97E-06
γ-tocotrienol cyclase	11	35	6.36	0.903	1.17E-06
γ-tocotrienol methyltransferase	8	28	7	1	1.39E-09
Sesamin synthase	11	28	5.09	0.827	0.951
Sclareol synthase	11	24	4.36	0.816	0.000206

Collectively, the network analyses indicate that 3-beta-hydroxysteroid-4-alpha-carboxylate 3-dehydrogenase is the most densely connected and functionally related module, with a higher number of interactions than expected. Therefore, the corresponding protein is considered biologically meaningful. This enzyme is responsible for the synthesis of all sterols, which are an integral part of the metabolic repertoire of the plants under study.

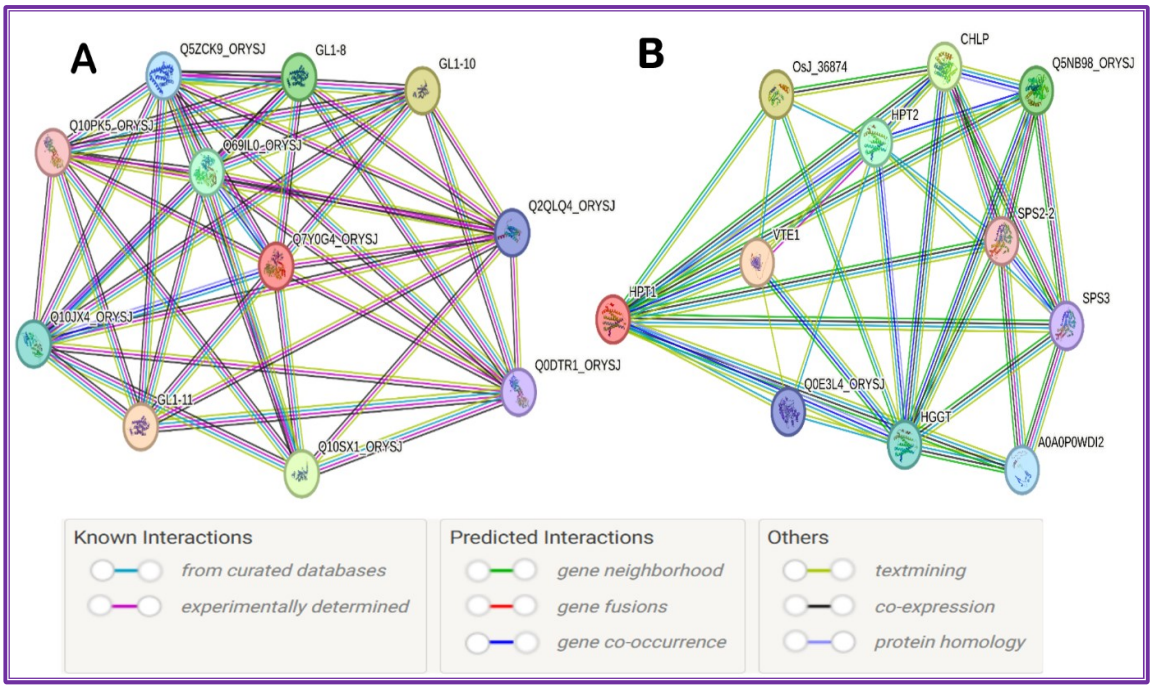

Figure 2:A- *Network Of 3-Beta-Hydroxysteroid-4-Alpha-Carboxylate 3-Dehydrogenase (Decarboxylating) Has 11 Nodes, 42 No of Edges, Average Node Degree Is 8.73 and Contains 2.14E-14 P Value. Here, Q7Y0G4_ORYSJ Is Query Protein.* ***B-*** *Network of Homogentisate Phytyl transferase has 11 Nodes, 38 No of Edges, Average Node Degree Is 6.91 and Contains 1.00E-10 P Value. Here, HPT1 Is Query Protein*

Phytochemical Profile Analysis of Sesamum indicum and Coriandrum sativum Seeds by GC-MS before and after elicitation:

The GC-MS analysis of the powdered samples dissolved in an aqueous medium from *Sesamum indicum* seeds and *Coriandrum sativum* seeds revealed a diverse array of bioactive compounds, as compared with NIST library spectra, highlighting their potential therapeutic applications.

Phytochemical Profile Analysis of Sesamum indicum Seeds

Initially, 22 compounds were identified in *Sesamum indicum*, including 3 substrates and 4 inhibitors of p-glycoprotein. Following elicitation, the number of detected compounds increased to 50, comprising 3 substrates and 19 p-glycoprotein inhibitors (Supplementary File 1). The control sesame seed extract produced a chromatogram (Figure 3) with approximately 22 observable peaks, spanning RT ~3.20 min to ~45.53 min. Key peaks include: glycerin, 1,3-dicyclohexylurea, and tetramethylphosphonium cation at ~3.20 min (~4.72%), representing small polar alcohols and derivatization residues; a significant trio at ~25.30 min (~16.32%) comprising n-hexadecanoic acid, pentadecanoic acid, and l-(+)-ascorbic acid 2,6-dihexadecanoate; poly- and monounsaturated fatty acid esters appear as peaks at ~28.63 min (~2.6%) and ~28.78 min (~3.37%); a dominant cluster at ~29.75 min (~38.9%) includes cis-vaccenic acid, cis-13-octadecenoic acid, and oleic acid; minor alcohol esters appear at ~35.90 min (~1.3%), and early eluting fatty alcohols at ~35.98 min (~1.36%); a prominent late-eluting cluster at ~45.53 min (~31.4%) consists of lignans- (+)-sesamin, asarinin, and episesamin.

These results align well with prior GC-MS investigations in sesame. For example, cis-9-hexadecenal and lignans like sesamol and sesamin are frequently reported as major peaks in ethanolic sesame

seed extracts, accounting for ~85% lipid derivatives and ~ 4-5% sesamin, respectively (Qadir *et al*., 2018). In addition, other studies identify glycerin and gamma-tocopherol as significant components in defatted seed meals (~32.8% glycerin), supporting your glycerin-rich early peak (Yeasmin *et al*., 2021).

The chromatogram of elicited sesame seed extract (Figure 4) reveals early eluting short-chain acids at RT ~3.34 min (~1.98%), specifically propanedioic propyl ester, hexanoic acid, and heptanoic acid, which were absent in the control, indicating a stress-responsive metabolite induction. In the mid-region (~8.5-11.7 min, ~2%), several medium-chain compounds appear: 2-sec-butylcyclohexanone (substrate), cyclohexanone derivatives, medium-chain aldehydes and alcohols (e.g., 2-nonen-1-ol, nonanal), and an organophosphate (2-nonylmethylphosphonofluoridate), marking elicitor-induced secondary metabolism. At ~25.48 min (7.18 %), a mixed cluster of n-hexadecanoic acid, pentadecanoic acid, and l-(+)-ascorbic acid 2,6-dihexadecanoate occurs, similar to the control but slightly reduced in intensity. The most dominant peak (~29.99 min, 57.32 %) comprises long-chain unsaturated fatty acids—cis-13-octadecenoic acid and cis-vaccenic acid—higher than in the control (~38.9 %), suggesting enhanced oleic acid accumulation. Adjacent at ~30.36 min (2.97%), octadecanoic acid and it ethoxylated ester appears exclusively in the elicited sample, along with a suite of inhibitory fatty acid esters and alcohols between 32 and 35.7 min (~2%). Finally, late-eluting lignans at ~45.54 min included (+)-sesamin (16.75%), episesamin (4.84%), and a benzo-dioxol lignan derivative (4.84%), collectively accounting for approximately 26.4% of the detected phytochemicals. Although this proportion was lower than that observed in the control sample, lignans remained a prominent phytochemical class following SA elicitation. Compared with the control, SA elicitation was associated with an increase in the relative abundance of unsaturated fatty acids from approximately 39% to 57%, while lignans remained among the major phytochemical constituents (~26–31%), suggesting retention of antioxidant-related compounds. Previous studies have reported that sesame seeds typically contain approximately 35–46% oleic acid and 38–50% linoleic acid, with lignan contents ranging from 2–6 mg/g seed, primarily sesamin and sesamolin (Qadir *et al*., 2018; Dar *et al*., 2019). Furthermore, sesamin has been reported to influence fatty acid oxidation and lipid metabolism at the molecular level (Pham *et al*., 2023). The observed increase in the relative abundance of unsaturated fatty acids following SA elicitation is consistent with these reported effects of sesamin and may indicate a role for lignan-associated pathways in lipid biosynthesis. Overall, SA elicitation preserved the nutritional and bioactive characteristics of sesame seeds while being associated with increased unsaturated fatty acid content and the continued presence of lignan constituents.

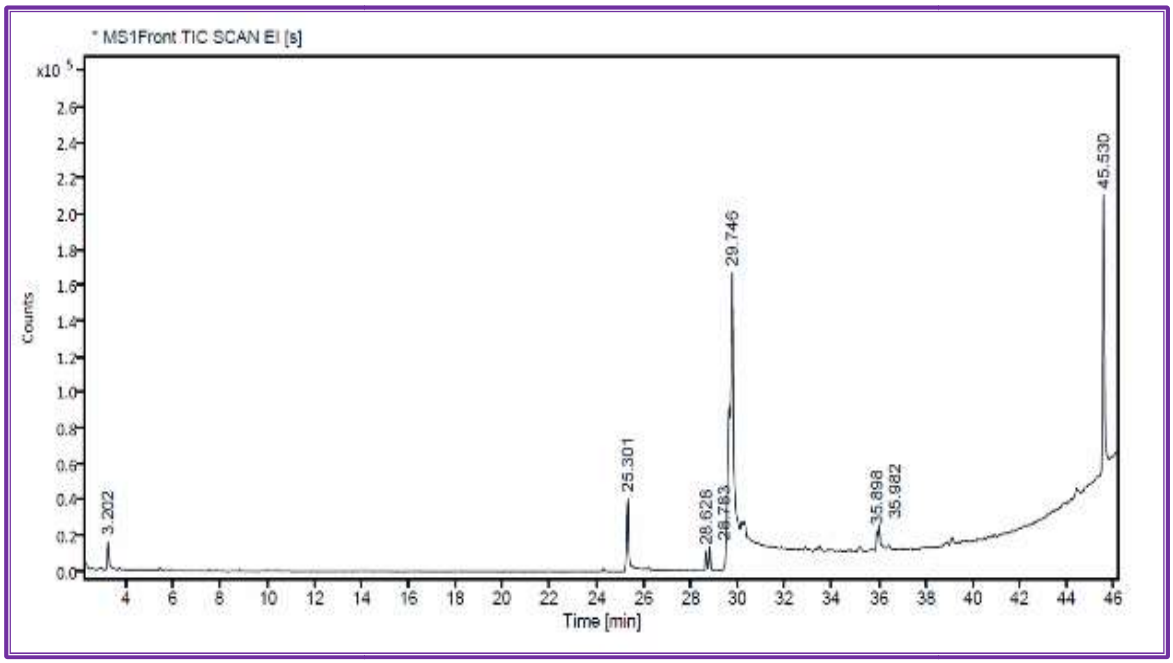

Figure 3: *GC-MS Chromatogram of S. Indicum Seed Extract (Control)*

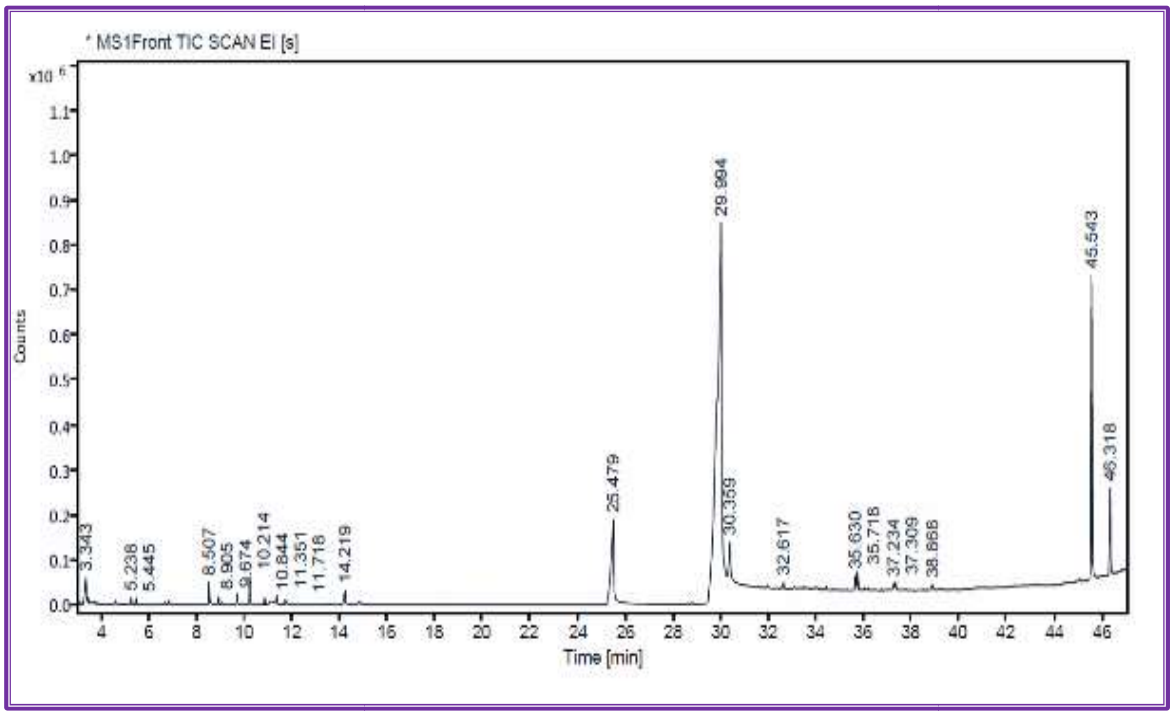

Figure 4: *GC-MS Chromatogram of S. Indicum Seed Extract Following SA Elicitation*

Phytochemical Profile Analysis of Coriandrum sativum Seeds

The GC-MS analysis of Coriandrum sativum seeds demonstrated marked alterations in their phytochemical composition following treatment with SA. In the untreated sample, 42 distinct compounds were identified, including 5 components with inhibitory activity and 2 recognized as substrates of p-glycoprotein. The chromatogram (Figure 5) reveals a complex metabolite profile, beginning with early-eluting low-molecular components such as N-substituted amines (e.g., cyclohexanamine derivatives), pyrimidines like thymine, and heterocyclic acids and amines—indicative of primary nitrogenous metabolism. Around 6-9 min, a cluster of pyrone/furanone derivatives, aromatic acids (benzoic acid), and amino sugars emerges, alongside sulfide-containing compounds like thiocyanates. Mid-chromatogram (13-18 min) is dominated by storage carbohydrates: Sucrose, melezitose, trehalose, and sugar alcohols such as cyclohexanetetrol are consistent with typical seed-reserve profiles. Later retention times (25-30 min) bring saturated and unsaturated fatty acids (palmitic, pentadecanoic, polyunsaturated C18 derivatives), and larger esterified molecules such as ascorbic acid dihexadecanoate. The late-eluting region (36-42 min) shows a striking presence of phenolic and bioactive secondary metabolites: a brominated antihistamine analog, naphthoquinones, xanthones, methoxycoumarins, decussatin, and anthocyanin glycosides. The study reveals high phenolic content in methanolic coriander extracts, with a mid-chromatogram dominating sugars. This derivatized extract highlights polar metabolites and secondary phenolic compounds, showcasing coriander's biochemical versatility compared to monoterpene-rich essential oil profiles.

The elicited seed extract showed an altered chromatographic profile (Figure 6) compared to the control, with 44 detectable components, including 8 p-glycoprotein inhibitors and 7 substrates (Supplementary File 1). A sharp and dominant peak at ~6.365 min- representing linalool, linalyl acetate, and linalyl butyrate-accounts for approximately 37% of the total ion current. This is accompanied by additional early eluting monoterpenes (~3.56-5.58 min), including α-pinene, 3-carene, γ-terpinene, terpinolene, D-limonene, and camphor, none of which were present in control samples. The dominant appearance of linalool and its esters confirm the elicitor effect of SA on linalool synthase, consistent with studies on methyl jasmonate enhancing linalool levels in coriander leaves (Kianersi*et al*., 2022; Puthusseri*et al*., 2013). This suggests a similar activation by SA, prioritizing aromatic oil accumulation. Mid-RT peaks (9-14 mi) indicate the presence of sugar-related compounds such as glycerin, sugar alcohols, trisaccharides (melezitose), and disaccharides (maltose,

lactose). The elicited sample sees a notable rise in fatty acid methyl esters, particularly monounsaturated (methyl elaidate, methyl oleate) and polyunsaturated fatty acids, and they are flagged as potential inhibitors. The control only had these at low levels. Their high abundance (~29% at ~30.45 min) suggests SA-induced activation of lipid metabolism, possibly defence- or signalling-related fatty acids. A triglyceride (trielaidin) appears at ~40.7 min, representing stress-related long-chain lipid accumulation. Early peaks of diketones and thioguanidine derivatives also point to stress- or defense-related metabolism. While melezitose appears in both profiles, new sugars- maltose and lactose- emerge post-elicitation (4.61 % at ~14.13 min), replacing sucrose and trehalose seen in the control. This change implies a shift in carbohydrate metabolism, likely supporting energy requirements for secondary metabolite biosynthesis.

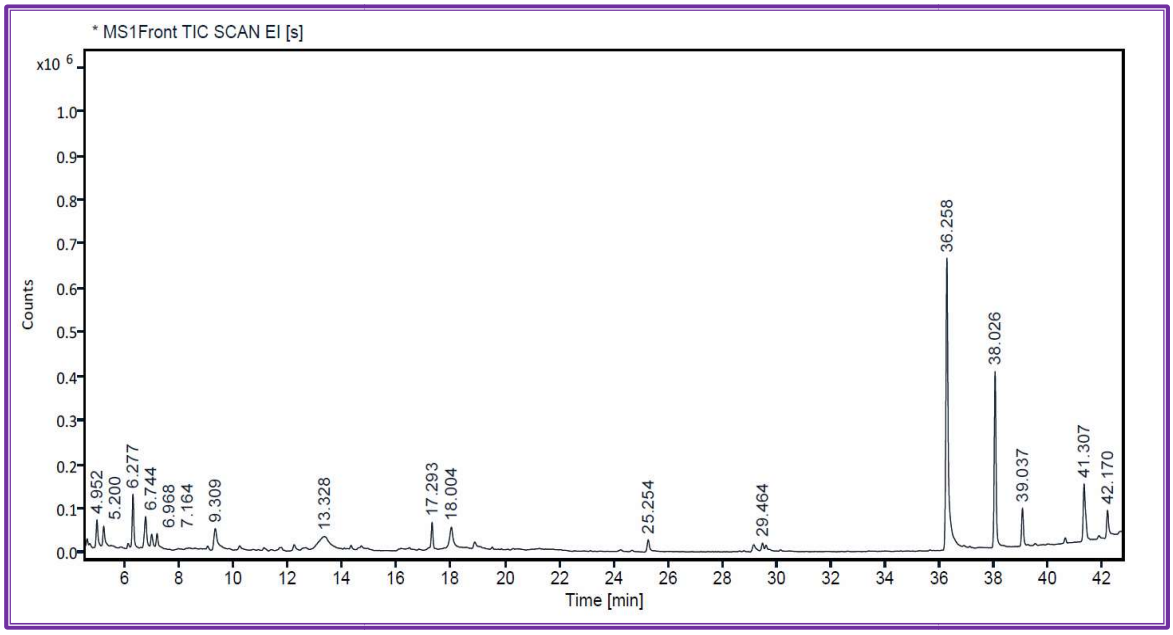

Figure 5: *GC-MS Chromatogram of C. Sativum Seed Extract (Control)*

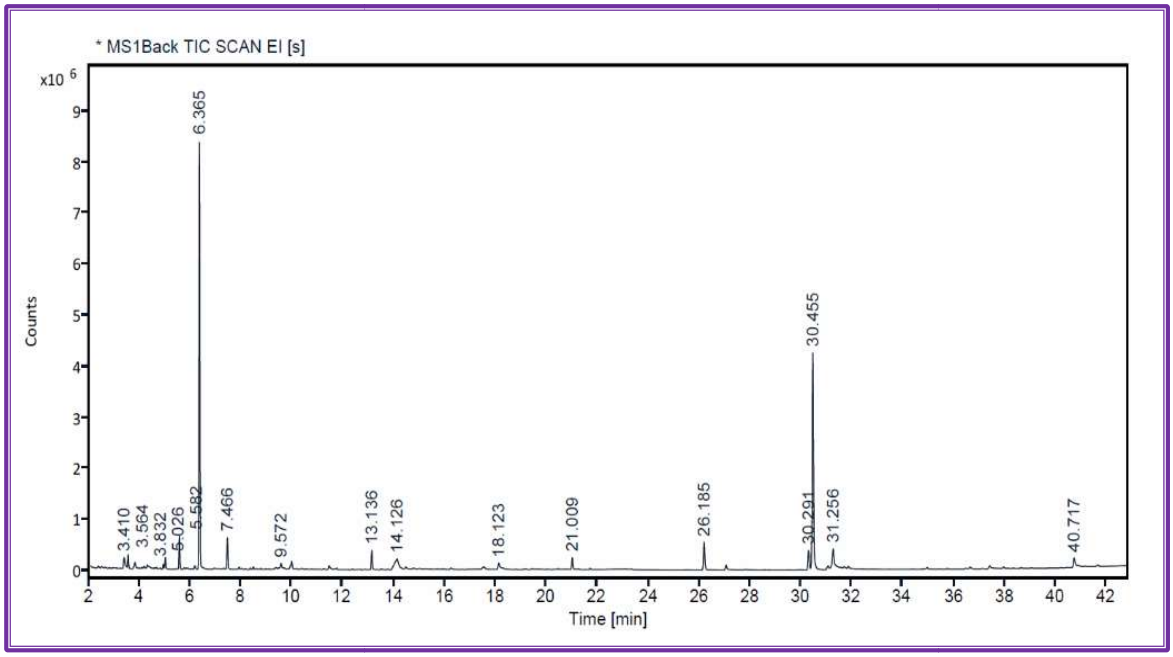

Figure 6: *GC-MS Chromatogram of C. Sativum Seed Extract Following SA Elicitation*

Discussion

P-glycoprotein inhibitors play a crucial role in pharmacology and medicine by modulating the activity of the membrane transporter, thereby influencing drug absorption, distribution and bioavailability. They also contribute to drug-drug interactions, as numerous therapeutic agents are substrates of P-glycoprotein (Veiga-Matos *et al*., 2023; Harish *et al*., 2025). Inhibition of this transporter can increase intracellular drug concentrations, potentially enhance therapeutic efficacy but also increase the risk of toxicity. However, not all naturally occurring compounds reported to inhibit P-glycoprotein are suitable for therapeutic application. To be developed into established drug candidates, such compounds must undergo extensive preclinical and clinical evaluation to determine their safety, efficacy, pharmacokinetic properties and potential adverse effects (Gandla *et al*., 2023; Yu *et al*., 2016). Once identified, these bioactive compounds must be produced in sufficient quantities through chemical synthesis, metabolic engineering, plant biotechnology or plant cell culture systems. Enhancing the biosynthetic pathways responsible for producing such compounds in plants may provide a cost-effective, environmentally sustainable and scalable strategy for obtaining valuable phytochemicals (Guo *et al*., 2024). Various chemical stimuli can effectively enhance the phytochemical content of different plant species.

Extensive research on SA has revealed its critical role in regulating a broad range of physiological, biochemical and developmental processes, as well as its capacity to induce substantial alterations in plant secondary metabolism. Exogenous SA application has been reported to improve plant growth performance while simultaneously stimulating the production of secondary metabolites in several horticultural and medicinal crops (Khan *et al*., 2015; Ali, 2021 & Wu *et al*., 2026). Treatment of plant species such as Salvia, Ginkgo, Carthamus, Ajuga, Capsicum, Zingiber and Thymus with SA has been associated with increased accumulation of phenolic compounds, including caffeic acid, rosmarinic acid, chlorogenic acid, apigenin, rutin and anthocyanins (Abbasi *et al*., 2020; Ejtahed *et al*., 2015 & Ni *et al*., 2018). In the present study, foliar application of SA was associated with an increase in phytochemical diversity and abundance in *Sesamum indicum* and *Coriandrum sativum* after 100 days of treatment, indicating that SA may serve as an effective elicitor for stimulating secondary metabolite biosynthesis in these species.

Elicitors induce oxidative stress in plants by promoting the generation of reactive oxygen species (ROS), which function as signaling molecules that activate plant defense responses. The accumulation of ROS initiates a cascade of molecular events leading to the biosynthesis of protective secondary metabolites and stress-responsive compounds (Rao *et al*., 2025; Munir *et al*., 2025). Among the various elicitors studied, SA functions as a key signaling molecule that promotes the accumulation of antioxidant compounds, including phenolic acids, polyphenols and flavonoids, which contribute to plant adaptation and defense mechanisms. Their accumulation is frequently associated with oxidative stress responses triggered by elicitors and other signaling agents (Kumar *et al*., 2023; Hatta *et al*., 2026).

In the present study, phytochemicals detected in sesame and coriander seeds were systematically screened through literature mining, database searches and bioinformatic analyses to identify compounds that have previously been reported as potential P-glycoprotein inhibitors. The study further identified biosynthetic pathways associated with these compounds and revealed 14 common enzymes involved in their biosynthesis. Among these, 3β-hydroxysteroid-4α-carboxylate 3-dehydrogenase emerged as the most interconnected enzyme, suggesting a potentially important role in sterol biosynthesis. GC-MS profiling revealed changes in phytochemical diversity and composition in both sesame and coriander seeds following SA elicitation. These observations are consistent with previous reports describing SA as a potent elicitor that activates stress-responsive signaling pathways and promotes the biosynthesis of defense-related secondary metabolites. However, the biological activities of the identified compounds, including their reported P-glycoprotein inhibitory properties, require validation through dedicated biochemical, cellular and pharmacological studies.

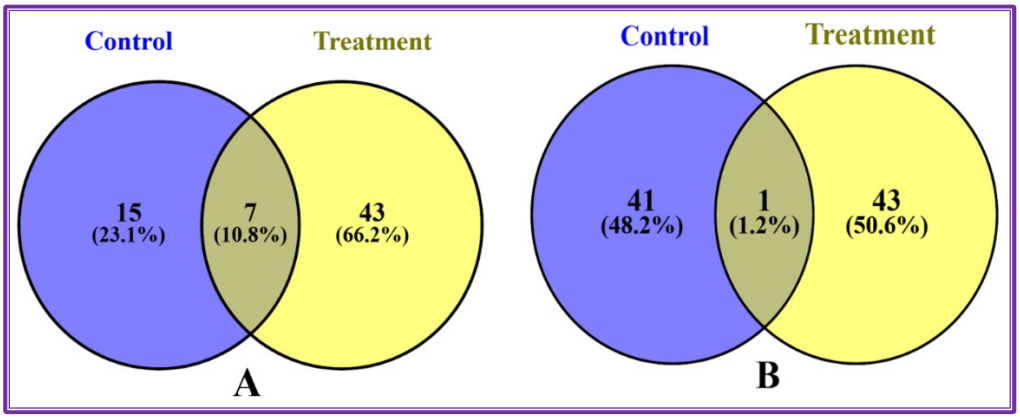

Figure 7: *The Venn Diagram Illustrates the Distribution of Compounds Detected By GC-MS In Sesamum Indicum (A) And Coriandrum Sativum (B) Under Both Control and Elicited Conditions. For Sesamum Indicum, 65 Compounds Were Identified, With 43 Unique to The Treatment, 15 Unique to the Control And 7 Common to Both. For Coriandrum Sativum, 85 Compounds Were Identified, With 43 Unique to The Treatment, 41 Unique to the Control And 1 Shared by Both Conditions*

GC-MS metabolite profiling and the Venn diagrams presented in Figure 7 for *Sesamum indicum* and *Coriandrum sativum* illustrate striking shifts in phytochemical production following SA elicitation. Specifically, sesame showed a doubling of detected compounds—65 in total—with 66% treatment-specific compounds, 23% control-specific compounds, and only 11% overlap. Coriander displayed even more dramatic reprogramming, with 85 compounds in total: 50% treatment-specific, 48% control-specific, and only 1% shared.These distributions suggest that SA is associated with extensive metabolic reorganization, particularly in coriander, where nearly all detected compounds differ between conditions. In sesame, elicitation enriches existing pathways, as evidenced by increased fatty acid esters and lignans, while maintaining some baseline metabolites. In contrast, the almost complete turnover in the coriander metabolome underscores a more radical shift, aligning with the emergence of essential terpenoids such as linalool and γ-terpinene. Taken together, the Venn diagrams are consistent with the proposed role of SA in influencing metabolic equilibrium toward defense-associated secondary metabolism, corroborating earlier statements about species-specific responses and metabolic flux redistribution.

In sesame, elicitation appeared to enrich pathways associated with fatty acid derivatives and lignan biosynthesis while retaining a subset of metabolites present under control conditions. Conversely, the pronounced reduction in overlap between control and treated coriander samples suggests a broader restructuring of the metabolome, coinciding with the appearance of characteristic terpenoid compounds such as linalool and γ-terpinene. These observations are consistent with the notion that SA influences metabolic equilibrium and promotes the biosynthesis of defense-associated secondary metabolites. The contrasting responses observed between sesame and coriander further indicate that elicitor-induced metabolic reprogramming may be strongly influenced by species-specific regulatory mechanisms, metabolic architecture and pathway responsiveness.

Collectively, the findings of this study suggest that SA elicitation can influence phytochemical composition and enhance the occurrence of compounds previously reported in the literature as potential P-glycoprotein inhibitors. Recent studies have further highlighted the importance of synergistic interactions among phytochemicals in influencing biological activity and health-related outcomes, emphasizing the value of characterizing diverse phytochemical mixtures in plant-derived products (Fahey & Liu, 2026). The integration of pathway analysis, protein interaction networks and GC-MS profiling provides a framework for understanding the biosynthetic origins of these phytochemicals and their possible regulation under elicitor-induced stress conditions. However, future studies incorporating independent biological replicates, quantitative metabolomic analyses and direct experimental evaluation of P-glycoprotein inhibitory activity will be necessary to validate the pharmacological relevance of the identified compounds and confirm their functional roles.

Limitations

Phytochemical identification in this study was based on GC-MS profiling and NIST library matching without validation using authentic standards or biological assays; therefore, the reported compounds and their associated activities should be considered tentative. The study was conducted under natural environmental conditions, and parameters such as temperature, relative humidity and photoperiod were not continuously monitored. In addition, GC-MS analysis was performed on pooled seed samples from each treatment group, with a single analytical run per sample, preventing assessment of biological variability and statistical evaluation of treatment effects. Furthermore, the study was limited to two plant species and did not include gene or protein expression analyses. Consequently, the observed phytochemical differences should be interpreted as descriptive trends that require validation through future studies employing independent biological replicates, replicated metabolomic analyses and functional biological assays.

Future Scope

Future studies should focus on experimentally validating the reported P-glycoprotein inhibitory activities of the identified phytochemicals through appropriate biological assays. The inclusion of independent biological replicates, quantitative metabolomic analyses, and gene or protein expression studies would provide deeper insight into the mechanisms underlying salicylic acid-induced phytochemical production. Additionally, exploring other elicitors and medicinal plant species may further enhance the sustainable production of valuable bioactive compounds.

Conclusion

This study demonstrates the utility of integrating cheminformatic screening, metabolic pathway analysis and GC-MS profiling to investigate phytochemicals associated with reported P-glycoprotein inhibitory activity in *Sesamum indicum* and *Coriandrum sativum*. The identification of 3β-hydroxysteroid-4α-carboxylate 3-dehydrogenase as a highly interconnected enzyme highlights its potential importance as a regulatory node in sterol biosynthesis. SA elicitation was associated with notable alterations in phytochemical composition, including an increased occurrence of unsaturated fatty acids, esters, alcohols, lignans and terpenoids. These findings indicate that SA-mediated elicitation can influence metabolic pathways involved in the biosynthesis of diverse phytochemicals and may represent a useful strategy for enhancing phytochemical diversity in medicinal and aromatic plants.

The identified compounds represent potential P-glycoprotein inhibitor candidates reported in the literature; however, their inhibitory activities were not experimentally evaluated in the present study and should therefore be validated through dedicated biochemical, cellular and pharmacological investigations. Future studies should also incorporate independent biological replicates, quantitative metabolomic analyses, and detailed gene and protein expression studies to better understand the molecular mechanisms underlying elicitor-induced phytochemical production. The findings presented here provide a foundation for future investigations into plant-derived compounds with potential relevance to multidrug resistance and other pharmacological applications.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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