สารทุติยภูมิในพืช: การผสานพฤกษเคมี คอมพิวเตอร์ และนวัตกรรมเทคโนโลยีชีวภาพสู่อนาคต

พืชเป็นแหล่งทรัพยากรธรรมชาติที่มีความสำคัญต่อมนุษย์มาอย่างยาวนาน โดยเฉพาะในฐานะแหล่งของตัวยาและสารออกฤทธิ์ทางชีวภาพ สารเหล่านี้เรียกว่า **"สารทุติยภูมิในพืช" (Plant Secondary Metabolites: PSMs)** ซึ่งเป็นสารโมเลกุลขนาดเล็กที่พืชสร้างขึ้นจากกระบวนการเมแทบอลิซึมขั้นปฐมภูมิ แม้ว่าสารทุติยภูมิจะไม่ได้รับบทบาทโดยตรงในการเจริญเติบโตหรือการสืบพันธุ์ของพืชเหมือนคาร์โบไฮเดรต โปรตีน หรือลิพิด แต่สารกลุ่มนี้ทำหน้าที่เสมือน "เกราะป้องกันตัว" และ "สารสื่อสาร" ที่ช่วยให้พืชสามารถปรับตัว รอดพ้นจากความเครียดในสิ่งแวดล้อม เช่น อุณหภูมิ ภัยแล้ง รังสี UV รวมถึงการเข้าทำลายของแมลงและเชื้อโรคต่าง ๆ ได้อย่างมีประสิทธิภาพ

การจัดกลุ่มสารทุติยภูมิในพืชและความสำคัญทางการแพทย์

จากการศึกษาพฤกษเคมี พืชสร้างสารทุติยภูมิออกมาหลากหลายรูปแบบ ซึ่งสามารถจำแนกกลุ่มใหญ่ ๆ ได้ตามโครงสร้างทางเคมีและจุดกำเนิดทางชีวสังเคราะห์ ดังนี้

1. **กลุ่มสารที่มีซัลเฟอร์เป็นองค์ประกอบ (Sulfur-containing compounds):** เช่น กลูโคสิโนเลต (Glucosinolates) และกลูตาไธโอน (Glutathione) มักพบในพืชตระกูลกะหล่ำ (Brassicaceae) และพืชสกุลกระเทียม (*Allium*) สารกลุ่มนี้เด่นในด้านการต้านอนุมูลอิสระ ต้านการอักเสบ และต้านจุลชีพ
2. **กลุ่มสารที่มีไนโตรเจนเป็นองค์ประกอบ (Nitrogen-containing compounds):** กลุ่มที่โดดเด่นที่สุดคือ **อัลคาลอยด์ (Alkaloids)** เช่น มอร์ฟีน (Morphine) จากฝิ่นที่ใช้เป็นยาแก้ปวดระดับรุนแรงทางการแพทย์ ควิโนน (Quinine) ที่ใช้ต้านมาลาเรีย และวินคริสทีน (Vincristine) จากแพงพวยฝรั่งที่ใช้เป็นยารักษามะเร็ง
3. **ฟีนอลิกและฟลาโวนอยด์ (Phenolics & Flavonoids):** สารกลุ่มนี้มีวงแหวนเบนซีนและหมู่ไฮดรอกซิล มีความสามารถในการต้านอนุมูลอิสระสูงมาก เช่น เคอร์ซิทีน (Quercetin) อะพิจีนิน (Apigenin) และเรสเวอราทรอล (Resveratrol) ซึ่งมีศักยภาพในการต้านการอักเสบ ป้องกันมะเร็ง และปกป้องระบบหลอดเลือดหัวใจ
4. **เทอร์พีนอยด์ (Terpenoids):** เป็นกลุ่มสารที่มีความหลากหลายทางโครงสร้างสูงที่สุด เช่น อาร์ทีมิซินิน (Artemisinin) ที่ใช้รักษาโรคมาลาเรีย ลิโมนีน (Limonene) จากเปลือกส้มที่มีฤทธิ์ต้านไวรัส รวมถึงสารกลุ่มถังเช่าหรือซาโปนินต่าง ๆ

ข้อจำกัดแบบดั้งเดิม สู่ "การสกัดสีเขียว" (Green Extraction)

ในอดีต การนำสารทุติยภูมิมาใช้ประโยชน์มักเผชิญกับข้อจำกัดเรื่องปริมาณสารที่พืชสร้างขึ้นตามธรรมชาติซึ่งมีอยู่น้อยมาก การสกัดด้วยวิธีดั้งเดิม เช่น การแช่ยุ่ย (Maceration) หรือการกลั่นด้วยไอน้ำ (Hydrodistillation) มักต้องใช้ตัวทำละลายอินทรีย์ในปริมาณมาก ซึ่งอาจทิ้งสารตกค้างและก่อให้เกิดมลพิษต่อสิ่งแวดล้อม อีกทั้งความร้อนที่ยาวนานอาจทำลายโครงสร้างของสารที่ไวต่ออุณหภูมิได้

ด้วยเหตุนี้ เทคโนโลยี **"การสกัดสีเขียว" (Green Extraction Systems)** จึงเข้ามามีบทบาทสำคัญ โดยการนำนวัตกรรม เช่น การสกัดด้วยคลื่นไมโครเวฟช่วย (Microwave-Assisted Extraction: MAE) การสกัดด้วยคลื่นอัลตราซาวด์ช่วย (Ultrasound-

Assisted Extraction: UAE) หรือการใช้ตัวทำละลายที่เป็นมิตรต่อสิ่งแวดล้อม เช่น ตัวทำละลายยูเทกติกประหยัดลึกธรรมชาติ (Natural Deep Eutectic Solvents: NaDES) และน้ำหรือเอทานอล วิธีการเหล่านี้ไม่เพียงแต่ช่วยลดระยะเวลาและพลังงานที่ใช้ แต่ยังรักษาสภาพโครงสร้างและเพิ่มประสิทธิภาพในการเก็บเกี่ยวสารออกฤทธิ์ทางชีวภาพได้ดียิ่งขึ้น

พลิกโฉมการค้นหายาด้วยคอมพิวเตอร์ (In Silico Approaches)

กระบวนการพัฒนาและค้นหายาในปัจจุบันได้เปลี่ยนผ่านจากวิธี "หนึ่งโรค-หนึ่งยา" แบบดั้งเดิมที่ต้องอาศัยการทดลองในห้องปฏิบัติการซึ่งใช้เวลานานและมีค่าใช้จ่ายสูง ไปสู่การบูรณาการเทคโนโลยีคอมพิวเตอร์และชีววิทยาระบบ (Computational & Systems Biology) หรือที่เรียกว่า **การศึกษาระดับ In Silico**

เทคนิคการจำลองการจับกันของโมเลกุล หรือ **Molecular Docking** ร่วมกับการคัดกรองเสมือนจริง (Virtual Screening) ช่วยให้นักวิจัยสามารถนำโครงสร้างของสารทุติยภูมิจากพืชนับพันชนิดในฐานข้อมูลเปิด (Open-access databases) มาทดสอบความสามารถในการเข้าจับกับโปรตีนเป้าหมายของโรคได้อย่างรวดเร็ว

ตัวอย่างความสำเร็จ: ในช่วงที่มีการแพร่ระบาดของ COVID-19 นักวิจัยได้ใช้เทคนิค In Silico คัดกรองสารทุติยภูมิในพืชจำนวน 4,704 ชนิด จนพบว่าสารกลุ่มไตรเทอร์พีนอยด์มีฤทธิ์เด่นในการเข้าจับกับส่วนโมเลกุลส่วนปลาย (RBD) ของโปรตีนหนาม (Spike Protein) ของไวรัส SARS-CoV-2 ซึ่งช่วยยับยั้งการเข้าสู่เซลล์เจ้าบ้านได้ รวมถึงสารกลุ่มฟลาโวนอยด์ที่สามารถยับยั้งเอนไซม์ Main Protease (M^{pro}) ของไวรัสได้อย่างมีประสิทธิภาพ

นอกจากนี้ การใช้คอมพิวเตอร์ยังช่วยทำนายคุณสมบัติทางเภสัชจลนศาสตร์และความเป็นพิษ (ADMET Profiling) เช่น การดูดซึม การกระจายตัว และการเปลี่ยนสภาพของยาในร่างกาย ช่วยตัดสารที่ไม่ผ่านเกณฑ์ออกไปก่อนที่จะก้าวเข้าสู่กระบวนการทดลองจริงในสัตว์หรือในมนุษย์

ความเชื่อมโยงระหว่างพืชและไมโครไบโอม (The Plant-Microbiome Connection)

ความก้าวหน้าทางวิทยาศาสตร์ทำให้เราพบว่า การสร้างสารทุติยภูมิของพืชไม่ได้เกิดขึ้นอย่างโดดเดี่ยว แต่มีความสัมพันธ์อย่างลึกซึ้งกับจุลินทรีย์ในดินและบริเวณรากพืช (Rhizosphere Microbiome) พืชสามารถปล่อยสารทุติยภูมิ เช่น กลูโคสิโนเลต เบนโซซาซินอยด์ (Benzoxazinoids) หรือสารกลุ่มคูมาริน (Coumarins) ออกมาทางราก เพื่อทำหน้าที่เลือกสรรและดึงดูดกลุ่มจุลินทรีย์ที่มีประโยชน์ (Plant Growth-Promoting Rhizobacteria: PGPR) ให้เข้ามาอยู่อาศัย

ในทางกลับกัน จุลินทรีย์เหล่านี้จะช่วยส่งเสริมการเจริญเติบโต ช่วยให้พืชดูดซึมสารอาหารได้ดีขึ้น และกระตุ้นระบบภูมิคุ้มกันของพืช ส่งผลให้พืชผลิตสารทุติยภูมิที่มีคุณค่าทางยาเพิ่มมากขึ้นและมีความหลากหลายทางโครงสร้างสูงขึ้นผ่านกระบวนการเปลี่ยนสภาพทางชีวภาพของจุลินทรีย์ (Microbial Biotransformation)

นวัตกรรมชีววิทยาสังเคราะห์และการผลิตอย่างยั่งยืน

เพื่อแก้ปัญหาความไม่แน่นอนของสภาพภูมิอากาศและการขาดแคลนทรัพยากรพืชในธรรมชาติ นวัตกรรม **ชีววิทยาสังเคราะห์ (Synthetic Biology)** และ **วิศวกรรมเมแทบอลิซึม (Metabolic Engineering)** ได้กลายมาเป็นคำตอบสำหรับการผลิตสารทุติยภูมิอย่างยั่งยืน

ปัจจุบันมีการนำเทคโนโลยีแก้ไขยีน เช่น **CRISPR-Cas9** และเทคโนโลยีการทำให้ยีนเงียบ (Gene Silencing) มาใช้ปรับแต่งเส้นทางชีวสังเคราะห์ในพืชอย่างแม่นยำ ตัวอย่างเช่น:

- การใช้ CRISPR-Cas9 ตัดยีน SQS ในต้นอาร์ทีมิเซีย (*Artemisia annua*) สามารถเพิ่มผลผลิตของสารอาร์ทีมิซินินได้ถึง 3.2 เท่าในการเพาะเลี้ยงราก
- การกระตุ้นยีน SmMYB1 ในกลุ่มพืชสมุนไพรช่วยเพิ่มปริมาณสารแทนชิโนน (Tanshinone) ได้สูงถึง 8.5 เท่า

นอกจากนี้ การเพาะเลี้ยงเนื้อเยื่อพืช (Plant Tissue Culture) การเพาะเลี้ยงรากฝอย (Hairy Root Cultures) โดยใช้เชื้อ *Agrobacterium rhizogenes* ตลอดจนการขยายขนาดการผลิตในถังหมักชีวภาพ (Bioreactors) ล้วนเป็นระบบควบคุมสภาพแวดล้อมที่ช่วยให้มนุษย์สามารถผลิตสารออกฤทธิ์ทางยาได้อย่างยืดหยุ่น ปลอดภัย และไม่ทำลายความหลากหลายทางชีวภาพในธรรมชาติ

บทสรุปและทิศทางในอนาคต

สารทุติยภูมิในพืชเปรียบเสมือนขุมทรัพย์ทางยาที่ยังรอการค้นพบอีกเป็นจำนวนมาก การก้าวข้ามขีดจำกัดแบบเดิมจำเป็นต้องอาศัยการบูรณาการความรู้แบบสหวิทยาการ ตั้งแต่การถอดรหัสพันธุศาสตร์ฟังก์ชัน (Functional Genomics) การคัดกรองความแรงของสารด้วยคอมพิวเตอร์ (In Silico Screening) ไปจนถึงการใช้ระบบชีววิทยาสังเคราะห์ในการผลิต แนวทางหลากมิตินี้ไม่เพียงแต่จะช่วยเร่งอัตราการค้นพบตัวยาใหม่ ๆ ที่มีประสิทธิภาพในการรักษาโรคอุบัติใหม่และโรคที่มีความซับซ้อนของมนุษย์ แต่ยังเป็นรากฐานสำคัญสู่การเกษตรที่ยั่งยืนและการพัฒนาผลิตภัณฑ์ฐานชีวภาพเพื่อคุณภาพชีวิตที่ดีขึ้นในอนาคต

เอกสารอ้างอิงเชิงวิชาการ:

- Priyanka, Sharma, P., Gupta, M., Bansal, P., Singh, R., Sharma, A. K., & Datta, S. (2026). Plant secondary metabolites: Integrating phytochemical characterization, in silico approaches, and biotechnological innovations for future applications. *Rhizosphere*, 39, 101392.

Rhizosphere 39 (2026) 101392

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

Plant secondary metabolites: Integrating phytochemical characterization, *in silico* approaches, and biotechnological innovations for future applications

Priyanka [a], Pooja Sharma [a], Mahiti Gupta [a], Poonam Bansal [a], Raj Singh [a], Anil Kumar Sharma [b], Sonal Datta [a,*]

[a] Department of Bio-Sciences and Technology, MMEC, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, 133207, India
[b] Department of Biotechnology, Amity University of Punjab, Mohali, 140306, India

ARTICLE INFO

Keywords:
Drug discovery
Plant secondary metabolites
Extraction
Purification
In silico analyses
Microbiome
Metabolic engineering
Synthetic biology

ABSTRACT

Plant secondary metabolites (PSMs) are specialized compounds that are primarily involved in plant defense mechanisms, adaptation, and interactions with the natural environment. In addition to their ecological roles, these molecules have been of significant interest due to their broad pharmacological applicability and long history of importance to human health. Many natural products derived from plants have yielded useful therapeutic agents and continue to serve as templates for designing new bioactive molecules. Nevertheless, their full use has been restricted by the difficulties associated with low natural abundance, the complexity of the pathway, and the effective characterization of the pathway. This review highlights the emerging importance of PSMs that incorporate recent discoveries in phytochemistry, computational methods, and biotechnology. We discuss how *in silico* tools, such as molecular docking, virtual screening, and artificial intelligence-assisted analyses, are accelerating the discovery of promising candidates and their molecular targets. Simultaneously, metabolic engineering, synthetic biology, tissue culture, and controlled production systems are opening up fresh prospects of scalable, sustainable production of metabolites. The review also highlights the role of increasing prominence of plant-microbiome interactions in the development of PSM biosynthesis, diversification, and functional activity.

Altogether, there is growing evidence to indicate that the combination of computational tools, systems biology models, and innovative production technologies would greatly accelerate the discovery, optimization, and translational use of plant secondary metabolites. It is expected that such multidisciplinary strategies will have transformative impacts on improving therapeutics, sustainable agriculture, and the development of functional bio-based products.

1. Introduction

Various secondary metabolites found in plants have been valuable sources of insecticides, food additives, and human medication due to their extensive biological activity (Han and Miao, 2024). Thousands of plant secondary metabolites are known, which are classified based on their structure, function, and biosynthesis (Thirumurugan et al., 2018). Plant metabolites are broadly classified into two categories: primary and secondary. Primary metabolites, including proteins, lipids, and carbohydrates, are essential for plant growth, development, and reproduction. In contrast, secondary metabolites are low-molecular-weight compounds derived from primary metabolic pathways. Under specific conditions, these compounds perform specialized functions, including protecting plants against insect herbivory and pathogenic infections, as well as enhancing tolerance to diverse environmental stresses (Upadhyay et al., 2024). Although they are not directly involved in plants' primary metabolic activity, they are considered defense compounds that protect plants under environmental stress. They play a pivotal role in modulating complex stress-response signaling pathways and regulating diverse metabolic processes (Rao et al., 2025). In addition to their defensive roles, plant secondary metabolites act as signaling molecules, mediating critical ecological interactions, symbiotic associations, and metal ion chelation pathways (Selwal et al., 2025). For centuries, secondary metabolites have been exploited in both drug design (Atanasov et al., 2021) and agriculture to enhance human health and improve crop productivity (Clemensen et al., 2020).

* Corresponding author. Department of Bio-Sciences and Technology, M. M. Engineering College, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala, 133207, India.
E-mail addresses: sdatta.sonal@gmail.com, sonaldatta@mmumullana.org (S. Datta).

https://doi.org/10.1016/j.rhisph.2026.101392
Received 5 December 2025; Received in revised form 14 May 2026; Accepted 7 June 2026
Available online 10 June 2026

Historical records, particularly from Ayurvedic medicine, document extensive knowledge of plants, their interactions with microorganisms, and their therapeutic applications. Plants have long held a central place in human societies due to their capacity to yield natural remedies (Sofowora et al., 2013). However, the advent of organic chemistry during the Industrial Revolution introduced synthetic compounds into the pharmaceutical market (Seidel, 2020). Over time, the emergence of adverse side effects and the rapid development of antimicrobial resistance to synthetic drugs renewed interest in natural sources, such as bacteria, fungi, and plants, as sources of novel bioactive agents.

Ethnopharmacological studies reveal that secondary metabolites, produced by bacteria, fungi, and plants under biotic and abiotic stress, are responsible for diverse bioactivities and therapeutic properties (Dou and Weathers, 2022). Plants, in particular, represent an abundant reservoir of such metabolites. They maintain intricate symbiotic relationships with endophytic bacteria and fungi, facilitating mutual survival and resilience under environmental pressures. Communication between plants and their endophytes occurs via chemical signaling or, in some cases, horizontal gene transfer (Howard et al., 2022). The resulting plant endophyte consortia synthesize a wide array of bioactive compounds, many of which have been harnessed as medicinal agents for the treatment of various diseases. In this respect, the combination of traditional knowledge and contemporary drug discovery methods has become a topic of great interest. Recent developments in computational biology, high-throughput screening, and green extraction technologies have enabled a more systematic exploration of plant secondary metabolites as therapeutic agents. However, despite the increasing number of studies, a comprehensive synthesis of phytochemical diversity in relation to computational strategies, sustainable extraction techniques, technologies, and translational drug development remains limited. Thus, this review aims to provide an integrative overview of plant secondary metabolites, including their therapeutic potential, current advances in *in silico* drug discovery, advances in biotechnological methods, and the challenges and future research directions. Although there is a rich literature on plant secondary metabolites and their pharmacological interest, current reviews tend to focus primarily on discrete facts of bioactivity, classification, or extraction methods and do not incorporate new interdisciplinary methodologies. Specifically, no integration of phytochemical diversity with more sophisticated computational approaches, green extraction methods, and translational drug discovery systems has been achieved.

In this regard, the current review offers a comprehensive and integrative approach by synthesizing classic information on plant secondary metabolites with contemporary computational methods, including molecular docking, network pharmacology, and *in silico* ADMET profiling. Moreover, this review focuses on sustainable and green extraction practices, critically analyzes structure-activity relationships, and indicates contemporary problems and future research opportunities of phytochemical-based therapy. With these domains filled, the work provides a futuristic model for accelerating the discovery and optimization of plant-based bioactive substances as future drug targets (Barthwal and Mahar, 2024).

2. Plant secondary metabolites

Plants synthesize a diverse array of organic compounds, collectively termed secondary metabolites, most of which are not directly involved in primary physiological processes such as growth and development (Kohnen-Johannsen and Kayser, 2019). In contrast, primary metabolites, including organic acids, acyl lipids, carbohydrates, and phytosterols, are universally present in plant tissues and are essential for metabolic activities that sustain plant growth and reproduction. Historically, primary metabolites have been extensively studied, whereas secondary metabolites have often been overlooked by plant biologists due to their perceived lack of biological importance. However, secondary metabolites are now recognized for their critical roles in shaping the distinct taste, color, and aroma of plant organs, as well as for their ecological functions.

Numerous studies have reported diverse ecological functions of plant secondary metabolites, particularly their role in protection against environmental stressors and in defense mechanisms against microbes, herbivores, and insects (Adedayo et al., 2025). In addition to conferring biotic stress tolerance, plant secondary metabolites also play a crucial role in mitigating abiotic stresses such as temperature fluctuations, ultraviolet (UV) exposure, and drought (Muhammad et al., 2025).

Certain compounds have also been shown to play a role in providing defense against herbivores, luring pollinators, acting as allelopathic agents, providing protection against toxicity, blocking UV light, and facilitating signal transduction (Edreva et al., 2007; Grassmann et al., 2002; Osbourn et al., 2003; Vasconsuelo and Boland, 2007). They are incredibly diverse; thousands have been identified in several classes. It is known that each plant family, genus, and species exhibit distinct characteristics based on the products they produce or release. Many secondary metabolites are small molecules with a molecular weight of less than 1500 Da, and their biosynthesis occurs in specific cellular structures, cell types, and organelles specialized for their production and storage (Li et al., 2024).

Furthermore, the plant secondary metabolites are categorized into four major classes: sulfur-containing compounds, including Glutathione (GSH), Glucosinolates (GSL), phytoalexins, thionins, defensins, and lectins; Nitrogen-containing compounds (non-protein-amino-acids, cyanogenic glucosides, and alkaloids); phenolics (lignans, phenolic acid, tannins, coumarins, lignins, stilbenes, and flavonoids), terpenoids (carotenoids, sterols, cardiac glycosides, and plant volatiles), as shown in Fig. 1. (Erb and Kliebenstein, 2020).

Secondary metabolites are valuable resources for pharmaceuticals, nutraceuticals, food additives, flavoring agents, and other food industry applications (Zhao et al., 2005). Owing to their extensive applications in research, medicine, and industry, the global demand for these compounds is steadily increasing. Their commercial uses span drug formulation, dye and polymer production, waxes, glues, fibers, antibiotics, herbicides, and insecticides. In plants, their ecological importance is well established, particularly in defense against pathogen attack, mitigation of environmental stress, and facilitation of interspecific protection (Samuni-Blank et al., 2012). Given their multifunctional roles, substantial commercial value, and generally low toxicity to living cells, secondary metabolites are considered highly advantageous. Their contributions extend beyond plant defense to encompass ecological interactions and beneficial impacts on human health, highlighting their multidimensional importance in both plant and human life (Agostini-Costa et al., 2012; Crozier et al., 2006; Wawrosch and Zotchev, 2021).

2.1. Extraction, purification, characterization, and green extraction strategies of plant secondary metabolites

Several conventional and traditional methodologies have been standardized for the extraction and purification of plant secondary metabolites (Wawrosch and Zotchev, 2021). Historically, techniques such as steam or hydrodistillation, maceration, percolation, infusion, decoction, and reflux extraction have been the primary approaches employed to isolate bioactive compounds (Wulandari, 2021). These methods typically utilize a range of organic and inorganic solvents, including methanol, hexane, petroleum ether, acetone, ethanol, and water, selected based on the solubility profile of the target metabolites (Abubakar and Haque, 2020). Depending on their physicochemical properties, secondary metabolites can dissolve in specific solvents at ambient temperature (Nielsen et al., 2019; Zhang et al., 2018). Maceration is a conventional extraction method that involves soaking plant materials in a suitable solvent at room temperature for an extended period (Wulandari, 2021). This technique enables the passive diffusion of plant secondary metabolites into the solvent (Chongo, 2025).

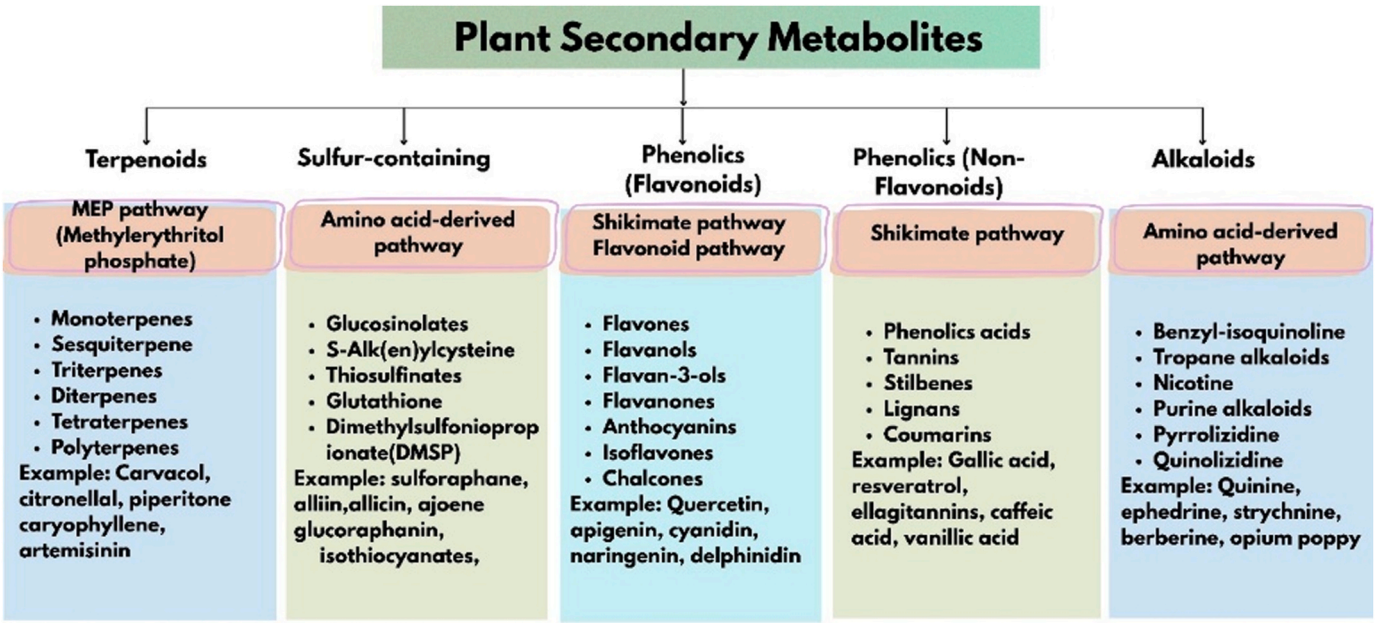

Fig. 1. Showing classification of Plant Secondary Metabolites. As depicted in the figure, there are four major types of Secondary Metabolites present in plants, i.e., Terpenoids (derived mainly from the MEP pathway), Sulfur-containing metabolites (amino acid-derived compounds), phenolics, subdivided into flavonoids and non-flavonoids (primarily originating from the shikimate and flavonoid pathways), and Alkaloids (largely amino acid-derived metabolites). Representative examples of each class are also shown (Erb and Kliebenstein, 2020).

Hydrodistillation is an essential, straightforward technique that uses the Soxhlet apparatus to isolate active compounds. The hydrodistillation method involves boiling plant materials in water or exposing them to steam, thereby facilitating the release of bioactive volatile compounds. These compounds are subsequently condensed and collected (Perović et al., 2024), making the technique widely used for the extraction of essential oils. However, hydrodistillation presents several challenges, including the loss of thermolabile compounds, prolonged processing time, and emulsification issues (Pheko and Makhzoum, 2024). Although widely used, traditional extraction methods are constrained by several factors that limit their effectiveness and applicability. Thermally labile Bioactive secondary metabolites are susceptible to degradation during extended exposure to heat, resulting in reduced yield and loss of biological activity. Moreover, the use of large amounts of organic solvents raises concerns about toxicity, environmental impacts, and the subsequent purification of the end product. The biological assays and pharmaceutical use may also be affected by solvent residues. Moreover, conventional extraction methods are often time-consuming and energy-intensive, making them less suitable for large-scale industrial use. The standardization and reproducibility are further complicated by variability in extraction efficiency due to variation in plant material, solvent polarity, and extraction conditions (El-Saadony et al., 2025).

The traditional PSM extraction methods have several limitations, including degradation of phytochemicals caused by excessive heat and the use of organic solvents. Additionally, these methods require the use of organic solvents at several steps, producing harmful byproducts and waste that can lead to environmental pollution. Therefore, green extraction methods and solvents are required for efficient, cost-effective extraction of PSM. Green extraction comprises a process that can reduce energy intake and increase the use of green solvents. The most promising methods are Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), Voltage-Assisted Extraction (VAE), Pressurized Liquid Extraction (PLE), and, recently, Shockwave-Assisted Extraction (SWAE). Additionally, by removing organic solvents from the extraction process, the use of green solvents lowers environmental pollution. The first example of a green solvent is Deep Eutectic Solvents (DES), a term first introduced by Abbott in 2002 (Perna et al., 2019). The DES led to the identification of Natural Deep Eutectic Solvents (NaDES); NaDES are large concentrations of naturally occurring DES in the cell that can be separated into various solvent types, including lipids and water. In addition to DES and NaDES, water is also considered a green solvent because it does not cause air or soil pollution. The production costs of distilled water are also very low. Furthermore, water-based formulations are commonly found in traditional medicine, such as macerations, infusions, decoctions, percolations (Torres-Ortiz et al., 2024). Moreover, ethanol is also regarded as a green solvent because it is easily and affordably obtained, purified, and biodegraded. In addition to alcohols, especially ethanol, green solvents include ketones and esters (Torres-Ortiz et al., 2024). Innovative green extraction techniques enhance extraction yields by rupturing cells, minimizing heat exposure, and protecting secondary metabolites from structural damage. Thus, green extraction aims to create environmentally friendly chemistry by using less harmful, safer reagents, cleaner extraction techniques, and producing safer products. Compared with organic solvents, extraction yields can be readily achieved and even surpassed with green solvents such as DES and NaDES, regardless of the extraction technique. Additionally, DES and NaDES are excellent substitutes for organic solvents in the extraction process since they are safer for biological applications, highly reusable, and non-toxic (Brito et al., 2025). Furthermore, new assisted extraction techniques, such as probe UAE and SWE, which rely on physical principles, such as cavitation, to collect secondary metabolites in the extract and reduce the risk of heat-degradable molecules breaking down, are a reliable alternative to conventional extraction techniques (Torres-Ortiz et al., 2024). Furthermore, the extraction period is shortened, resulting in a quicker extraction process and reduced overall energy use. Finally, coupling green extraction methods with green solvents can result in greater yields of secondary metabolites from plants. These methods improve the yield of secondary metabolite extraction from plants compared to conventional extraction techniques. Green extraction has a long way to go to improve and develop assisted extraction methods, where innovative steps may help increase extraction efficiencies, including the use of conventional solvents such as water or ethanol (Xi et al., 2023). Green solvents, nevertheless, have proven to be a competitive participant in the ongoing quest for safer

plant extracts with a vast diversity of secondary metabolites, and are even more efficient when extractants are designed specifically for the phytochemical expected to be obtained.

Other than conventional methods, some of the novel high-throughput techniques, such as Microwave-Assisted Extraction (MAE), Enzyme-Assisted Extraction (EAE), and Supercritical Fluid Extraction (SFE), particularly using supercritical carbon dioxide, are effective techniques for isolating polar compounds such as lipophilic antioxidants and terpenoids (Dashtian et al., 2024), Ultrasound-Assisted Extraction (UAE), and Pressurized Liquid Extraction (PLE) (Tena and Asuero, 2022).

Compared with traditional procedures, these modern techniques offer significant advantages, including reduced extraction time, lower solvent consumption, and improved yields and purities of secondary metabolites (Veer and Gopalakrishnan, 2016). A comparative analysis of traditional and green extraction methods reveals significant differences in efficiency, cost, and environmental impacts. Maceration and hydrodistillation are relatively simple and inexpensive methods that require longer extraction times, large volumes of solvent, and may produce lower yields, especially of thermosensitive compounds. Conversely, green extraction methods, including Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), and Supercritical fluid extraction (SFE), offer high extraction efficiency, shorter processing time, and reduced solvent use. These approaches are generally more environmentally friendly as they consume less energy and produce less hazardous waste. But their application could be associated with higher initial equipment and technical skills costs. Although green methods are the best in terms of yield and selectivity, scalability and economic viability are the major issues that need to be addressed to scale them to the industrial level. Hence, the preferred extraction method depends on a tradeoff among efficiency, cost, compound stability, and environmental factors (Cao et al., 2025; Wawrosch and Zotchev, 2021).

Crude plant extracts represent complex mixtures of diverse natural compounds. To isolate individual bioactive constituents, fractionation procedures are employed based on differences in metabolite polarity and molecular size (Gao et al., 2019). Purification can be achieved through a range of chromatographic and extraction techniques, including Column Chromatography (CC), Vacuum Liquid Chromatography (VLC), Size-Exclusion Chromatography (SEC), liquid-liquid extraction, and solid-phase extraction (Srivastava et al., 2021). High-Performance Liquid Chromatography (HPLC) is one of the most widely adopted advanced techniques for the efficient purification of secondary metabolites, peptides, flavonoids, and other small to medium-sized organic molecules. HPLC serves as a fundamental analytical platform for separating and quantifying compounds from crude soluble extracts (Dwivedi et al., 2021). Application-specific HPLC systems utilize tailored stationary phases, such as Normal-Phase (NP-HPLC) and reversed-phase HPLC (RP-HPLC), to optimize separation efficiency (Kanavarioti, 2019). High-performance Thin-Layer Chromatography (HPTLC) is an advanced form of conventional TLC that offers superior resolution, improved reproducibility, and enhanced sensitivity. The technique incorporates automated sample application, precisely controlled development chambers, and multi-wavelength detection, enabling both qualitative and quantitative analysis (Balekundri et al., 2025; Khorshed et al., 2025). HPTLC is particularly valuable for generating characteristic fingerprints of plant extracts, detecting adulteration, and ensuring the standardization of herbal formulations. In phytochemical characterization and quality control, it serves as a complementary technique to HPLC (Rabadiya et al., 2025; Vázquez and Tabanca, 2025).

The choice of column is determined empirically, based on the physicochemical characteristics of the target compound, to ensure high accuracy and reproducibility. Following purification, compounds are structurally characterized using a suite of analytical tools, including Nuclear Magnetic Resonance (NMR) spectroscopy, High-Resolution Mass Spectrometry (HRMS), X-ray crystallography, Infrared (IR) spectroscopy, Ultraviolet-Visible (UV-Vis) spectroscopy, and Optical Rotary Dispersion (ORD) for the evaluation of stereochemistry and isomeric mixtures (Maurer and Muddiman, 2012; Zarrouk et al., 2021).

NMR is a versatile, reproducible, and highly informative technique for elucidating metabolite structures (Rehman et al., 2022), applicable to both metabolomics and structural analysis. Metabolomics enables the mechanistic exploration of biochemical and cellular processes, with modern analytical techniques capable of detecting trace-level metabolites crucial for cellular signaling (Klassen et al., 2017). Once purified, natural products can be identified and assessed for pharmacological activity, enabling drug discovery studies. Additionally, purified metabolites are valuable in investigating the chemical diversity of plants or microbial species from different geographical regions, conducting translational metabolomics of biofluids to identify disease-specific biomarkers, analyzing cellular pathways, and performing organelle-targeted studies (Trivedi et al., 2020).

HRMS provides accurate molecular mass measurements and facilitates the determination of unambiguous molecular formulas for thousands of metabolites in complex mixtures. IR spectroscopy provides insights into functional group composition, while UV-vis spectroscopy distinguishes conjugated systems. ORD measurements determine optical activity and absolute configuration, and X-ray crystallography provides a complete three-dimensional structural resolution of crystalline compounds (Yousf et al., 2017). Methodologies for obtaining pure and biologically active compounds from plant extracts are illustrated in Fig. 2.

2.2. *In vitro* production of secondary metabolites

Secondary metabolites have long been exploited in medicine, drug development, and semi-synthetic modification by research institutions and the pharmaceutical industry. However, large-scale production directly from natural sources is limited, as excessive harvesting can have adverse impacts on floral biodiversity. Overexploitation of plants rich in essential secondary metabolites has already driven certain species into marginal habitats or placed them at risk of endangerment (Pandith et al., 2018). Furthermore, the quality and yield of these metabolites are influenced by geographical location, seasonal variation, and environmental factors. Consequently, reliance on natural harvesting may lead to the depletion of rare medicinal plants. Compounding this issue is the complexity of conventional purification and extraction procedures, which are often multistep and resource-intensive.

To address these limitations, advanced biotechnological *in vitro* strategies, such as micropropagation, organ culture, tissue culture, adventitious root culture, and cell culture, have emerged as viable alternatives for large-scale plant biomass production, germplasm conservation, and the synthesis of bioactive compounds (Fazili et al., 2022; Nalawade and Tsay, 2004). Metabolites obtained from *in vitro* cultures can be purified in substantial quantities using streamlined extraction methods (Biswas et al., 2023).

Among these approaches, cell suspension culture offers a rapid and scalable method for producing biomass for pharmaceutical applications, often yielding higher secondary metabolite yields than micropropagation or organ/tissue culture. Nonetheless, organ cultures can be more effective than suspension systems for producing certain target metabolites (Baque et al., 2012; Giri and Narasu, 2000; Urquiza-López et al., 2025). Hairy root culture represents another efficient platform for the biosynthesis of secondary metabolites in specific plant species. This method utilizes *Agrobacterium rhizogenes*, a natural genetic engineer that can transfer Root-Inducing (Ri) genes directly into the plant genome. Researchers have exploited its Ri-plasmid as a vector to enhance and stabilize metabolite production. Recent developments in plant technologies, especially hydroponics and controlled-environment cultivation, offer a promising avenue for improving PSM production (Chandran et al., 2020; Kaur et al., 2021). These systems can induce the production of desirable phytochemicals by carefully controlling nutrients and environmental factors to promote PSM production. An example

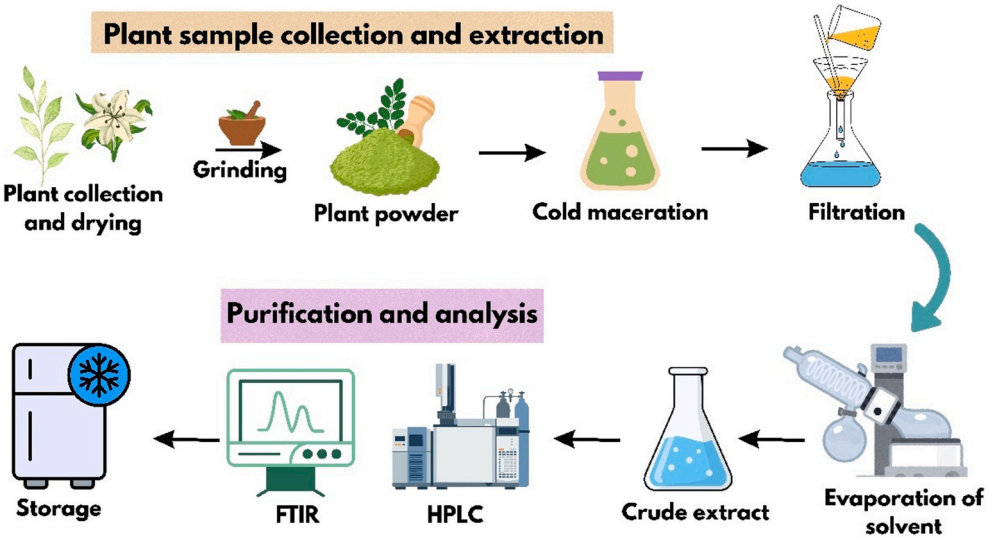

Fig. 2. Basic procedure to obtain pure and active compounds from plant extract. Starting with the selection of a plant and its specific part as a starting material. Extraction is performed by cold maceration with an appropriate solvent, followed by filtration and solvent evaporation to obtain the crude extract. The crude extract must undergo single or multiple rounds of chromatographic purification. The structural characterization can also be done by using high-performance liquid chromatography (HPLC) and FTIR for separation, phytochemical profiling, and functional group characterization. Extracts were stored under appropriate conditions for further experimental applications.

is nutrient modulation of hydroponically grown lettuce, which has been demonstrated to enhance certain phenolics, including flavones, anthocyanins, and phenolic acids, thereby enhancing antioxidant activity. These methods offer a sustainable, scalable platform for optimizing the production of PSMs for nutraceutical and pharmaceutical applications (D. T. P. Nguyen et al., 2020; Senizza et al., 2020).

2.3. Secondary metabolites as drug candidates

2.3.1. Plants as green gold of pharmacophores

Approximately 400,000 species of terrestrial plants have been documented (Cheek et al., 2020), of which only 50,000–80,000 species have been investigated for their medicinal potential (Chen et al., 2016). Between 1990 and 2016, approximately 1500 compounds were approved by the U.S. Food and Drug Administration (FDA) as therapeutic agents, available either over the counter or via prescription (Newman and Cragg, 2016). Of these, only 326 compounds were derived exclusively from natural sources, while the remainder were synthetic molecules or chemically modified derivatives of natural products (Lautié et al., 2020). In the absence of a definitive treatment for COVID-19, global interest in plant-derived therapeutics has intensified, as a substantial proportion of existing drugs originate from plants or their bioactive constituents. This suggests a promising potential for medicinal plants to yield compounds with anti-COVID-19 activity (England et al., 2023). Consequently, systematic exploration of the vast botanical diversity is essential to identify novel plant secondary metabolites with significant pharmacological activities.

2.3.2. Different classes of secondary metabolites

The biosynthetic pathway for the production of plant secondary metabolites remains incompletely elucidated. Advances in analytical and molecular techniques have enabled the identification of ultra-small phytochemical compounds and facilitated comprehensive metabolomic profiling. Plant secondary metabolites are generally produced in low concentrations and can be broadly classified into four major groups based on their chemical composition and biosynthetic origin: alkaloids, terpenoids, phenolic compounds, and sulfur-containing metabolites (González Mera et al., 2019). Many of these bioactive molecules possess significant pharmaceutical and industrial relevance.

2.3.3. Terpenes

Terpenes represent the most structurally and functionally diverse class, comprising approximately 42,000 known molecules. They are non-saponifiable lipids that participate in both primary and secondary metabolic processes in plants. Terpenes are widely used as food additives, nutraceuticals, and therapeutic agents. Among them, monoterpenes are predominantly produced by aromatic plants and are major constituents of essential oils. For instance, cannabinoidal compounds such as Δ^9-tetrahydrocannabinol, derived from *Cannabis* species, exhibit antidepressant and antitumor activities (Zielińska-Błajet and Feder-Kubis, 2020). β-myrcene, a monoterpenoid identified in the essential oil of *Syzygium aromaticum*, exhibits antimalarial and *Trypanosoma* species activity, suggesting its potential role in the management of sleeping sickness. Limonene, a cyclic monoterpene obtained from citrus peel, exhibits pancreatic lipase-inhibitory properties and potent antiviral effects, particularly against Herpes simplex virus and SARS-CoV-2, and is incorporated into several ayurvedic formulations (Kvittingen et al., 2021). Within the sesquiterpenoid subgroup, compounds such as gossypol and avarol display notable antitumor and anti-HIV activities, respectively, and also serve as part of plant defense mechanisms (Imperatore et al., 2020). Artemisinin, a sesquiterpene lactone derived from *Artemisia annua*, is currently undergoing clinical trials for the treatment of malaria (J. Wang et al., 2019).

Recent studies have demonstrated that terpene-based essential oils exhibit strong antibacterial and preservative properties, making them valuable in the food industry. Recent studies reported that the combination of essential oils and terpenes with supercritical carbon dioxide (sc-CO_2) effectively extended the shelf life of chicken meat (Fabio et al., 2025). Owing to their hydrophobic and lipophilic properties, many terpenoids can disrupt microbial cell membranes and inhibit key enzymes involved in energy production and DNA replication, thereby reducing bacterial viability. Specific compounds such as α-pinene and limonene have been shown to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. Furthermore, certain terpenoids also exhibit antioxidant activity by scavenging free radicals, thus protecting food from oxidative damage (Wang et al., 2025).

Recently, tanshinones, a class of diterpenes derived from dried roots and rhizomes, have garnered attention for their therapeutic potential in cardiovascular disorders, arthritis, and cancer (Zhou et al., 2021). Additionally, diterpenes cafestol and kahweol, found in coffee beans,

exhibit antidepressant, anti-inflammatory, antidiabetic, and antioxidant properties, contributing to reduced prostate cancer risk, improved liver function, and increased HDL cholesterol levels (Makino et al., 2021). Furthermore, the triterpenoid saponin glycyrrhizin from licorice exhibits potent antiviral activity against HIV and SARS-CoV-2 (Huan et al., 2021).

2.3.4. Alkaloids

Alkaloids have always been alluring to researchers as they behave in very different ways. These are generally nitrogen-containing cyclic compounds constituting a small group of secondary metabolites. Approximately 18,000 alkaloids have been isolated from various plant species, and only 27% have been evaluated for their diverse activities. Most renowned and ill-famed compounds belong to alkaloids like cocaine, quinine, atropine, caffeine, nicotine, and morphine, but they play an essential role in curing some human diseases. Morphine is a widely used plant-derived analgesic obtained from the opium poppy (*Papaver somniferum*) and has been prescribed for decades to alleviate pain, particularly in cases of injury or post-surgical trauma. In modern medicine, morphine remains indispensable in pain management, especially for patients with advanced or terminal illnesses such as cancer (Stermitz and Rapoport, 1961). This unique plant-derived compound provides unparalleled analgesic efficacy due to its strong binding affinity to opioid receptors in the human body. The isolation of morphine not only represented a milestone in pharmacology but also paved the way for the identification of additional plant-based compounds with significant therapeutic potential (Nasim et al., 2022).

Some are non-addictive analgesics, some possess local anesthetic activity, and others are chemotherapeutic agents for some cancers. The main ingredient responsible for tobacco addiction, smoked in cigarettes, is nicotine, a principal alkaloid present in heavy amounts. Some alkaloids are unapproved drugs, like psilocybin extracted from *Psilocybe mexicana* and mescaline obtained from *Lophophora*, commonly used as hallucinogenic drugs (Twaij and Hasan, 2022). The significant secondary metabolites known as Benzylisoquinoline alkaloids (BIAs) are found in opium poppies (*Papaver somniferum L.*). Benzylisoquinoline Alkaloids (BIAs) are used as drugs and in pharmaceutical research. In genome-edited plant lines, BIAs, such as morphine and thebaine, were significantly reduced following CRISPR-Cas9-mediated knockdown of the 4′OMT and 4′OMT2 genes. Both genes are essential for the biosynthesis of morphine, thebaine, and codeine. Thus, the potential of the CRISPR system to manipulate metabolic pathways was demonstrated, paving the way for a comprehensive understanding of the PSM production pathway and a strategy to selectively increase the production of desired beneficial compounds (Mipeshwaree Devi et al., 2023). A well-known oncolytic alkaloid, Vincristine, produced by *Catharanthus roseus*, is being used as a therapeutic agent for Hodgkin's disease, uterine and cervical cancers, and many other cancers (Tyagi et al., 2010). Many alkaloids have reached the market for their effectiveness in treating diseases, such as Galantamine, an acetylcholinesterase inhibitor derived from the bark of *Galanthus woronowii*, an FDA-approved drug for Alzheimer's disease (Nunes et al., 2022). Hydroxychloroquine, a derivative of Chloroquine, turned out to be a life-saving drug against SARS-CoV-2 (Colson et al., 2020). Extended utilization of alkaloids leads to drug addictions with mental and physical dependence on these drugs, and withdrawal from these leads to severe side effects.

2.3.5. Phenolic compounds

Plant phenolics are aromatic compounds characterized by the presence of a benzene ring and are known to exhibit strong antioxidant activity, often surpassing that of other classes of secondary metabolites. They play a crucial role in regulating plant growth, physiological processes, and structural integrity (Hilal et al., 2024).

Polyphenols represent a broad and structurally diverse class of phytochemicals characterized by the presence of a benzene ring substituted with at least one hydroxyl group. This complex group encompasses flavonoids, flavones, flavonols, anthocyanidins, and lignins, among others. Among these, flavonoids constitute the largest subgroup and are widely recognized for their potent antioxidant and anti-inflammatory properties. Owing to their significant health benefits, polyphenols have long been the focus of scientific investigation and are also commonly consumed as natural dietary supplements. To date, approximately 8000 distinct polyphenols have been identified, of which nearly 200 are exported from India to major global markets, including the USA, UK, and Dubai.

Phenolic compounds, the most abundant and heterogeneous subclass of polyphenols, are widely distributed in cereals, legumes, fruits, and vegetables. They occur in free, soluble, and insoluble bound forms and exhibit a broad spectrum of biological activities, including antimicrobial, antioxidant, and anti-inflammatory effects (Nasim et al., 2022). For example, the anthocyanin cyanidin, extracted from *Ipomoea batatas* (sweet potato), has been reported to possess antioxidant, anticlastogenic, antiproliferative, and antitumorigenic properties.

Recent studies demonstrate that phenolic compounds can effectively counteract free radical-mediated damage, suppress acute inflammatory responses, and mitigate mutagenic activity. These bioactivities are attributed to the presence of catechol and pyrogallol functional groups, which readily interact with and neutralize reactive oxygen and nitrogen species. Collectively, these mechanisms underpin the therapeutic potential of phenolics in preventing or attenuating non-communicable and degenerative diseases, particularly those with long-term pathological progression.

Thymol, a monoterpenoid phenol derivative of *p*-cymene and an isomer of carvacrol, is a major constituent of thyme oil. This essential oil is produced by several members of the *Lamiaceae* family, with *Thymus vulgaris* (garden thyme) being the most common source. Traditionally, thyme oil has been widely recognized for its role as a natural food preservative and is extensively used in cosmetics, toiletries, and mouthwash formulations. Beyond these applications, thymol exhibits diverse biological activities, including antifungal, anti-inflammatory, and antibacterial properties, highlighting its potential for emerging therapeutic and industrial applications (Marchese et al., 2016). In the food packaging sector, natural antimicrobials and preservatives are being actively explored as sustainable alternatives to synthetic agents.

A notable study demonstrated that the utilization of a fusion protein comprising β-casein and the antimicrobial peptide E50-52 resulted in significant antimicrobial activity. In this system, β-casein functions as a carrier or fusion partner to express the E50-52 peptide in *Escherichia coli*, thereby producing an antimicrobial polymeric monomer with potential application in food packaging. Importantly, the synergistic activity of this fusion protein with thymol exhibited enhanced antimicrobial effects against common foodborne pathogens (Fahimirad et al., 2017). Mechanistically, antimicrobial peptides act by destabilizing bacterial membranes, leading to pore formation and subsequent cell lysis, whereas thymol disrupts lipid bilayers by penetrating lipid assemblies and altering membrane integrity (Trombetta et al., 2005).

Recent investigations have also highlighted the role of flavonoids in combating infectious diseases. For instance, quercetin and apigenin have demonstrated inhibitory activity against the SARS-CoV-2 Main Protease (Mpro), thereby showing therapeutic promise against COVID-19 (Jo et al., 2020). Similarly, Kobophenol-A, a plant-derived phenolic compound, has been shown to bind effectively to the SARS-CoV-2 spike protein receptor, suggesting that natural products may be promising therapeutic candidates for the treatment of viral diseases (Kaliaperumal et al., 2023).

Several widely used Indian medicinal plants, such as *Phyllanthus emblica* (Indian gooseberry, amla), *Terminalia chebula* (harad), *Terminalia bellirica* (baheda), *Tinospora cordifolia* (amrita), *Tribulus terrestris* (gokshura), and *Withania somnifera* (ashwagandha), are rich sources of polyphenols and flavonoids. These plants contain bioactive compounds, including kaempferol, quercetin, ellagitannins, shikimic acid, tannic acid, caffeic acid, ascorbic acid, vanillic acid, p-coumaric acid, and gallic

acid, all of which contribute to their therapeutic benefits for immunity, skin, hair, cardiovascular health, digestion, vision, and respiratory function (Ahmad et al., 2020; Vallish et al., 2022).

Resveratrol, a stilbenoid derived from *Vitis vinifera*, exerts chemotherapeutic effects by inducing cell cycle arrest through the activation of p21 and p27 while concurrently downregulating oncogenic microRNAs (Abazari et al., 2021; Meshram et al., 2022; Shankar et al., 2017). Similarly, flavopiridol, a semisynthetic flavonoid originally derived from the bark of *Dysoxylum binectariferum*, has been developed as a leading anticancer drug with established efficacy in the treatment of leukemia and lymphomas. Apigenin, another well-characterized flavonoid found in red cherries, grapes, barley, and chamomile tea, is widely used in sunscreen formulations due to its ability to scavenge reactive oxygen species generated upon Ultraviolet (UV) exposure. In addition, apigenin exhibits chemopreventive activity by inhibiting the development of cancers such as breast, leukemia, and prostate malignancies (Shankar et al., 2017).

Collectively, these findings underscore that polyphenols and flavonoids possess both chemopreventive and chemotherapeutic potential, establishing them as promising scaffolds for the development of novel drug candidates. Given their multifaceted bioactivities, increased research attention is warranted to systematically explore these compounds for translational applications in human health and disease management.

2.3.6. Sulfur-containing compounds

There are very few plant-based sulfur-containing secondary metabolites. They are generally sulfur-containing amino acids, like cysteine and methionine, produced by animals (Hill et al., 2023). However, some plants in the *Brassicaceae* family and the *Allium* genus produce sulfur-containing secondary metabolites, including glutathione, allicin, phytoalexins, and glucosinolates (V. P. T. Nguyen et al., 2020). Sulfur is sequestered from the environment and then assimilated into plant tissues. This sequestered sulfur is essential for human health as it plays a role in anti-inflammatory and antioxidant pathways. The development of biosynthetic pathways illustrates how genetic variation and environmental pressures shape plant metabolism (Younkin et al., 2024). This phenomenon was investigated in *Erysimum cheiranthoides* (*Brassicaceae*), which independently evolved the ability to produce cardiac glycosides while retaining GLS biosynthetic pathways. Their findings demonstrated that cardiac glycosides emerged as an additional defense mechanism against herbivores, particularly in cases where Glucosinolates (GSLs) were less effective against specialist feeders. By employing isogenic plant lines, the study distinguished the ecological and evolutionary roles of these compounds, providing evidence that newly evolved metabolic traits can function in concert with ancestral ones to modulate plant-herbivore interactions. The authors further emphasized that plant metabolism reflects a balance between innovation and redundancy, underscoring the importance of integrating genomic, biochemical, and ecological approaches to fully elucidate evolutionary adaptations in plants (Younkin et al., 2024).

In *Allium* species such as garlic and onion, the phytoanticipin compounds exhibit potent antimicrobial activity, providing defense against bacterial, fungal, and viral pathogens. Alliin, a sulfur-rich organosulfur compound in garlic, has been shown to reduce arterial cholesterol plaque formation in arteriosclerosis and also demonstrates neuroprotective potential through its anti-inflammatory effects (Sánchez-Sánchez et al., 2020). Similarly, sinigrin, a glucosinolate found in broccoli, exhibits anti-arteriosclerotic, anti-carcinogenic, and antioxidant properties (Jang et al., 2017; Lee et al., 2017). Ajoene, another key organosulfur compound from garlic, possesses hepatoprotective activity by preventing thiol depletion and restoring normal liver enzyme function (Subramanya et al., 2018).

Sulfur-containing amino acids, primarily methionine and cysteine, GSLs, and the byproducts of their hydrolysis, such as isothiocyanates, as well as other sulfur-containing substances, including phytoalexins or cysteine sulfoxides, have been the subject of research in recent years. Because of their unique biological characteristics, including antibacterial, anti-inflammatory, anticancer, and antioxidant qualities, GSLs are considered an important phytochemical group. Functional foods, nutraceuticals, and cosmeceutical products thus use sulfur-containing metabolites as preventative and therapeutic ingredients (Castro et al., 2023).

2.4. Pharmacokinetic and toxicological limitations of natural products

Although plant secondary metabolites possess a wide range of bioactivities, including antioxidant, anti-inflammatory, and anticancer properties, their clinical use is often constrained by adverse pharmacokinetic properties. Most phytochemicals have poor water solubility, low gastrointestinal absorption, and undergo first-pass metabolism, leading to reduced systemic bioavailability and decreased therapeutic efficacy. Moreover, the lack of predictability in their metabolic stability and high rate of clearance also limit their efficacy in target sites. Toxicity is also a major concern because some of the compounds can be dose-dependent, cytotoxic, off-target, and organ-specific (Vaou et al., 2025; Yuan et al., 2024).

Pharmacokinetic challenges in Absorption, Distribution, Metabolism, and Excretion (ADME) pose serious hurdles to the translational potential of these compounds. Recent computational data suggest that a significant percentage of phytochemicals exhibit changing ADME/Tox profiles, including restricted blood-brain barrier permeability and intermediate gastrointestinal absorption. *In silico* ADMET prediction models, such as SwissADME and pkCSM, are increasingly used in early-stage screening and lead compound optimization to overcome these limitations. Also, new methods to achieve bioavailability, stability, and targeted delivery are being investigated, including novel delivery systems, nanoformulations, and structural modifications. Together, a combination of pharmacokinetic and toxicity analyses and bioactivity research is necessary to develop plant secondary metabolites into useful therapeutic agents (Odhiambo et al., 2025).

3. Computational approaches as promising tools for drug development

Rapid lifestyle changes and accelerated mutation rates in pathogenic microorganisms, such as bacteria, fungi, and viruses, have rendered traditional “one-disease-one-drug” approaches increasingly ineffective. Consequently, modern drug discovery has shifted toward integrative strategies, including computational biology, *in silico* molecular docking, and systems biology, to identify novel therapeutic targets. The emergence of Omics sciences, including genomics (functional, comparative, and structural), transcriptomics (gene-level analysis), proteomics, and metabolomics (phenotypic profiling), has become pivotal for elucidating interactions among secondary metabolites, their molecular targets, and associated regulatory genes (van Santen et al., 2021).

Researchers are investigating new approaches, such as multitarget drug designing and artificial intelligence-based virtual screening, to improve the efficacy and accuracy of drug discovery from medicinal plants through Computer-Aided Drug Discovery and Development (CADD). The drug discovery process is impacted extensively by CADD, as it can save time and resources needed for each step of drug discovery and development (Javid et al., 2024). Understanding and applying these advanced Omics-based tools is critical for identifying novel natural secondary metabolites, predicting their potential molecular targets, elucidating structural analogs, and optimizing pharmacokinetic profiles (Romano and Tatonetti, 2019). Plant-derived bioactive compounds generally exhibit superior polypharmacological properties compared to their fully synthetic counterparts. Recent advances in machine learning driven virtual screening have enabled high throughput docking of natural products and their analogs with specific molecular targets, thereby accelerating drug discovery (Fang et al., 2018). The continual

development of specialized databases and advanced bioinformatics tools will further enhance polypharmacology-focused research.

The term *in silico* refers to experiments conducted and analyzed using computer-aided tools. A related discipline, *in silico* pharmacology, also termed computational pharmacology or computational therapeutics, involves the application of software-based techniques to collect, process, and interpret complex biological and medical datasets, ultimately driving discoveries in drug development (Ekins et al., 2007). Historically, plants and their extracts have served as vital resources for human healthcare for over 5000 years (Brown and Wright, 2016), providing therapeutic agents with cardioprotective, antibiotic, analgesic, and antineoplastic properties (Chen et al., 2015). In developing nations, 70-90% of the population continues to rely on traditional plant-based medicines (Chin et al., 2006). Secondary metabolites represent the most valuable phytochemical constituents, offering a diverse range of pharmacological benefits (Robinson and Zhang, 2011). More than 50% of FDA-approved drugs are derived from natural products or their derivatives (Anand et al., 2019). According to the World Health Organization, over 30% of plant species have documented medicinal uses (Yi et al., 2018).

However, the traditional process of medicinal plant research is time-consuming and expensive. However, the conventional process can be modified to improve the efficiency of medicinal plant research (Yi et al., 2018). Therefore, scientists first predict a large number of chemical compounds or ligands for a particular target by the *in silico* method, then, for verification, *in vitro* and *in vivo* experiments are performed; this process improves the efficiency of evaluation of natural compounds from plants, and this method relatively takes less time for drug designing. Nowadays, computer-aided drug design methods are frequently used to discover drugs from natural compounds.

3.1. In silico drug discovery: A new approach

Plant secondary metabolites encompass a diverse array of bioactive compounds that confer adaptive advantages to plants, enhancing their survival and facilitating various aspects of growth and development (Birchfield and McIntosh, 2020). The composition and physicochemical properties of these metabolites vary considerably across plant species, rendering them promising drug candidates due to their structural diversity, bioactivity, and potency. Secondary metabolites have demonstrated efficacy in combating antimicrobial resistance and may serve as alternative therapeutic agents, particularly in resource-limited settings where access to conventional healthcare is restricted (Bhatti et al., 2022). The biochemical properties of these compounds underpin their ability to interact with a wide range of molecular targets within biological systems (Mohammed et al., 2023).

Extensive information on PSMs has been systematically archived in various commercial and open-access databases, significantly accelerating natural product-based drug discovery. Notable commercial databases include the Dictionary of Natural Products (DNP) (https://dnp.chemnetbase.com/chemical/ChemicalSearch.xhtml?dswid=-3497), Reaxys (https://www.reaxys.com), the National Institute of Standards and Technology (NIST) database (https://www.nist.gov/srd/nist-standard-reference-database-1a), NaprAlert (Loub et al., 1985), AntiBase (https://application.wiley-vch.de/stmdata/antibase.php), MarinLit (https://marinlit.rsc.org/), the Natural Product Discovery System (NADI) (Ikram et al., 2015), ChemTCM, The Natural Products Library (NPL) (Quinn et al., 2008), The Natural Product Discovery System (NADI) (Vickers, 2017), The Ayurveda dataset (Lagunin et al., 2016), The Berdy's Bioactive Natural Products Database (Berdy and Kertesz, 1989). While comprehensive, these resources are often more costly than freely available platforms. In contrast, the literature reports at least 92 open-access databases dedicated to natural products (Sorokina and Steinbeck, 2020). Such databases, curated by computational chemists, have substantially advanced the virtual screening and selection of natural products as potential therapeutics (Baxevanis and Bateman, 2015), providing essential resources for *in silico* pharmacognosy and phytochemical chemoinformatics (Prachayasittikul et al., 2015). Medicinal plant drug discovery aims to identify and optimize lead molecules from the vast pool of phytochemicals. These lead compounds must also undergo extensive preclinical and clinical trials to be approved as effective medications. In the absence of computational tools, both procedures are time-consuming, expensive, and laborious (Javid et al., 2024).

In silico high-throughput screening (HTS) is a new door for screening different natural compounds in significantly less time to identify potential drugs. HTS based on molecular docking minimizes the ligands for *in vitro* and *in vivo* screening (Anand et al., 2019). Various FDA-approved medicines derived from natural products were developed using *in silico* docking approaches, such as Dozamide in 1995, Imatinib in 2001, Dasatinib in 2006, and Ponatinib in 2012 (Stocks, 2015). Likewise, Saquinavir was the first FDA-approved HIV-1 enzyme inhibitor developed through *in silico* docking (Zaman et al., 2002). Moreover, virulent proteins from several diseases (SARS-CoV, Diabetes, Cancer, Monkeypox, and Dengue) were docked with various plant secondary metabolites via HTS, as listed in Tables 1 and 2.

3.2. Steps involved in computational drug designing

3.2.1. Preparation of a plant secondary metabolites library and QSAR analysis

The PSM library can be prepared through a literature survey or by identifying phytochemicals via GC-MS of an optimized plant extract. Plant Secondary Compound (PSC) is a freely available database with 3-D structural information/details of various/different natural products and their physicochemical and pharmaceutical properties (Valdés-Jiménez et al., 2021). After preparing the PSM library, Quantitative Structure-Activity Relationship (QSAR) analysis could be performed in which the PSM structure is quantitatively correlated with its biological activity. QSAR-Co and Prediction of Activity Spectra for Substances (PASS) are QSAR analysis software that are freely available online (Ambure et al., 2019; Pagadala et al., 2017).

3.2.2. Molecular docking analysis

The compounds selected in the previous steps will proceed to molecular docking studies. Molecular docking is an *in silico* method used to study the interactions and binding affinities of target molecules, i.e., proteins and their ligands or peptides. Several tools are available for docking studies, including PyrX, AutoDock, FRODOCK, ZDOCK, MEGADOCK, and SOFTDOCK (Pagadala et al., 2017). Researchers can select any docking analysis tools, followed by results analysis by visualization tools, i.e., Pymol or Discovery Studio Visualizer. After docking, Molecular Dynamics Simulation (MDS) could be performed, which is a computational method for analyzing the physical motion of proteins and ligands. AMBER (Assisted Model Building with Energy Refinement), GROMACS (Groningen Machine for Chemical Simulations), CHARMM (Chemistry at Harvard Macromolecular Mechanics), and NAMD (Nanoscale Molecular Dynamics) are the software available for MDS (Lee et al., 2017). The advancement of molecular docking has facilitated drug discovery and development, particularly for viral diseases such as COVID-19 and SARS (Quimque et al., 2023). Advances in molecular docking and MDS have significantly accelerated drug discovery and development, particularly in the identification of potential therapeutics against viral pathogens such as SARS-CoV and SARS-CoV-2, and veterinary pathogen *Pasteurella multocida* (Soni et al., 2023, 2025).

Although molecular docking has been widely used for screening plant secondary metabolites, there are several limitations that must be taken into consideration. Many of the studies included in this review's summary are based solely on docking scores, without additional confirmation from molecular dynamics simulations or experimental validation. Although molecular dynamics has been proposed as a follow-up measure, its implementation has been limited and rare. Moreover, there is variability in the methodologies used: different docking

Table 1
In silico efforts at natural product drug discovery for various diseases: molecular docking of phytochemical ligands with potential protein targets.

Target	Plant secondary metabolites	Binding Interaction	Method	Key Target Site	References
SARS-CoV-2 spike protein	Coagulins K and N	Compounds that strongly bind (BE low) and are in the top rank.	Molecular docking (virtual screening)	Receptor-Binding Domain (RBD) (ACE2 binding interface)	Puttaswamy et al. (2020)
	Saponins	Positive association; a component of triterpenoid-rich compounds with the highest rankings.	Molecular docking	RBD	(Puttaswamy et al., 2020)
	Graecunin E	Favorable binding interaction (virtual screening)	Molecular docking	RBD	(Puttaswamy et al., 2020)
	Stigmasteryl glucoside	High binding affinity	Molecular docking	RBD	(Puttaswamy et al., 2020)
	Yuccagenin	Favorable interaction (triterpenoid-related)	Molecular docking	RBD	Puttaswamy et al. (2020)
	Bismahanine	Interaction reported	Molecular docking	RBD	(Puttaswamy et al., 2020)
	Tannic acid	Favorable interaction with spike protein	Molecular docking	RBD	Puttaswamy et al. (2020)
	Hesperidin	Positive binding interaction; possible inhibitor.	Molecular docking	Spike protein	Tallei et al. (2020)
	Cannabinoids	Interaction reported	Molecular docking	Spike protein	(Tallei et al., 2020)
	Cinnamtannin-B1	Interaction that was reported (screened compound; not a top hit)	AutoDock Vina, PyRx; MD (GROMACS)	Mpro active site	Prasanth et al. (2021)
	Lantacin	Possible inhibitor.	Molecular docking	RBD	Amparo et al. (2021)
	Apocynin E	Reported interaction; possible inhibitor.	Molecular docking	RBD	(Amparo et al., 2021)
	Solanine	Positive binding behavior and stability of complexes with MD simulation.	Molecular docking; MD simulation	Mpro active site	Teli et al. (2020)
	Oleocanthal	Reported interaction; possible inhibitor.	Molecular docking	Mpro active site	Bansal et al. (2022)
SARS-CoV-2 Main Protease (M^{pro})	Theaflavin 3-gallate	Positive binding interaction; MD simulation stability.	Molecular docking; MD simulation	Mpro active site	C et al. (2022)
	Delphinidin 3,5-diglucoside	Desirable binding interaction; stability assisted by MD simulation.	Molecular docking; MD simulation	Mpro active site	Sharma and Shanavas (2021)
	Withanoside V	A stable protein-ligand interaction is shown during the simulation, indicating a possible inhibitory effect.	Molecular docking; MD simulation	Mpro active site	Shree et al. (2022)
	Peonidin-3-glucoside	During simulation, the exhibits remained in contact with the protease's active site, indicating stable binding.	Molecular docking coupled with MD simulation	Mpro active site	Majumder and Mandal (2022)
	Bonducellin D	Maintains stable interactions with active site residues during simulation, implying a steady binding profile	Molecular docking followed by MD simulation	Mpro active site	Gurung et al. (2020)
	Procyanidin A_3, A_4, B_4, A_1 and B_2	Shows several interactions with protease residues, which means it may have binding ability.	Molecular docking with MD-based assessment	Mpro active site	Teli et al. (2020)
	Acetoside	Interacts with catalytic site residues; binding stability was simulated.	Molecular docking and MD simulation	Mpro active site	(Teli et al., 2020)
	Rutin	Alters contact with the protease-binding pocket; stability is tested under dynamic conditions.	Molecular docking coupled with MD simulation	Mpro active site	(Teli et al., 2020)
	Dicaffeoylquinic acid	Interacts with spike protein residues, suggesting potential interference with viral binding.	Molecular docking	Mpro active site	Amparo et al. (2021)
	Taraxerol acetate	Exhibits binding in the spike protein region, suggesting the potential to interfere with host-virus interactions.	Molecular docking	Mpro active site	(Amparo et al., 2021)
	14-deoxy-11,12-didehydroandrographolide	Interacts with residues in the protease catalytic region, suggesting a possible inhibitory interaction.	Molecular docking	Mpro active site	Majumdar et al. (2022)
	Andrographolide	Displays binding within the protease pocket, suggesting possible interference with enzymatic function	Molecular docking	Mpro active site	(Majumdar et al., 2022)
	Hypericin	Interacts with the active-site residues, suggesting potential binding.	Molecular docking	Mpro active site	(Majumdar et al., 2022)
	Sotetsuflavone	Demonstrates occupancy in the protease binding site, suggesting that	Molecular docking	Mpro active site	(Majumdar et al., 2022)

(*continued on next page*)

Table 1 (*continued*)

Target	Plant secondary metabolites	Binding Interaction	Method	Key Target Site	References
		there could be a ligand-protein interaction.			
	Proanthocyanidins	Has several contacts with the protease-binding pocket, suggesting possible inhibitory relevance.	Molecular docking	Mpro active site	(Majumdar et al., 2022)
SARS-CoV-2 RNA-Dependent RNA Polymerase (RdRp)	Cyanidin 3-(6″-malonylglucoside)	Remains continuously associated with the protease binding pocket during the entire simulation, indicating stable ligand binding.	Molecular docking integrated with MD simulation	RdRp binding site	Shawky et al. (2020)
	Chrysophanol 8-(6-galloylglucoside)	Displays binding behavior to the polymerase binding region, which suggests that it may be a good partner to the enzyme	Molecular docking	RdRp binding site	Alamri et al. (2020)
	Eriodictyol-7-O-rutinoside	Publishes its compatibility with the polymerase pocket, indicating potential interaction with catalytic regions.	Molecular docking	RdRp binding site	(Alamri et al., 2020)
	Myricetin 3-rutinoside	Fills the polymerase-binding cavity, a possible interaction site for the ligand with the enzyme.	Molecular docking	RdRp binding site	(Alamri et al., 2020)
	Rotundioside B	Shows activity with residues of the polymerase domain.	Molecular docking	RdRp binding site	(Alamri et al., 2020)
	Hippomanin A	Binding to the enzyme region with displays denoting possible applicability to polymerase inhibition.	Molecular docking	RdRp binding site	(Alamri et al., 2020)
Dengue virus NS2B/NS3 protease	2R-Acetoxymethyl-1,3,5-trimethyl	Shows contact with protease binding sites, which shows a possible inhibitory effect.	Molecular docking (HEX), E-min: −199.90 kcal/mol	Protease active site	Menaga et al. (2021)
	3,7,11,15-Tetramethyl-2-hexadece	Compatible with the protease pocket, which may imply interaction with catalytic residues.	Molecular docking (HEX), E-min: −198.28 kcal/mol	Protease active site	(Menaga et al., 2021)
	Palmitic Acid	Fills the enzyme-binding site, suggesting it may bind protease residues.	Molecular docking (HEX), E-min: −209.63 kcal/mol	Protease active site	(Menaga et al., 2021)
	Agathisflavone	Demonstrates a stable binding in the protease active pocket, which suggests possible enzymatic inhibition.	Molecular docking (with simulation validation)	Active site (His41–Cys145 region)	Bhattarai et al. (2022)
	Epigallocatechin gallate	Shows a high degree of interaction with catalytic residues, which may indicate the possibility of disrupting the protease activity.	Molecular docking (with simulation validation)	Catalytic dyad (His41–Cys145	(Bhattarai et al., 2022)
NS5 polymerase	Pectolinarin	Interacts with the polymerase-binding pocket, suggesting it may interfere with RNA synthesis.	Molecular docking	RdRp catalytic region	(Bhattarai et al., 2022)
	acacetin-7-O-rutinoside	Gives a positive reaction with the polymerase active region, suggesting that enzyme activity is inhibited.	Molecular docking	Polymerase active site	(Bhattarai et al., 2022)
Type 2 nonstructural protein (NS1)	kaempferol-3-O-rutinoside	Exhibits stable binding in the protease active pocket, which suggests the possibility of disrupting the enzyme processing.	Molecular docking	Active site (His41–Cys145 region)	Agrawal and Murti (2022)
	lithospermic acid	Shows that it interacts with the catalytic residues, indicating that it may inhibit protease activity.	Molecular docking	Catalytic dyad (His41–Cys145)	(Agrawal and Murti, 2022)
	Jaceidin, Centaureidin, Artecanin	These flavonoid compounds into the protease active site and make contacts with residues involved in catalysis, suggesting they may interfere with proteolytic activity.	Molecular docking	Active site (His41–Cys145 region)	Qaddir et al. (2020)
Diabetes					
α-amylase enzyme	Alpha-farnesene	Ligands to the enzyme binding site, which means that they may modulate carbohydrate digestion pathways.	Molecular docking	Catalytic pocket	Rao and Hariprasad (2021)
	Catechin	Interacts with residues of the catalytic pocket, which suggests a possible inhibition of the hydrolysis of carbohydrates.	Molecular docking	Active site residues	Bohara et al. (2022)
Protein tyrosine phosphatase (PTP)	Delphinidin 3-O-glucoside	Forms contacts at the catalytic site of the enzyme, suggesting the possibility of inhibiting carbohydrate-digesting activity.	Molecular docking	Active site residues	Damián-Medina et al. (2020)
	Pelargonidin 3-O-glucoside	Interacts in the enzyme binding pocket, implying that it may be	Molecular docking	Active site residues	Nguyen-Vo et al. (2016)

(*continued on next page*)

Table 1 (*continued*)

Target	Plant secondary metabolites	Binding Interaction	Method	Key Target Site	References
		modulated to alter carbohydrate hydrolysis			
11β-HSD1	Taraxerol	Binds with the catalytic site of the enzyme, which suggests that it may have an inhibitory effect on binding with the substrate	Molecular docking	Catalytic pocket	(Nguyen-Vo et al., 2016)
PTP1B (4Y14)	Corilagin	Forming contacts with the residues that are engaged in the enzymatic activity, which indicates that it may inhibit the pathways of glucose metabolism	Molecular docking	Enzyme active region	(Nguyen-Vo et al., 2016)
GFAT (2ZJ3)	Cosmosiin	Shows some compatibility with the enzyme binding site, which means that it may interfere with the carbohydrate digestion processes.	Molecular docking	Active pocket	(Nguyen-Vo et al., 2016)
Cardiovascular disease					
Malonyl Coenzyme A	Morin	Shows communication in functional binding sites of cardiac-related proteins, which is indicative of the possible regulation of pathways that mediate cardioprotection.	Molecular docking	Functional Binding site	Laskar et al. (2014)
Chemokine receptor CXCR4	Curcumin	Ca2+ ions Interact with functional domains of target proteins, which implies that they may modulate pathways of inflammation and oxidative stress.	Molecular docking	Active sites	Murad et al. (2022) (Murad et al., 2022)
Chemokine receptor CXCR7	Quercetin	Interacts with the binding pockets of target proteins, suggesting potential control of mechanisms associated with vascular activity and inflammation.	Molecular docking	Active/functional sites	
Cancer					
CDK-2	epigallocatechin gallate	Forms contacts with functional domains on target proteins, which may indicate the modulation of pathways involved in tumor progression.	Molecular docking (with quantum chemical analysis)	Functional binding regions	Sarkar et al. (2021)
Human topoisomerase IIa	daidzein	Incorporates into the binding pockets of target proteins, which means potential disruption of molecular processes associated with cancer formation.	Molecular docking (with quantum chemical analysis)	Active/Functional binding site	(Sarkar et al., 2021)
VEGFR-2	quercetin	Bonds with residues of major protein targets, which implies the possibility of controlling signaling pathways related to carcinogenesis	Molecular docking (with quantum chemical analysis)	Functional binding regions	(Sarkar et al., 2021)
HIF-1α receptor	1′,4-dihydroxy-2,3′-dimethyl-1,2′-binaphthyl-5,5′,8,8′-tetramine tetraone	Evidence of interplay with functional domains of target proteins that promote tumor progression, suggesting that it may modulate cancer-associated pathways.	Molecular docking	Active/functional binding region	Ngbolua et al. (2022)
Human androgen receptor	6′-ethoxy-1,′3′-dihydroxy-4,6-dimethyl-1,2′-binaphthyl-2,5′,8,8′-tetramine tetraone	Bonds with the binding sites of cancer-related proteins, which may indicate a possible interference with molecular processes related to cell proliferation.	Molecular docking	Active/functional sites	(Ngbolua et al., 2022)
Epidermal Growth Factor Receptor (EGFR)	2H-Benzo(cd) pyrene-2,6(1H)-dione	Binds to functional domains of target proteins, indicating possible disruption of molecular pathways related to cancer development.	Molecular docking	Functional Binding region	Jaikumar et al. (2017)
ErBb2	Corynan-17-ol,18,19-dihydrodidehydro-10-methoxy, Acetate	Binds functional domains of target proteins implicated in cancer development, suggesting it could regulate cancer-related pathways.	Molecular docking	Functional Binding region	Athiraja et al. (2019)
Monkeypox envelope protein	Silibinin	Binds to functional sites of microbial proteins, which suggests the possibility of disrupting the survival and virulence of pathogens.	Molecular docking (with biological context)	Functional Binding region	Gulati et al. (2022)
	oleanolic acid	Mostly hydrophobic interactions of stabilizing van der Waals forces in the binding pockets of the proteins.	Molecular docking + molecular dynamics (MD) simulation + MM/PBSA binding energy analysis *in silico*.	Envelope structural proteins (D13, A26, H3) of Monkeypox virus (MPXV)	Zhou et al. (2020)
	ursolic acid	Interacts with binding sites on microbial proteins, potentially interfering with bacterial physiological functions.	Molecular docking	Functional Binding Site	Bansal et al. (2023)

Table 2
Plant secondary metabolites showing different activities, which are under clinical trials or are approved by the FDA to be used as OTC drugs.

Name of drug	Type of secondary metabolite	Source plant	Activity	Mechanism of Action	Limitations/ Resistance	Status	References
Vincristine	Alkaloid	*Catharanthus roseus* (leaves)	Anti-cancer activity against acute lymphoblastic leukemia	Inhibits microtubule polymerization	Neurotoxicity, drug resistance (P-gp efflux)	FDA approval in 1963 under the name Oncovin	Nourozi et al. (2025)
Paclitaxel	Terpenoid	*Taxus brevifolia*/ Himalayan Yew (Bark)	Anti-cancer activity for breast, ovarian, and lung cancers	Stabilizes microtubules	Low solubility, resistance through tubulin mutation	FDA approval in 1993 under the name Taxol	(Bernabeu et al., 2017; Sati et al., 2024)
Homoharringtonine (Structural analog of cephalotaxine)	Alkaloid	*Cephalotaxus* species (Bark, Leaves, Seeds)	Anti-cancer activity for chronic myeloid leukemia	Inhibits protein synthesis	Myelosuppression, limited specificity	FDA approval in 2012 under the name Synribo	(Kantarjian et al., 2013; Razzaq et al., 2025)
Ingenol mebutate (Structural analog of Ingenol)	Diterpene	*Euphorbia peplus* (latex sap)	Anti-cancer activity against non-melanoma skin cancer	Induces apoptosis & inflammation	Dermatitis irritation, low penetration	Clinical trials II/III	(Madkour et al., 2024; Ogbourne and Parsons, 2014)
Irinotecan and Topotecan (Structural analogs of Camptothecin)	Alkaloid	*Camptotheca acuminate* (leaves)	Anti-cancer activity against pancreatic, ovarian, and colorectal cancers	Topoisomerase I inhibition	Toxicity, resistance through DNA repair mechanisms	FDA-licensed semi-synthetic drugs	(Choudhari et al., 2019; Madkour et al., 2024)
Lycopene	Carotenoid (Terpene)	Red fruits and vegetables like tomatoes	Prostate cancer	Antioxidant activity	Poor bioavailability, reliable absorption	Completed phase II clinical trials	(Kapała et al., 2022; López-Solís et al., 2025)
Berberine, Jatrorrhizine, Palmatine, Coptisine	Alkaloid	*Berberis vulgaris*	Anti-diabetic, Anti-cancer, Anti-bacterial, Analgesic, Anti-inflammatory, and Cardiovascular	DNA intercalation, enzyme inhibition	Absorption impairment, fast metabolism	Phase II clinical trials	Harrison et al. (2021)
Quinine	Alkaloid	*Cinchona* species (Bark)	Anti-malarial drug	Inhibits heme detoxification	Resistance in parasites, toxicity	FDA approval in 2005	Mohammadi et al. (2020)
Salvinorin A	Terpenoid	*Salvia divinorum*	Hallucinogen and analgesic	Kappa-opioid receptor agonist	Psychoactive effects, short half-life	Phase I clinical trial	Listos et al. (2011)
Silymarin	Flavonoid	*Silybum marianum*	Hepatoprotective activities	Antioxidant, membrane stabilization	Poor bioavailability, inconsistent efficacy	Phase II clinical trial	Avelar et al. (2025)
Capsaicin	Alkaloid	*Capsicum annuum*	Pain relievers	TRPV1 receptor activation	Desensitization effects, skin irritation	FDA approval in 2009 under the name QUTENZA	Pasierski and Szulczyk (2022)
Huperzine A	Sesquiterpene alkaloid	*Huperzia serrata*	Restores cognitive deficits by reversibly inhibiting AChE	AChE inhibition	Scarcity of clinical information, dosing issues	Phase III clinical trials	Friedli and Inestrosa (2021)
Tiotropium (Structure analog of atropine)	Alkaloid	*Atropa belladonna*	In the treatment of Chronic Obstructive Pulmonary Disease (COPD)	Muscarinic receptor antagonist	Dry mouth, cardiovascular effects	FDA approved in 2005 under the name Spiriva	Kohnen-Johannsen and Kayser (2019)
Camptothecin	Alkaloid	*Nothapodytes nimmoniana*	Topoisomerase I inhibitor leading to anticancer activity	Topoisomerase I inhibition	Lack of solubility toxicity	Phase II clinical trials	Mohiuddin et al. (2021)
Reserpine	Alkaloid	*Rauvolfia serpentina*	A drug for hypertension and a tranquilizer	VMAT inhibition	Depression, CNS side effects	FDA approved in 1955	Mukherjee et al. (2021)
Morphine	Alkaloid	*Opium*	Analgesic	μ-opioid receptor agonist	Addiction, intolerance, pulmonary intoxication	FDA approved in 2013	Carlin et al. (2020)
Acetylsalicylic acid (Structure analog of Salicin)	Phenolics	*Salix alba* (inner bark)	Pain relievers	COX inhibition	Gastric irritation, risk of bleeding	FDA approved in 1988 under the name Aspirin	Mishra and Baek (2021)

platforms (e.g., AutoDock, PyRx, ZDOCK) and scoring functions are employed, which reduces reproducibility and complicates cross-study comparisons. Moreover, docking methods only give a fixed picture of ligand protein interaction and fail to fully model protein dynamics, solubility, and biology. Therefore, the methods are useful for preliminary screening; however, their predictive capability is limited and needs to be supported by experimental research (K and Venugopal, 2024).

In a one comprehensive virtual screening study, 4704 plant secondary metabolites were screened against key targets of SARS-CoV-2,

including the spike protein Receptor-Binding Domain (RBD), human transmembrane serine protease 2 (TMPRSS2), Mpro, and RNA-dependent RNA polymerase (RdRp). Molecular docking studies revealed a structure-dependent pattern of interactions, with different phytochemical classes selectively interacting with distinct proteins. It is important to note that triterpenoids (>50%) have a high binding affinity with the spike RBD, and may therefore disrupt viral host cell recognition through the hACE2 interface. Conversely, flavonoids and flavonoid glycosides (>32%), flavonol glycosides, and anthocyanidins (>16) were found to interact effectively with TMPRSS2 and Mpro, respectively. Also, flavonol glycosides (>13%) were identified as targeting RdRp, indicating they inhibit viral replication.

The binding energy of several lead compounds, such as Coagulin K, Kamalachalcone C, Ginkgetin, Isoginkgetin, and 3,3 2-biplumbagin, was low, and their drug-likeness properties were favorable, which was in line with the Lipinski rule, and their bioavailability scores were acceptable. Notably, the article emphasized that bioavailability and the metabolic transformation of phytochemicals are key factors that determine *in vivo* effects, because bio-transformed products can still contain pharmacologically active moieties. All in all, the present study highlights the efficiency of large-scale virtual screening and molecular docking in the discovery of structurally diverse phytochemicals as putative antiviral agents, thereby providing a solid basis for *in vitro* and *in vivo* validation (Puttaswamy et al., 2020).

Moreover, in the other study, integrative research involved the bioactive compounds of *Uvaria scheffleri*, *Clematis burgensis,* and *Euphorbia schimperiana*, using phytochemical isolation, antimicrobial testing, and computer modeling. Silica gel chromatography yielded five compounds, one of which was an indole analog, N-methyl-2,3-bis(2-hydroxybenzyl)-1H-indole, and the other four were known (uretin, lupeol, and a prenylated coumarin derivative). *U. scheffleri* has a strong bioactivity, as the dichloromethane root extract demonstrated a high level of antibacterial activity against *Staphylococcus aureus* with a minimum inhibitory concentration of 6.25 μg/mL, which is similar to gentamicin (5 μg/mL).

In silico molecular docking experiments revealed that the identified compounds (1, 3–5) bind with high affinity to *Escherichia coli* DNA gyrase B, with binding energies ranging from −6.9 to −6.0 kcal/mol, similar to the standard antibiotic ciprofloxacin (−7.2 kcal/mol). Further, molecular docking of human DNA topoisomerase II alpha showed binding energies of −5.9 and 5.3 kcal/mol, indicating potential anticancer properties. It is interesting to note that the prenylated coumarin derivative and lupeol showed particularly strong interactions, suggesting their potential as antibacterial leads. Complementary ADMET analysis was used to confirm good drug-likeness profiles, and DFT calculations (B3LYP/6-31G) showed low energy gaps and high chemical reactivity of the compounds. Of significance, the reliability of combining computational methods with biological validation of *in vitro* antimicrobial activity can be highlighted/demonstrated by the consistency between *in vitro* and, *in silico* results (Anza et al., 2021).

3.2.3. Drug likeness prediction based on Lipinski's rule of five and ADMET profile

The ligands that showed the highest binding energy were subjected to Lipinski's rule of five (Jayaram et al., 2012) and the SWISS ADME (Daina et al., 2017), a web tool for pharmacokinetics study. These tools predict drug-like properties, including molecular weight (>500 Da), molar refractivity, H-bond donor (>5), H-bond acceptor (>10), Log P (>5), and absorption, distribution, metabolism, and excretion (ADME).

3.2.4. Bioactive prediction score

The bioactive score indicates the potential of ligands to serve as drugs. The bioactive score is evaluated by using the Molinspiration online server and calculated from ligand SMILES (https://www.molinspiration.com, Slovensky Grob, Slovakia). This tool calculates ligand scores against regular human receptors, including enzyme inhibitors (EI), G-Protein Coupled Receptors (GPCR), Kinase Inhibitors (KI), Ion Channel modulators (ICM), and Nuclear Receptor Ligands (NRL).

4. Plant secondary metabolites and microbiome connection

The synthesis of secondary metabolites in the host plant can be influenced by specific microbes connected to specific plants (Huang et al., 2018). Despite growing interest in plant secondary metabolites as modulators of microbial communities, the existing literature has significant limitations and inconsistencies. Many of the reported findings are based on controlled laboratory experiments or model plant systems, which may not accurately reflect real environmental conditions. Furthermore, the effects of these metabolites on microbiomes are usually concentration-dependent, and some have both positive and negative effects on microbial populations. Contradictory results have also been observed, especially regarding antimicrobial activity compared to microbiome stability, suggesting that these interactions are highly context-specific. Moreover, there are methodological differences, such as variations in sequencing technologies, experimental design, and environmental parameters, which further contribute to differences across studies. These issues demonstrate the need to have standardized experimental models and integrative methods that incorporate metabolomics, microbiome profiling, and functional validation (Huang et al., 2018).

Methylobacterium regulates the synthesis of phytometabolites, such as Volatile Organic Compounds (VOCs), that are connected to the metabolism of the host plant (Brader et al., 2017). In a balanced microbial state (eubiosis), beneficial microorganisms enhance plant growth by promoting photosynthesis and mitigating the detrimental effects of both environmental and biotic stresses. Under such conditions, plants often release increased levels of secondary metabolites; however, the associated energetic costs are offset by microbial production of auxins, as well as improved photosynthetic efficiency and nutrient acquisition (Tsavkelova et al., 2024). The nutrient properties of microbes in association with plants may lead to the formation of a specific microbial colony (Zhalnina et al., 2018). Using beneficial microbes has been shown to increase plants' resistance to various stresses. Microbial secondary metabolites have been the subject of several investigations on their efficacy as biological control agents against weeds, diseases, and pests (Dwisandi et al., 2024). In mycorrhizal symbiosis in soybeans, isoflavones and strigolactones serve as signal molecules. During the period of symbiosis, the roots of legume plants exude a range of 144 proteins in the mould *Laccaria* bicolor (Hartman et al., 2017; Li et al., 2016). The number of endophytes and ectomycorrhizal fungi colonizing maize and poplar tree roots decreased when the release of flavonoids and the phenylpropanoid pathway was suppressed (Mehmood et al., 2020). Thus, microbes control the production of plant secondary metabolites by regulating hormone levels, nutrient absorption, precursor substance availability, enzyme and gene expression, and by sensing environmental changes(Lv et al., 2024).

4.1. Plant secondary metabolites affect microbiome

4.1.1. Glucosinolates

Most studies suggest that glucosinolates are present in the *Brassicaceae* family as a defense chemical (Halkier and Gershenzon, 2006). The GSL-myrosinase system can release multiple bioactive compounds in response to pathogen infection, environmental stress, or mechanical damage to plants. Although many GSLs benefit human health, some have harmful side effects when consumed (Cheng et al., 2024). This is because following tissue damage, myrosinase converts these sulfur-containing metabolites into hazardous and deterrent isothiocyanates, nitriles, or other compounds. Previously, these compounds were thought to confer herbivore defense. Because they are present in root exudates, they also possess antifungal and antibacterial mechanisms that affect the soil microbiota (Bednarek et al., 2009;

Mönchgesang et al., 2016). Numerous studies link phenylethyl isothiocyanate levels, which is the breakdown product of glucosinolates by myrosinase hydrolysis in the rhizosphere to bacterial community structures (Rumberger and Marschner, 2004).

Glucosinolates also demonstrate how endophytic fungi interact with *Arabidopsis*. Tryptophan-derived indolic glucosinolates accumulate following the inoculation of *Arabidopsis* roots with *Serendipita indica* or *Sebacina vermifera*, which naturally colonize them (Lahrmann et al., 2015). For its plant growth promoter (PGP) function and disease prevention, the naturally occurring *Arabidopsis* colonizer *Colletotrichum tofieldae* requires indolic glucosinolates (Hiruma et al., 2016). Moreover, recent work shows that seed-transmitted microorganisms, potentially interacting with glucosinolate-derived compounds, can significantly influence early root microbiome assembly via niche partitioning and facilitation (Garrido-Sanz and Keel, 2025).

4.1.2. Camalexin

One of the primary phytoalexins, like phytoalexin camalexin, 3-thiazol-2′-yl-indole generated by *Arabidopsis* (Groβkinsky et al., 2024). Camalexin must be used as a barrier against necrotrophic infections brought on by *Alternaria brassicicola*, oomycete *Phytophthora brassicae*, and *Botrytis cinerea* (Rowe and Kliebenstein, 2008; Schlaeppi et al., 2010).

Using Genome-wide association studies (GWAS), variations in microbial sulfatase activity in rhizosphere soil were found to be related to the cytochrome P450 isoform gene. Camalexin biosynthetic genes and their loss are associated with reduced sulfatase activity and altered camalexin accumulation in plant roots. Exogenous application of camalexin may partially restore these phenotypes by modulating rhizosphere chemical interactions (Koprivova et al., 2019). The increase in biomass suggests that the PGP effects exhibited by different strains of mutualistic bacteria in a specific location on wild-type plants were absent in these camalexin-deficient *Arabidopsis* mutants. The loss of indole secondary metabolites in mutants with defective CYP79B2 and CYP79B3 function altered the vast number of diverse strains in SynCom; nevertheless, camalexin's direct impact on the emergence of microbial communities has not yet been established (Voges et al., 2019). Emerging efforts conceptualize the phytobiome as a complex communication network, suggesting that specific metabolites (like camalexin) may operate as molecular signals coordinating microbiome structure and resilience in smart-agriculture systems (Gulec et al., 2026).

4.1.3. Benzoxazinoids

Benzoxazinoids, a class of indole-derived defense compounds, are predominantly found in maize (*Zea mays*) and other grasses, where they contribute to resistance against herbivorous insects (Baumeler et al., 2000). They have been employed in agriculture for centuries to either encourage beneficial heterospecific feedback or lessen the negative effects of monocropping. For instance, legumes' symbiotic relationship with nitrogen-fixing bacteria can improve soil fertility, increasing the yield of succeeding crops (Gfeller et al., 2024).

These compounds also act as allelochemicals and antimicrobial agents, functionally analogous to camalexin and glucosinolates (de Bruijn et al., 2018). Studies on benzoxazinoid-deficient maize mutants have demonstrated altered rhizosphere bacterial and fungal community composition, suggesting a role in shaping the soil microbiome. Furthermore, benzoxazinoids contribute to plant soil feedback mechanisms that can influence the disease resistance of subsequent crop generations (Hu et al., 2018).

4.1.4. Coumarins

Many plants contain a specific type of secondary metabolite known as coumarins. Most of these chemicals are made via the phenylpropanoid pathway. Numerous biological actions, such as antibacterial and antioxidant qualities that support plant disease resistance, have been demonstrated by coumarins (Zaynab et al., 2024).

Coumarins represent a diverse group of plant-derived phenolic compounds with both beneficial and deleterious biological effects. While extensively studied in the context of human health, coumarins also influence plant-microbe interactions. For example, dietary exposure to ochratoxin A, a mycotoxin with a coumarin backbone, has been shown to increase the prevalence of specific *Lactobacillus* strains in the gut microbiome of rats (Guo et al., 2014). In plants, the root-associated bacterium *Pseudomonas simiae* plays a key role in inducing systemic resistance against pathogens, and the transcription factor MYB72 is essential for regulating genes involved in coumarin biosynthesis (Zamioudis et al., 2014). Under iron-deficient soil conditions, coumarin-mediated interactions have been observed to modulate microbial community composition, enabling beneficial plant growth-promoting *rhizobacteria* (PGPR) to suppress pathogenic fungi (Stringlis et al., 2018).

Coumarins exert their antibacterial effects through several mechanisms. They prevent enzymatic activity, damage pathogen cell membranes, and inhibit nucleic acid synthesis. Additionally, by inducing the generation of reactive oxygen species (ROS) and promoting the expression of genes linked to immunology and signaling pathways, including the salicylic acid-dependent pathway, coumarins enhance plant defense responses. Coumarins play an important part in defense mechanisms, making them suitable for use in sustainable agriculture methods that prioritize ecologically friendly integrated pest management techniques (Zamioudis et al., 2014).

4.1.5. Triterpenes

A type of secondary metabolite known as triterpene esters is created by applying a sequence of acylation, glycosylation, and oxidation changes on triterpene skeletons. The pharmaceutical, cosmetic, and pesticide industries have all found uses for the numerous triterpene esters identified for their significant bioactivities. Furthermore, they are crucial for controlling root microbes and protecting plants from pests, illnesses, and physical harm (Liu et al., 2024).

Arabidopsis species synthesize a diverse array of triterpenes in their roots. Multiple biosynthetic genes are induced by jasmonate signaling, indicating a potential role in mediating plant-microbe interactions (Huang et al., 2019). Mutations in key genes required for the biosynthesis of triterpenes, including those influencing Thalianin, Arabidin, and various triterpene fatty acid esters, result in pronounced alterations in microbiome assembly when plants are cultivated in natural soils (Huang et al., 2019). Disruption of triterpene production alters the composition of root-associated microbial communities, with non-*Arabidopsis*-associated bacterial taxa becoming more prevalent. This suggests that the presence of specific triterpenes in plant roots may selectively influence the recruitment of microbial strains during root microbiome formation. The growing accessibility of untargeted metabolomics datasets enables correlation analyses between root metabolomic profiles and the taxonomic composition of rhizosphere microbiomes (Cotton et al., 2019).

4.2. Production of volatile organic compounds (VOCs)

Evidence indicates that plant- and soil-associated microorganisms producing Volatile Organic Compounds (VOCs) can suppress pathogenic microbes, highlighting their potential as biocontrol agents against plant diseases (Ossowicki et al., 2017). VOC secretion by *Bacillus amyloliquefaciens* FZB42 not only enhances plant growth but also triggers systemic resistance and exhibits antibacterial activity (Compant et al., 2005). VOCs from *Bacillus subtilis* GB03, particularly acetoin and 2,3-butanediol, induce systemic resistance (ISR) in *Arabidopsis* seedlings, significantly reducing disease severity caused by the soft rot pathogen *Erwinia carotovora* (Ryu et al., 2004). Microbial VOCs have also demonstrated strong efficacy in controlling postharvest fruit diseases. Bacterial VOCs encompass a wide chemical diversity, including alcohols, aldehydes, alkenes, alkynes, benzenes, esters, heterocycles, ketones, sulfides, and

terpenoids (Song et al., 2024).

VOC production by *B. amyloliquefaciens* NJN-6 inhibits mycelial growth and spore germination of *Fusarium oxysporum*, reducing fungal growth by 30-40% (Yuan et al., 2012). Additionally, VOCs inhibit the activity of catalase and superoxide dismutase in pathogens, thereby attenuating their virulence. The regulatory role of VOCs in controlling phytopathogens is further exemplified by *B. amyloliquefaciens* SQR9, whose VOCs significantly reduce biofilm formation, motility, and *Ralstonia solanacearum* colonization in tomato roots (Raza et al., 2016). Microorganisms inhibited under specific environmental conditions often possess unique metabolic pathways and a high potential for producing novel bioactive metabolites. This expands the likelihood of discovering novel biologically active alkaloids through targeted exploration (Zhu et al., 2023).

4.2.1. Antibacterial molecules

Bacterial leaf streak and bacterial blight in rice are caused by *Xanthomonas oryzae pv. oryzae* and *Xanthomonas oryzae pv. oryzicola*, respectively. Biocontrol agents such as *B. amyloliquefaciens* FZB42 can effectively suppress these economically significant diseases (Wu et al., 2015). The wild-type FZB42 strain exhibits stronger inhibitory activity against *Erwinia amylovora* compared to mutant derivatives that lack difficidin but still produce macrolactin or bacillaene. The antibiotic bacilysin, synthesized by *B. amyloliquefaciens* FZB42, has also been reported to inhibit *E. amylovora* (Chen et al., 2009).

Bacteriocins are ribosomally synthesized peptide toxins that can be produced in the plant rhizosphere in response to environmental cues, functioning to suppress pathogenic nematodes and bacteria (Scholz et al., 2011; White et al., 2017). Genome analyses of *B. amyloliquefaciens* FZB42 have identified non-ribosomally encoded gene clusters responsible for the biosynthesis of novel antibacterial and nematicidal compounds, such as plantazolicin (Scholz et al., 2011). Amylocyclicin, a hydrophobic cyclic peptide, is encoded by a six-gene cluster spanning approximately 4.5 kb that governs biosynthesis, post-translational modification, export, and self-immunity (Scholz et al., 2014).

4.2.2. Antifungal molecules

Non-ribosomal peptide synthetase (NRPS)-derived lipopeptides produced by *Bacillus amyloliquefaciens* FZB42 are the primary determinants of its antifungal activity(Chen et al., 2006). Mutant strains of *B. amyloliquefaciens* FZB42 lacking bacillomycin-D exhibited a marked reduction in antifungal efficacy, indicating that bacillomycin-D is critical for the antifungal potential of this strain (Chen et al., 2006). Treatment of *Fusarium graminearum* hyphae and conidia with bacillomycin-D induces distinct morphological alterations in the plasma membrane and cell wall structures (Gu et al., 2017). Furthermore, approximately 115 secondary metabolites from about 9 taxonomic groups have been isolated from *Biscogniauxia species*. Of these, the terpenoid class and its derivatives represented the most prevalent group, with 43 compounds identified, followed closely by the coumarin class, comprising 21 compounds (Purbaya et al., 2023). Taken together, the emerging evidence indicates that plant secondary metabolites actively regulate microbiome recruitment, functional dynamics, and downstream bioactivity, thereby directly influencing their potential for drug discovery. For example is a structurally differentiated glucosinolate has been shown to selectively recruit specific groups of bacteria, such as *Comamonadaceae* and *Oxalobacteraceae,* and has shown that even subtle chemical differences can restructure microbial community assemblage by substrate selectivity and cross-feeding of metabolic pathways (Baatsen et al., 2026).

Similarly, camalexin expression is dynamically regulated by interactions with microbes, and there are distinct time-dependent observations between beneficial versus pathogenic bacteria, indicating that context within the microbiome determines the extent and stability of metabolite buildup. Benzoxazinoid derivatives, including 6-Methoxy-2-benzoxazolinone (MBOA), also have long-term effects on the microbial composition of the rhizosphere and on plant defense in grasses, whereas coumarins are bidirectional signaling molecules that not only regulate microbial communities but are also induced by beneficial microbes, thereby creating a feedback loop that improves plant fitness. The terpenes and other specialized metabolites also contribute to the establishment of below-ground interactions between microbes and ecological adaptation (Mucha et al., 2019).

From a drug discovery perspective, the results show that the microbiome-mediated shift in PSM availability might not be limited to availability itself but also to structural diversity and functional activity, including bioactive derivatives derived through microbial biotransformation. However, these interactions are highly context-dependent, and the assembly of the microbiome and the pattern of metabolites cannot be predicted; assembly and patterns differ across plant genotype, metabolite structure, and environment. The mentioned complication may be included in the description of discrepancies in reported bioactivities of plant-derived compounds. In this way, microbiome profiling should be combined with metabolomics, functional validation, and computational techniques to evaluate and optimize plant secondary metabolites as therapeutic leads with the desired level of precision (Huang and Osbourn, 2019).

These microbiome-induced variations also have important implications for *in silico* drug discovery plans, by microbial biotransformation and context-specific metabolic expression are capable of altering ligand structures and binding profiles. Such biological variability is not given much attention in existing docking methods, which could reduce their predictive capability. It was then possible to use the microbiome-inspired metabolite information in computational workflows to find more biologically interesting and translationally viable lead compounds (V. P. T. Nguyen et al., 2020).

4.3. Do plant secondary metabolites influence plant traits?

Environmental factors, such as soil type, temperature, bacterial and viral infections, growth season, or pests, influence the ultimate composition of microbes. In reaction to stimuli, certain compounds that are part of a plant's natural defense mechanism are undoubtedly synthesized more actively, providing chemical defense against herbivorous animals (Cotton et al., 2019). *Horse chestnut* leaf mining is evident by the presence of brown-yellow patches on leaves, fast withering, and early leaf fall before the end of the growing season (Myśkow et al., 2021; Weryszko-Chmielewska and Haratym, 2012a, 2012b). Many insects cause significant damage to plants by reducing their photosynthetic capacity, the size and weight of their fruit and seeds, the intensity of their flowering, and the size and vigour of their seedlings just beginning to sprout (Holoborodko et al., 2021; Percival et al., 2011; Tyapkina et al., 2022). Palisade parenchyma, damaged spongy parenchyma cells, and epidermal cell mortality on both sides of the leaf blade have previously been observed in anatomical and histological examinations of chestnut leaves (Bennett and Wallsgrove, 1994; Weryszko-Chmielewska and Haratym, 2012a).

Previous research indicates that plants produce more compounds in response to stress, potentially strengthening their chemical defenses against infections. Increased light exposure throughout the growing season may have contributed to the partial breakdown of flavonoids, which protect plants' photosynthetic organs. As a result of the activity of components, the antiradical activity of extracts has an impact on the plant's resistance to stress stimuli (Bennett and Wallsgrove, 1994; Falcone Ferreyra et al., 2012; Gourlay and Constabel, 2019).

Schultz shows how the combination of *Betula lutea* and *Acer saccharum* leaves impacts plant resistance to parasite damage (Schultz, 1983). Numerous studies have shown that the quantity of tannins and phenolic compounds in chestnut leaves significantly reduces *Cameraria ohridella* foraging activity (Głowacka et al., 2009; Pavan et al., 2003). According to Paterska et al., the concentration of tannins in healthy and

infested leaves did not differ in the most recent studies. However, their concentration impacts the plant's health (Paterska et al., 2017). *Horse chestnut* leaves that have been invaded have more phenolic compounds than could be observed under fluorescence microscopy. The walls of the vascular bundles and the outer cells of the adaxial epidermis of healthy leaves most noticeably revealed the blue light autofluorescence of phenolic chemicals. In response, phenolic compounds were shown to be present in the spongy parenchyma cells, adaxial and abaxial epidermal cells of leaves harmed by foraging *C. ohridella* larvae. They are mainly accumulated in tissues close to the pest's feeding areas. Palisade parenchyma, a dense cluster of cells with many chloroplasts, has been shown to be the primary food source for *C. ohridella* larvae infesting horse chestnut leaves in both earlier and more recent studies (Konarska et al., 2020). Palisade parenchyma has a high surface-to-volume ratio, which facilitates CO_2 absorption and speeds up photosynthesis in the area of the leaf that receives abundant light (Borsuk and Brodersen, 2019; Théroux-Rancourt et al., 2021).

4.4. Plant secondary metabolites influence the ecosystem

Secondary metabolites (SMs) play a pivotal role in mediating plant-environment interactions, making them particularly sensitive to climate change. Elevated atmospheric CO_2 (eCO_2) and elevated temperature (eT) frequently enhance the accumulation of plant phenolic compounds, such as rutin and chlorogenic acid (Ghasemzadeh et al., 2012; Jing Guo et al., 2020; Nguyen et al., 2019). The response of SMs to climate change is influenced by both metabolite type and specific climatic factors, with additional variation attributable to genetic and physiological differences among plant taxa and functional groups (Tshivhandekano et al., 2017). Liquorice shows beneficial effects on leaf phenolic compounds under Decreased Precipitation (DP) conditions, but negatively affects root phenolic compounds (Chang et al., 2020). Similarly, the ginsenoside content of *Panax ginseng* increases with moderate shifts in temperature or precipitation but declines under more extreme changes (Liao et al., 2017; S. Wang et al., 2019). The Growth-Defence Balance Hypothesis (GDBH) posits that under stress, plants allocate more resources to secondary metabolism than to primary metabolism, contributing to metabolic diversity (Monson et al., 2022). For instance, *Populus spp*. (poplars) increase lignin content under heat stress, a modification regulated by functional genes involved in SM biosynthesis, thereby enhancing environmental adaptability (Xu et al., 2023).

Given their importance in plant environment interactions and human health, the responsiveness of SMs to climate change has major implications for both ecological adaptation and medicinal value (Verma and Shukla, 2015). Meta-analyses of medicinal and aromatic plants (MAAPs) reveal that phenolic compounds, terpenoids, and alkaloids respond variably to different Global Change Factors (GCFs), with phenolics showing the most pronounced sensitivity (Sharma et al., 2019). Elevated temperature generally improves plant physiological capacity to withstand abiotic stress, including drought, UV-B radiation, and salinity (Sharma et al., 2019).

In contrast, eCO_2 often reduces alkaloid content while increasing terpenoid levels—likely because terpenoids depend on photosynthetic carbon assimilation, whereas alkaloid biosynthesis requires nitrogen-derived amines (Jamloki et al., 2021; Lichman, 2021). These patterns align with the Carbon-Nitrogen Balance (CNB) hypothesis, in which the carbon-to-nitrogen (C/N) ratio influences resource allocation between primary and secondary metabolism (Watanabe et al., 2021). Drought-induced reductions in metabolic activity suggest that SMs may also function as osmotic regulators, maintaining water balance under stress (Niinemets, 2016; Selmar, 2014). Terpenoid synthesis under combined elevated nitrogen (eN) and dP conditions indicates that drought can counteract the inhibitory effects of eN and potentially restore MAAP quality reduced by global nitrogen deposition (Selmar, 2014). The Growth-Differentiation Balance Hypothesis and the Optimal Defense Theory predict that plants with limited nitrogen allocate more carbon to carbon-based defensive SMs, particularly in tissues critical to fitness (Dilena et al., 2023). Observations indicate that climate change effects on SM metabolism, especially phenolic metabolism, require further multifactorial studies, as most current data originate from the Northern Hemisphere, introducing geographic bias (Bennett and Classen, 2020; Gerstner et al., 2014; Zamorano Miranda et al., 2020). Unavoidable geographic bias may result in restrictive search criteria or in the particular distribution of MAAPs (Gerstner et al., 2014).

The phenolic and terpenoid levels of woody plants responded favourably to eN than in herbaceous plants. This is likely because woody plants are less photosynthetically sensitive to eN than herbs or because shrubs like tea trees grow quickly and have unique plucking methods (Liang et al., 2020; Mudau et al., 2006). The root system responded to eCO_2 with higher phenolic content than the leaves, due to the sharing of carbon resources in the ground system compared to above-ground organs (Thompson et al., 2017). These findings demonstrate the significance of Nitrogen nutrition in plant development and its role as a key growth controlling factor (GCF). No organ-specific features were seen under dP and eT. This suggests that whole-plant metabolic processes regulated by signals may reduce the adverse effects of the stressor (Kollist et al., 2019). A recent meta-analysis of plant photosynthetic responses found that phenolic compounds exhibit strong positive or negative responses to elevated eCO_2 and eN levels driven by climate change (Dusenge et al., 2019; Liang et al., 2020). Fig. 3 depicts factors influencing plant-microbe interactions, including anthropogenic, environmental, host, and edaphic determinants that regulate plant health and defense, and is adapted from (Tsavkelova et al., 2024). Long-term CO_2 enrichment was predicted to improve plant photosynthesis and Carbon absorption, thereby providing carbon substrates for increased secondary metabolism; it was assumed that eCO_2 duration typically has a positive effect on secondary metabolite responses (Gamage et al., 2018; Royer et al., 2013). The high alkaloid content and low terpenoid and essential oil content in increasing evaluated Nitrogen amendments are both equally startling. This shows that Nitrogen nutrition has a positive effect on N-containing substances but a negative effect on C-based secondary metabolites. The fact that this action depends on the nitrogen threshold further underscores the significance of the C-N balance in plant secondary metabolism. Despite this, many studies show that terpenoid synthesis increases under heat stress, and extreme heat negatively affects morphology, physiology, and anatomy (Hassan et al., 2021). Long-lasting, mild warming was considered advantageous for terpenoid synthesis, as temperature fluctuation altered terpenoid production in MAAPs. In addition to dP levels or exposure periods, secondary metabolite levels were higher in MAAPs. This demonstrates the secondary metabolite's antioxidant and osmotic regulation functions in stress conditions, such as drought, and provides valuable information for enhancing MAAP quality through water changes (Niinemets, 2016).

It has previously been shown that when eCO_2 was present, a positive correlation existed between plant sensitivity and either mean annual temperature (MAT) or mean annual precipitation (MAP). In contrast to eCO_2, however, the varying secondary metabolite responses to eN demonstrate altered plant metabolite sensitivity (Baig et al., 2015). The conservation of plant secondary metabolite responses to drought and these responses are unrelated to environmental moderators. Since plants and soil are closely related, certain soil properties, such as Soil Organic Carbon (SOC), can significantly influence plant response (Van Nuland et al., 2016; de Vries and Wallenstein, 2017). An increase in soil total nitrogen and SOC typically reduces the reactivity of secondary metabolites to future eN and dP. This is due to improved plant health and favorable soil conditions, which correlate with rhizospheric bacteria diversity and stability (Junjie Guo et al., 2020). This phenomenon shows that a rise in SOC may lead to the production of a C-based secondary metabolite that enhances plant health by feeding a specific beneficial bacterium. It is crucial to remember that increased SOC also influences plant C allocation through rhizosphere C secretion (Kolton et al., 2017;

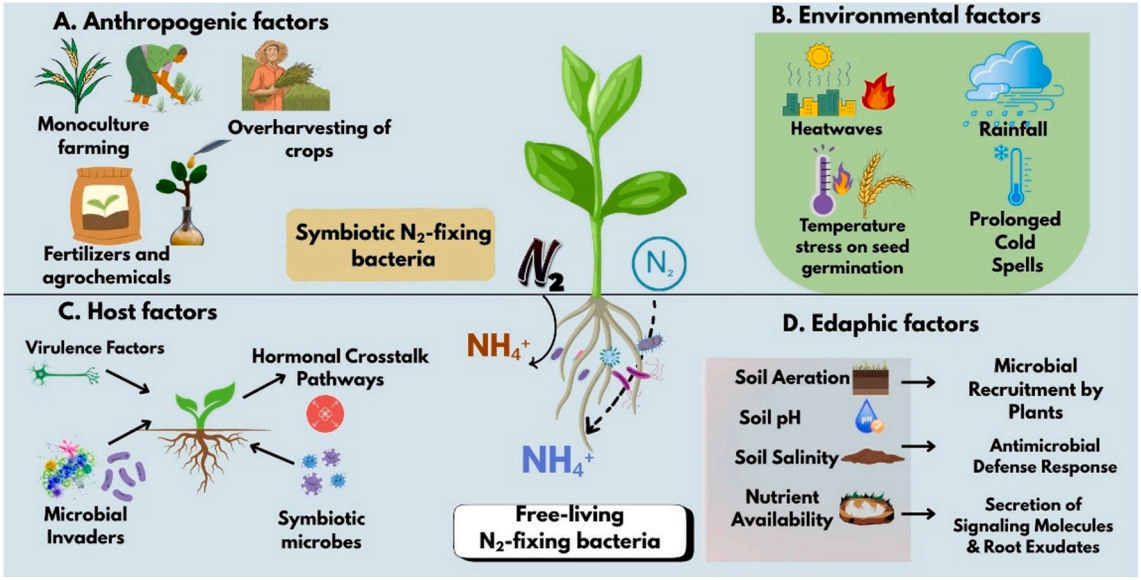

Fig. 3. Factors influencing plant-microbe interactions include (A) Anthropogenic determinants (monoculture farming, overharvesting of crops, fertilizers, and chemicals), (B) Environmental factors (heatwave, temperature stress, and rainfall), (C) Host-related traits (symbiotic microbes, hormonal crosstalk pathways, and microbial invaders) and (D) Edaphic conditions (soil pH, soil aeration, soil salinity, and nutrient availability). These elements collectively regulate plant health and defense while shaping microbial associations, including free-living nitrogen fixation and symbiotic interactions with nitrogen-fixing bacteria, which enhance nutrient acquisition and stress tolerance (Tsavkelova et al., 2024).

Prescott et al., 2020). eCO also made other plants more toxic, suggesting that the negative effects on people could be exacerbated. However, it's unclear whether CO_2, combined with other abiotic stressors, will have the same impact (Lv et al., 2024). Although computational strategies have contributed greatly to the discovery of promising bioactive plant metabolites, their functional importance on biological systems is eventually determined by the high complexity of interactions within microbial communities. The fact that *in silico* predictions have been very limitedly integrated with in-depth microbiome-level understanding is also a major gap, and the rationale for seeking multidisciplinary solutions to connect computational screening with ecological and functional validation.

5. Synthetic biology for secondary metabolites biosynthesis

Synthetic biology is a multidisciplinary field that integrates biological science, engineering, and computational sciences. It has become an advanced method for exploring and enhancing Biosynthetic processes. The primary objective of synthetic biology is to design and construct new biological components, tools, and networks, or to modify existing designs for beneficial applications.

In the biosynthesis of phytochemicals, synthetic biology provides new avenues for validating metabolic processes and modifying them to produce high-yielding, bioactive compounds. These modifications can be introduced in both plants and microbial systems, even for bioactive compounds that are basically found only in specific plant species (Qin et al., 2025).

Hairy root cultures are a reliable approach for enhancing the yield of

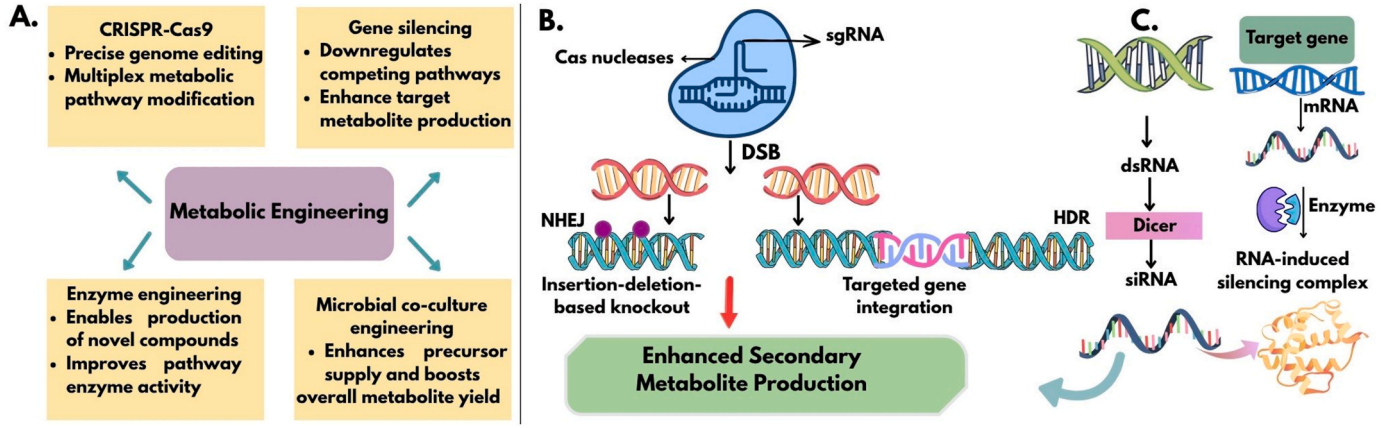

Fig. 4. Primary pathway modification techniques for boosting bioactive compounds production. (A) CRISPR-Cas9 modifies specific biosynthetic genes; gene silencing inhibits competing genes; enzyme engineering enhances catalytic steps; and mixed microbial cultures allocate pathway tasks to enhance precursor availability. (B) CRISPR-Cas9 process showing the formation of a specific double-strand break. NHEJ (non-homologous end joining) produces a small sequence change for gene disruption, while HDR (homology-directed repair) allows exact modification or substitution of pathway-associated genes, and (C) the RNA interference (RNAi) mechanism used for gene silencing. Double-stranded RNA is cleaved by Dicer into small interfering RNAs, which guide the RISC complex to cleave specific mRNAs, decreasing the expression of genes that shift metabolic routing (Gao et al., 2024).

plant-specific metabolites through metabolic modifications. By using targeted gene alteration, researchers have been able to alter metabolic processes, enhance the activity of essential regulatory genes, and introduce genes from different organisms to increase the accumulation of important phytochemicals (Singh and Dhar, 2020).

For example, the CRISPR-Cas9 gene modification system, which is part of metabolic engineering Fig. 4 has been efficiently applied and used in *Artemisia annua*, where knocking out the squalene synthase (SQS) gene rewired metabolic pathways to enhance artemisinin yield in transformed root culture, resulting in a 3.2-times enhancement in yield (Gao et al., 2024). Likewise, in *Salvia miltiorrhiza*, upregulation of the SmMYB1 gene triggered diterpenoid metabolic genes, resulting in an 8.5-fold rise in tanshinone level (Hao et al., 2020).

Alteration of the biosynthetic pathway by activating several genes has also been shown to be effective. For instance, in *Withania somnifera,* dual overexpression of squalene epoxidase and cytochrome P450 genes noticeably boosted withanolide levels, indicating the potential of multigene engineering in the root-based system (Sivanandhan et al., 2020).

Additionally, incorporating Rosmarinic acid synthase (RAS) and CYP98A14 genes originating from *S. miltiorrhiza* inside transformed root cultures enhanced phenolic acid biosynthesis up to threefold greater than in wild-type roots (Fu et al., 2020). Enhancing the Chalcone synthase (CHS) gene from *Glycyrrhiza uralensis* in its hairy roots also increased total flavonoid levels and enhanced the synthesis of liquiritigenin, isoliquiritigenin, and isoliquiritin (Yin et al., 2019). In transformed hairy root cultures of *Valeriana officinalis*, overexpression of Farnesyl pyrophosphate synthase, Valerendiene synthase, or Germacrene C synthase genes led to a 2-4-fold increase in valerenic acid, a 1.5-4 times rise in sesquiterpene hydrocarbons, and a 5-9-fold increase in oxygenated sesquiterpenoids compared to normal (non-transgenic) root lines (Ricigliano et al., 2016).

Plant microbiota can be modified using either a bottom-up or top-down approach. In the bottom-up approach, microbes naturally occurring on specific plants or plant organs are first extracted from natural microbiomes (Rodrigues et al., 2018). These primary microbes are then genetically modified to acquire the target characteristics and combined to form synthetic engineered microbial mixtures (SynComs) (Vorholt et al., 2017). The engineered strains are later reintroduced into plant hosts, where they can efficiently colonize them.

In the top-down approach, horizontal gene transfer (HGT) is used to directly transfer target characteristics into many microbial recipients within their natural ecosystem. One method is to utilize mobile genetic elements (MGEs), which can deliver and integrate foreign genes into diverse microbial organisms, enabling large-scale screening for plant growth promoting (PGP) traits. Another method involves using bacteriophages (phages) to specifically modify or eliminate certain microbial species within a population, thereby helping determine their functional roles (Ke et al., 2021).

5.1. Production of plant secondary metabolites using plant tissue culture

Haberlandt, in 1902, pioneered plant tissue culture, a technique been used for over a century. The most commonly used culture medium, developed by Murashige and Skoog in 1962, is still widely applied today, with some modifications (Sehgal and Khan, 2020). Model systems based on plant tissue culture are often utilized in research to overcome various plant-related problems, including physiological, biochemical, genetic, and structural issues (Anuradha et al., 2021).

5.1.1. Somatic embryogenesis

Somatic embryogenesis is a process in which somatic (non-reproductive) cells are reprogrammed to form embryogenic cells, which then develop into embryos through several structural and biochemical changes. This process enables efficient cloning of homogenous and superior plant lines. It is also useful in crop improvement methods such as genetic transformation and induced mutation (Abderrezag et al., 2024). During somatic embryogenesis, elevated levels of compounds, including phenolics, alkaloids, and terpenoids, are produced, making these cultures valuable for the production of secondary metabolites.

According to the review of literature, embryogenic cultures from various plant species (*Catharanthus roseus* and *Peucedanum japonicum)* have been utilized to produce secondary metabolites at different stages: embryo induction, maturation, and germination (Murthy et al., 2023).

In vitro adventitious root culture is employed for the large-scale production of metabolites, offering the benefit of producing compounds in greater amounts than cell suspension cultures. In some medicinal plants, these root cultures have been successfully grown at the bioreactor level (Murthy et al., 2008). Such cultures can be initiated from any plant part (explant) by using the appropriate concentration of phytohormone auxin.

5.1.2. Hairy root cultures

Hairy root cultures are created by infecting plant tissues with *Agrobacterium rhizogenes* and are a stable, reliable method for producing valuable secondary plant metabolites (Biswas et al., 2023; Liu et al., 2025). The use of elicitors further improves this process by activating the plant's defense system, which increases the production of secondary metabolites (Selwal et al., 2025). Recent developments in plant biotechnology and phytochemical research also point to strategies to improve the production of valuable secondary metabolites. The transcription factor SmMYB98 has been reported to positively regulate the production of both tanshinone and salvianolic acid in *Salvia miltiorrhiza*, demonstrating that transcription-factor engineering can be used to enhance the simultaneous production of terpenoid and phenolic compounds. Equally, *Rosmarinus officinalis* is a source of valuable bioactives, including rosmarinic acid and essential oil compounds with significant antibacterial activity against medically important pathogens. These results highlight that metabolic engineering in conjunction with phytochemical characterization can aid in the production and functional use of high-value plant metabolites in a sustainable manner (Anuradha et al., 2021; Sehgal and Khan, 2020).

5.1.3. Bioreactors

Plants are important sources of secondary compounds used to make medicines, flavors, fragrances, coloring agents, food additives, and agrochemicals (Wang et al., 2017). However, conventional plant-based syntheses of PSMs are often limited by low yields, variability, and reliance on particular growth factors. However, plant cell, tissue, and organ cultures, when used with bioreactors, provide a controlled platform that improves and scales up PSM production. Moreover, PSM production varies significantly among different parts of the same plant (Murthy et al., 2023).

Most PSMs shield plants protect them from unfavorable conditions. Plant cell and organ culture techniques have been developed for the efficient production of PSM, which in turn leads to significant advancements in bioreactor technology for plant cell and organ cultures (Murthy et al., 2008). However, several issues still limit their performance, including low cell productivity, slow growth rates, genetic instability in high-producing cell lines, poor control over cell differentiation, and difficulty in maintaining photoautotrophic (light-dependent) growth.

6. Challenges and research gaps, and future perspectives

Most bioactive compounds are found in plants at extremely low quantities, despite the enormous diversity and abundance of bioactive molecules in the plant kingdom. In addition to requiring a lot of land, large-scale farming is expensive and can be affected by pests and diseases. A less-than-ideal option is chemical synthesis, which often involves numerous steps, hazardous chemicals, and challenging conditions. Plant metabolic engineering and *in vitro* culture have been

employed for a long time to increase the production of secondary metabolites. It is clear that microbial chassis are more effective at creating structurally simple molecules or intermediates; however, they struggle to produce complex bioactive compounds in large quantities due to problems, including limited enzyme activity and interference with endogenous metabolism. The ability to precisely and specifically alter the plant genome through CRISPR-Cas9-based genome editing has transformed the area of genetic modification. With this cutting-edge technology, specific mutations, including insertions, deletions, nucleotide substitutions, and translocations, can be induced to confer improved traits in plants (Mipeshwaree Devi et al., 2023). Crop development and sustainable agriculture offer tremendous opportunities through CRISPR-Cas9-based genome editing and the optimization of plant secondary metabolite extraction and purification. To improve desired traits and overcome issues with off-target effects and limited yields of specific substances, genome editing enables the removal of undesirable genetic regions (Liu et al., 2025). Some of the challenges in the field include a limited understanding of biosynthetic pathways and regulatory mechanisms for many plant secondary metabolites, insufficient mapping of pathways for rare and novel compounds, the need for sustainable and ethical approaches to explore plant biodiversity, and the development of efficient green chemistry-based extraction and purification methods.

Although plant secondary metabolites are highly diverse and can be used therapeutically, they pose serious challenges and technical bottlenecks that hinder their use in drug discovery and development. The low natural concentration of bioactive compounds in plants is one of the main constraints, requiring extensive production. Nonetheless, this method is usually limited by high costs, space requirements, and vulnerability to environmental factors such as pests and disease. Alternatively, chemical synthesis is often inefficient, multistep, requires dangerous reagents, and is incapable of producing structurally complex molecules (Tawfeeq et al., 2018).

To overcome these constraints, plant metabolic engineering and *in vitro* culture methods have been investigated to improve metabolite production. Although microbial systems are beneficial for the production of simple intermediates, they remain limited in the production of structurally complex phytochemicals because they encounter difficulties with enzyme activity, pathway complexity, and metabolic interference. In this regard, new applications, such as CRISPR-Cas9-based genome editing are employed. Fig. 4 depicts the potential to selectively control biosynthetic pathways, enabling enhanced yield and quality of desirable metabolites. Nevertheless, the issues of off-target effects, pathway optimization, and scalability remain significant challenges (Hao et al., 2020).

Besides production-related constraints, a critical gap in the overall knowledge of biosynthetic pathways and regulatory networks of numerous plant secondary metabolites exists. Their systematic exploitation is limited by the lack of detailed pathway mapping, especially for rare and novel compounds. Future studies should focus on combining multi-omics strategies (genomics, transcriptomics, and metabolomics) to clarify these pathways and enable rational metabolic engineering (Wang et al., 2017).

Additionally, even with computational methodologies such as molecular docking and network pharmacology, there remains a pivotal need for robust experimental validation through *in vitro*, *in vivo*, and clinical studies. The second significant limitation is the low bioavailability and pharmacokinetic properties of most of the phytochemicals, which limit their therapeutic use. One way to address these challenges is to develop new drug delivery systems, such as nanoparticle-based carriers and nanoformulations (Putra et al., 2023).

Furthermore, standardizing extraction and purification procedures and scaling up green extraction technologies are major challenges for their industrial use. The solutions to these problems will involve finding sustainable, reproducible, and cost-effective extraction methods. Lastly, the ethical issues in the exploration of biodiversity, the regulatory approval process, and mass-scale production should also be addressed. In general, it will be necessary to address these cross-disciplinary issues to implement the findings of translation of plant secondary metabolites in laboratory studies into clinically useful therapeutic products (Putra et al., 2023).

7. Conclusion

There is an urgent need to identify novel potential plant-based drugs against various deadly diseases. Potential drugs could be analyzed using advanced computational methods, and only promising candidates could be subjected to *in vitro* and *in vivo* studies. This will provide a quick solution and speed up drug discovery against various diseases. Computational molecular docking and simulations can speed up and narrow down the potential drug target to be checked at preclinical stages. The plant secondary metabolites hold tremendous potential as therapeutic agents for treating even complex human diseases. Furthermore, there is still untapped potential in the vast plant diversity, which remains unexplored for novel plant secondary metabolites. The role of environmental influence in plant secondary metabolite synthesis and vice versa is also an interesting area worth exploring.

List of abbreviations

ABBREVIATIONS	FULL FORM
AMBER	Assisted model building with energy refinement
C	Carbon
CC	Column chromatography
CHARMM	Chemistry at Harvard Macromolecular Mechanics
CT	Condensed tannins
dP	Diffusion pressure
EAX	Enzyme assisted extraction
EI	Enzyme inhibitors
eCO2	Elevated carbon dioxide
eN	Elevated nitrogen
eT	Elevated temperature
FDA	Food and Drug Administration
GCF	Global change factors
GPCR	G-protein coupled receptors
GROMACS	Groningen machine for chemical simulation
HIV	Human immunodeficiency virus
HPLC	High-performance liquid chromatography
HRMS	High-resolution mass spectroscopy
HTS	High-throughput screening
ICM	Ion change modulators
IR	Infrared
KI	Kinetics inhibitors
MAE	Microwave-assisted extraction
MDS	Molecular dynamics simulations
N	Nitrogen
NAMD	Nanoscale molecular dynamics
NMR	Nuclear magnetic resonance
NRL	Nuclear receptors ligands
ORD	Optical rotary dispersion
PASS	Prediction of activity spectra for substances
PGP	Plant growth promoters
PLE	Pressurized liquid extraction
PSM	Plant secondary metabolites
QSAR	Quantitative structure-activity relationship
SARS COV2	Severe acute respiratory syndrome coronavirus 2
SEC	Size exclusion chromatography
SFE	Supercritical fluid extraction
SOC	Soil organic carbon
UAE	Ultraviolet-assisted extraction
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
VLC	Vacuum liquid chromatography
VOC	Volatile organic compound

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Funding

This review received no external funding.

CRediT authorship contribution statement

Priyanka: Visualization, Writing – original draft, Writing – review & editing. **Pooja Sharma:** Writing – original draft. **Mahiti Gupta:** Writing – original draft. **Poonam Bansal:** Writing – original draft. **Raj Singh:** Writing – original draft. **Anil Kumar Sharma:** Writing – review & editing. **Sonal Datta:** Conceptualization, Supervision, Visualization, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank Maharishi Markandeshwar (Deemed to be University), Mullana (Ambala), Haryana, India. Figures were prepared using Canva.

Data availability

No data was used for the research described in the article.

References

Abazari, M.F., Nasiri, N., Karizi, S.Z., Nejati, F., Haghi-Aminjan, H., Norouzi, S., Piri, P., Estakhr, L., Faradonbeh, D.R., Kohandani, M., Daliri, K., Sanadgol, N., Askari, H., 2021. Mini Rev. Med. Chem. 21, 2187–2201.
Abderrezag, N., Bragagnolo, F., Louaer, O., Meniai, A.-H., Cifuentes, A., Ibáñez, E., Mendiola, J., 2024. J. Supercrit. Fluids 207, 106211.
Abubakar, A.R., Haque, M., 2020. J. Pharm. BioAllied Sci. 12, 1–10.
Adedayo, A.A., Musser, R., Aanaenson, M., Babalola, O.O., 2025. Plant Signal. Behav. 20, 2439248.
Agostini-Costa, T., Vieira, R., Bizzo, H., Silveira, D., Gimenes, M., 2012. Secondary Metabolites.
Agrawal, K., Murti, Y., 2022. Int. J. Comput. Biol. Drug Des. 15, 96.
Ahmad, S., Zahiruddin, S., Parveen, B., Basist, P., Parveen, A., Gaurav, Parveen, R., Ahmad, M., 2020. Front. Pharmacol. 11, 578970.
Alamri, M.A., Altharawi, A., Alabbas, A.B., Alossaimi, M.A., Alqahtani, S.M., 2020. Arab. J. Chem. 13, 7224–7234.
Ambure, P., Halder, A.K., González Díaz, H., Cordeiro, M.N.D.S., 2019. J. Chem. Inf. Model. 59, 2538–2544.
Amparo, T.R., Seibert, J.B., Almeida, T.C., Costa, F.S.F., Silveira, B.M., da Silva, G.N., Dos Santos, O.D.H., de Souza, G.H.B., 2021. Phytother Res. 35, 4297–4308.
Anand, U., Jacobo-Herrera, N., Altemimi, A., Lakhssassi, N., 2019. Metabolites 9.
Anuradha, B., Appani, L., Shivaraj, D., 2021. Tissue Culture Techniques for Horticultural Crops, pp. 181–195.
Anza, M., Endale, M., Cardona, L., Cortes, D., Eswaramoorthy, R., Zueco, J., Rico, H., Trelis, M., Abarca, B., 2021. Adv. Appl. Bioinform. Chem. 14, 117–132.
Atanasov, A.G., Zotchev, S.B., Dirsch, V.M., Orhan, I.E., Banach, M., Rollinger, J.M., Barreca, D., Weckwerth, W., Bauer, R., Bayer, E.A., Majeed, M., Bishayee, A., Bochkov, V., Bonn, G.K., Braidy, N., Bucar, F., Cifuentes, A., D'Onofrio, G., Bodkin, M., Diederich, M., Dinkova-Kostova, A.T., Efferth, T., El Bairi, K., Arkells, N., Fan, T.-P., Fiebich, B.L., Freissmuth, M., Georgiev, M.I., Gibbons, S., Godfrey, K.M., Gruber, C.W., Heer, J., Huber, L.A., Ibanez, E., Kijjoa, A., Kiss, A.K., Lu, A., Macias, F. A., Miller, M.J.S., Mocan, A., Müller, R., Nicoletti, F., Perry, G., Pittalà, V., Rastrelli, L., Ristow, M., Russo, G.L., Silva, A.S., Schuster, D., Sheridan, H., Skalicka-Woźniak, K., Skaltsounis, L., Sobarzo-Sánchez, E., Bredt, D.S., Stuppner, H., Sureda, A., Tzvetkov, N.T., Vacca, R.A., Aggarwal, B.B., Battino, M., Giampieri, F., Wink, M., Wolfender, J.-L., Xiao, J., Yeung, A.W.K., Lizard, G., Popp, M.A., Heinrich, M., Berindan-Neagoe, I., Stadler, M., Daglia, M., Verpoorte, R., Supuran, C. T., Taskforce, the I.N.P.S., 2021. Nat. Rev. Drug Discov. 20, 200–216.
Athiraja, P., Arthi, S., Sudarmathi, B., 2019. Indian J. Pharmaceut. Sci. 81.
Avelar, B.A., Santos, A.P., Vieira, R.A.L., Mendonça, R.D.D., Menezes, M.C. de, 2025. Int. J. Environ. Res. Publ. Health 22.
Baatsen, J., Azevedo, J., Quecine, M., 2026. Rhizosphere 37, 101264.
Baig, S., Medlyn, B.E., Mercado, L.M., Zaehle, S., 2015. Glob. Change Biol. 21, 4303–4319.
Balekundri, A., Ahire, E., Shelke, R., Rishipathak, D., Kshirsagar, S., 2025. Green Anal. Chem. 13, 100234.
Bansal, P., Sharma, D., Panwar, A., Kumar, R., Sharma, Aanchal, Ramniwas, S., Dey, A., Chandran, D., Dhama, K., Sharma, Anil, 2023. J. Pure Appl. Microbiol. 17, 385–394.
Bansal, P., Tuli, H.S., Sharma, D., Mohapatra, R., Dhama, K.D., Sharma, A., 2022. J. Exp. Biol. Agric. Sci. 10, 396–404.
Baque, M.A., Moh, S.-H., Lee, E.-J., Zhong, J.-J., Paek, K.-Y., 2012. Biotechnol. Adv. 30, 1255–1267.
Barthwal, R., Mahar, R., 2024. Metabolites 14.
Baumeler, A., Hesse, M., Werner, C., 2000. Phytochemistry 53, 213–222.
Baxevanis, A.D., Bateman, A., 2015. Curr. Protoc. Bioinform. 50, 1.1.1–1.1.8.
Bednarek, P., Pislewska-Bednarek, M., Svatos, A., Schneider, B., Doubsky, J., Mansurova, M., Humphry, M., Consonni, C., Panstruga, R., Sanchez-Vallet, A., Molina, A., Schulze-Lefert, P., 2009. Science 323, 101–106.
Bennett, A.E., Classen, A.T., 2020. Ecology 101, e02978.
Bennett, R.N., Wallsgrove, R.M., 1994. New Phytol. 127, 617–633.
Berdy, J., Kertesz, M., 1989. In: Collier, H.R. (Ed.), Bioactive Natural Products Database: an Aid for Natural Products Identification BT - Chemical Information. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 237–251.
Bernabeu, E., Cagel, M., Lagomarsino, E., Moretton, M., Chiappetta, D.A., 2017. Int. J. Pharm. 526, 474–495.
Bhattarai, B., Adhikari, B., Basnet, S., Shrestha, A., Marahatha, R., Aryal, B., Rayamajhee, B., Poudel, P., Parajuli, N., 2022. J. Chem. 2022.
Bhatti, M., Ismail, H., Kayani, W., 2022. Plant Secondary Metabolites: Therapeutic Potential and Pharmacological Properties.
Birchfield, A., McIntosh, C., 2020. Curr. Plant Biol. 24, 100163.
Biswas, D., Chakraborty, A., Mukherjee, S., Ghosh, B., 2023. Front. Plant Sci. 14, 1197555.
Bohara, M., Ghaju, S., Sharma, K., Kalauni, S., Khadayat, K., 2022. J. Chem. 2022.
Borsuk, A.M., Brodersen, C.R., 2019. Plant Physiol. 180, 1406–1417.
Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L.-J., Sessitsch, A., 2017. Annu. Rev. Phytopathol. 55, 61–83.
Brito, T., Costa, F., Oliveira, R., Amaral, C., Labuto, G., Gonzalez, M., 2025. Food Chem. 464, 141922.
Brown, E.D., Wright, G.D., 2016. Nature 529, 336–343.
C, S., S, D.K., Ragunathan, V., Tiwari, P., A, S., P, B.D., 2022. J. Biomol. Struct. Dyn. 40, 585–611.
Cao, S., Liang, J., Chen, M., Xu, C., Wang, X., Qiu, L., Zhao, X., Hu, W., 2025. Front. Chem. 13, 1536590.
Carlin, J., Craig, C., Little, S., Donnelly, M., Fox, D., Zhai, L., Walters, L., 2020. Environ. Pollut. 264, 114633.
Castro, V., Rodríguez, M., Corral, M., Lopez-Soria, A., García-Pérez, P., Barral Martínez, M., Perez, R., Cao, H., Simal-Gandara, J., Prieto, M., 2023. Sulfur-Containing Compounds from Plants, pp. 363–402.
Chandran, H., Meena, M., Barupal, T., Sharma, K., 2020. Biotechnol. reports (Amsterdam, Netherlands) 26, e00450.
Chang, H., Chen, P., Ma, M., 2020. Sci. Rep. 10, 1534.
Cheek, M., Nic Lughadha, E., Kirk, P., Lindon, H., Carretero, J., Looney, B., Douglas, B., Haelewaters, D., Gaya, E., Llewellyn, T., Ainsworth, A., Gafforov, Y., Hyde, K., Crous, P., Hughes, M., Walker, B., Forzza, R., Wong, K., Niskanen, T., 2020. Plants People Planet 2, 371–388.
Chen, S.-L., Yu, H., Luo, H.-M., Wu, Q., Li, C.-F., Steinmetz, A., 2016. Chin. Med. 11, 37.
Chen, S.L., Song, J.Y., Sun, C., Xu, J., Zhu, Y.J., Verpoorte, R., 2015. Sci. Cust. Publ. Off, pp. S27–S29.
Chen, X.-H., Vater, J., Piel, J., Franke, P., Scholz, R., Schneider, K., Koumoutsi, A., Hitzeroth, G., Grammel, N., Strittmatter, A.W., Gottschalk, G., Süssmuth, R.D., Borriss, R., 2006. J. Bacteriol. 188, 4024–4036.
Chen, X.H., Scholz, R., Borriss, M., Junge, H., Mögel, G., Kunz, S., Borriss, R., 2009. J. Biotechnol. 140, 38–44.
Cheng, B., Ran, R., Qu, Y., Verkerk, R., Henry, R., Dekker, M., He, H., 2024. Agric. Commun. 2, 100040.
Chin, Y.-W., Balunas, M.J., Chai, H.B., Kinghorn, A.D., 2006. AAPS J. 8, E239–E253.
Chongo, Y., 2025. J. Food Sci. Gastron 3, 29–37.
Choudhari, A.S., Mandave, P.C., Deshpande, M., Ranjekar, P., Prakash, O., 2019. Front. Pharmacol. 10, 1614.
Clemensen, A.K., Provenza, F.D., Hendrickson, J.R., Grusak, M.A., 2020. Front. Sustain. Food Syst. 4.
Colson, P., Rolain, J.-M., Raoult, D., 2020. Chloroquine for the 2019 novel coronavirus SARS-CoV-2. Int. J. Antimicrob. Agents 55, 105923.
Compant, S., Duffy, B., Nowak, J., Clément, C., Barka, E.A., 2005. Appl. Environ. Microbiol. 71, 4951–4959.
Cotton, T.E.A., Pétriacq, P., Cameron, D.D., Meselmani, M. Al, Schwarzenbacher, R., Rolfe, S.A., Ton, J., 2019. ISME J. 13, 1647–1658.
Crozier, A., Clifford, M., Ashihara, H., 2006. Plant secondary metabolites: Occurrence, Structure and Role in the Human Diet. ISBN: 9781405125093.
Daina, A., Michielin, O., Zoete, V., 2017. Sci. Rep. 7, 42717.

Damián-Medina, K., Salinas-Moreno, Y., Milenkovic, D., Figueroa-Yáñez, L., Marino-Marmolejo, E., Higuera-Ciapara, I., Vallejo-Cardona, A., Lugo-Cervantes, E., 2020. Heliyon 6, e03632.
Dashtian, K., Kamalabadi, M., Ghoorchian, A., Ganjali, M.R., Rahimi-Nasrabadi, M., 2024. J. Chromatogr. A 1733, 465240.
de Bruijn, W.J.C., Gruppen, H., Vincken, J.-P., 2018. Phytochemistry 155, 233–243.
de Vries, F.T., Wallenstein, M.D., 2017. J. Ecol. 105, 913–920.
Dilena, E., Close, D., Hunt, I., Garland, S., 2023. Sci. Rep. 13.
Dou, J., Weathers, P., 2022. Plant Cell Tissue Organ Cult. 149, 105–111.
Dusenge, M.E., Duarte, A.G., Way, D.A., 2019. New Phytol. 221, 32–49.
Dwisandi, R., Miranti, M., Widiastuti, A., Prismantoro, D., Awal, M., Mispan, M., Joshi, R., Doni, F., 2024. Plant Stress 15, 100720.
Dwivedi, D., Sonter, S., Mishra, S., Singh, Priyanka, Singh, Prashant, 2021. Future J. Pharmaceut. Sci. 7.
Edreva, A.M., Velikova, V., Tsonev, T., 2007. Russ. J. Plant Physiol. 54, 287–301.
Ekins, S., Mestres, J., Testa, B., 2007. Br. J. Pharmacol. 152, 9–20.
El-Saadony, M.T., Saad, A.M., Mohammed, D.M., Alkafaas, S.S., Abd El-Mageed, T.A., Fahmy, M.A., Ezzat Ahmed, A., Algopishi, U.B., Abu-Elsaoud, A.M., Mosa, W.F.A., AbuQamar, S.F., El-Tarabily, K.A., 2025. Front. Nutr. 12, 1659743.
England, C., TrejoMartinez, J., PerezSanchez, P., Karki, U., Xu, J., 2023. Plants as biofactories for therapeutic proteins and antiviral compounds to combat COVID-19. Life 13, 617.
Erb, M., Kliebenstein, D.J., 2020. Plant Physiol. 184, 39–52.
Fabio, S., Elisa, L., Giulia, A., Valerio, G., Alessandro, Z., Sara, S., 2025. LWT 215, 117270.
Fahimirad, S., Abtahi, H., Razavi, S.H., Alizadeh, H., Ghorbanpour, M., 2017. Molecules 22.
Falcone Ferreyra, M.L., Rius, S.P., Casati, P., 2012. Front. Plant Sci. 3, 222.
Fang, J., Liu, C., Wang, Q., Lin, P., Cheng, F., 2018. Brief. Bioinform. 19, 1153–1171.
Fazili, M.A., Bashir, I., Ahmad, M., Yaqoob, U., Geelani, S.N., 2022. Bull. Natl. Res. Cent. 46, 35.
Friedli, M.J., Inestrosa, N.C., 2021. Molecules 26.
Fu, R., Shi, M., Deng, C., Zhang, Y., Zhang, X., Wang, Y., Kai, G., 2020. Food Chem. 331, 127365.
Gamage, D., Thompson, M., Sutherland, M., Hirotsu, N., Makino, A., Seneweera, S., 2018. Plant Cell Environ. 41, 1233–1246.
Gao, H., Pei, X., Song, X., Wang, S., Yang, Z., Zhu, J., Lin, Q., Zhu, Q., Yang, X., 2024. Front. Plant Sci. 15, 1477894.
Gao, Y., Wang, W., He, C., Fang, Z., Zhang, Y., Shi, Q., 2019. Anal. Bioanal. Chem. 411.
Garrido-Sanz, D., Keel, C., 2025. Nat. Microbiol. 10, 1130–1144.
Gerstner, K., Dormann, C.F., Stein, A., Manceur, A.M., Seppelt, R., 2014. J. Appl. Ecol. 51, 1690–1700.
Gfeller, V., Thoenen, L., Erb, M., 2024. New Phytol. 241, 2575–2588.
Ghasemzadeh, A., Jaafar, H.Z., Karimi, E., Ibrahim, M.H., 2012. BMC Complement. Altern. Med. 12, 229.
Giri, A., Narasu, M.L., 2000. Biotechnol. Adv. 18, 1–22.
González Mera, I., González Falconí, D.E., Morera, V., 2019. Bionatura 4, 1000–1009.
Gourlay, G., Constabel, C.P., 2019. Tree Physiol. 39, 345–355.
Głowacka, B., Lipiński, S., Tarwacki, G., 2009. For. Res. Pap. 70, 317–328.
Grassmann, J., Hippeli, S., Elstner, E.F., 2002. Plant Physiol. Biochem. 40, 471–478.
Großkinsky, D., Primisser, S., Reichenauer, T., Pfeifhofer, H., 2024. J. Gen. Plant Pathol. 90.
Gu, Q., Yang, Y., Yuan, Q., Shi, G., Wu, L., Lou, Z., Huo, R., Wu, H., Borriss, R., Gao, X., 2017. Appl. Environ. Microbiol. 83.
Gulati, P., Chadha, J., Harjai, K., Singh, S., 2022. Front. Microbiol. 13, 1073419.
Gulec, F., Awan, H., Wallbridge, N., Eckford, A.W., 2026. IEEE Commun. Mag. 64, 120–126.
Guo, Jing, Zhou, X., Wang, T., Wang, G., Cao, F., 2020b. Plant Physiol. Biochem. 147, 133–140.
Guo, Junjie, Ling, N., Chen, Z., Xue, C., Li, L., Liu, L., Gao, L., Wang, M., Ruan, J., Guo, S., Vandenkoornhuyse, P., Shen, Q., 2020a. New Phytol. 226, 232–243.
Guo, M., Huang, K., Chen, S., Qi, X., He, X., Cheng, W.-H., Luo, Y., Xia, K., Xu, W., 2014. Toxicol. Sci. 141, 314–323.
Gurung, A.B., Ali, M.A., Lee, J., Farah, M.A., Al-Anazi, K.M., 2020. Life Sci. 255, 117831.
Halkier, B.A., Gershenzon, J., 2006. Annu. Rev. Plant Biol. 57, 303–333.
Han, T., Miao, G., 2024. Molecules 29.
Hao, X., Pu, Z., Cao, G., You, D., Zhou, Y., Deng, C., Shi, M., Nile, S.H., Wang, Y., Zhou, W., Kai, G., 2020. J. Adv. Res. 23, 1–12.
Harrison, R., Fischer, S., Walpola, R.L., Chauhan, A., Babalola, T., Mears, S., Le-Dao, H., 2021. J. Healthc. Leader 13, 85–108.
Hartman, K., van der Heijden, M.G., Roussely-Provent, V., Walser, J.-C., Schlaeppi, K., 2017. Microbiome 5, 2.
Hassan, M., Rasool, T., Iqbal, C., Arshad, A., Abrar, M., Abrar, M.M., Habib Ur-Rahman, M., Noor, M.A., Sher, A., Sh, F., 2021. J. Plant Growth Regul. 41.
Hilal, B., Khan, M.M., Fariduddin, Q., 2024. Plant Physiol. Biochem. 211, 108674.
Hill, C.R., Shafaei, A., Balmer, L., Lewis, J.R., Hodgson, J.M., Millar, A.H., Blekkenhorst, L.C., 2023. Crit. Rev. Food Sci. Nutr. 63, 8616–8638.
Hiruma, K., Gerlach, N., Sacristán, S., Nakano, R.T., Hacquard, S., Kracher, B., Neumann, U., Ramírez, D., Bucher, M., O'Connell, R.J., Schulze-Lefert, P., 2016. Cell 165, 464–474.
Holoborodko, K.K., Seliutina, O.V., Ivanko, I.A., Alexeyeva, A.A., Shulman, M.V., Pakhomov, O.Y., 2021. Regul. Mech. Biosyst, 12, pp. 346–352.
Howard, M.M., Bass, E., Chautá, A., Mutyambai, D., Kessler, A., 2022. ISME J. 16, 5–9.
Hu, L., Robert, C.A.M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M.G.A., Schlaeppi, K., Erb, M., 2018. Nat. Commun. 9, 2738.
Huan, C., Xu, Y., Zhang, W., Guo, T., Pan, H., Gao, S., 2021. Front. Pharmacol. 12, 680674.
Huang, A.C., Jiang, T., Liu, Y.-X., Bai, Y.-C., Reed, J., Qu, B., Goossens, A., Nützmann, H.-W., Bai, Y., Osbourn, A., 2019. Science 364.
Huang, W., Long, C., Lam, E., 2018. Trends Plant Sci. 23, 559–562.
Huang, A.C., Osbourn, A., 2019. Pest Manag. Sci. 75, 2368–2377.
Ikram, N.K.K., Durrant, J.D., Muchtaridi, M., Zalaludin, A.S., Purwitasari, N., Mohamed, N., Rahim, A.S.A., Lam, C.K., Normi, Y.M., Rahman, N.A., Amaro, R.E., Wahab, H.A., 2015. J. Chem. Inf. Model. 55, 308–316.
Imperatore, C., Gimmelli, R., Persico, M., Casertano, M., Guidi, A., Saccoccia, F., Ruberti, G., Luciano, P., Aiello, A., Parapini, S., Avunduk, S., Basilico, N., Fattorusso, C., Menna, M., 2020. Drugs 18.
Jaikumar, K., Mohamed, S., Wyson, J., Deventhiran, M., Babu, A., Anand, D., Periyasamy, S., 2017. Asian J. Pharm. Clin. Res. 10, 214.
Jamloki, A., Bhattacharyya, M., Nautiyal, M.C., Patni, B., 2021. Heliyon 7, e07709.
Jang, Y.J., Park, B., Lee, H.-W., Park, H.J., Koo, H.J., Kim, B.O., Sohn, E.-H., Um, S.H., Pyo, S., 2017. Chem. Biol. Interact. 272, 28–36.
Javid, A., Fatima, A., Hamad, M., Ahmed, M., 2024. South Afr. J. Bot. 173, 159–174.
Jayaram, B., Singh, T., Mukherjee, G., Mathur, A., Shekhar, S., Shekhar, V., 2012. BMC Bioinf. 13 (Suppl. 1), S7.
Jo, S., Kim, S., Shin, D.H., Kim, M.-S., 2020. J. Enzym. Inhib. Med. Chem. 35, 145–151.
K, D., Venugopal, S., 2024. Front. Bioinforma. 4, 1463750.
Kaliaperumal, K., Salendra, L., Liu, Y., Ju, Z., Sahu, S.K., Elumalai, S., Subramanian, K., M Alotaibi, N., Alshammari, N., Saeed, M., Karunakaran, R., 2023. Front. Microbiol. 14, 1216928.
Kanavarioti, A., 2019. Sci. Rep. 9, 1019.
Kantarjian, H., Thomas, D., Jorgensen, J., Kebriaei, P., Jabbour, E., Rytting, M., York, S., Ravandi, F., Garris, R., Kwari, M., Faderl, S., Cortes, J., Champlin, R., O'Brien, S., 2013. Cancer 119, 2728–2736.
Kapała, J., Lewandowska, J., Placek, W., Owczarczyk-Saczonek, A., 2022. Int. J. Environ. Res. Publ. Health 19.
Kaur, G., Prakash, P., Srivastava, R., Verma, P.C., 2021. In: Ramawat, K.G., Ekiert, H.M., Goyal, S. (Eds.), Enhanced Secondary Metabolite Production in Hairy Root Cultures Through Biotic and Abiotic Elicitors BT - Plant Cell and Tissue Differentiation and Secondary Metabolites: Fundamentals and Applications. Springer International Publishing, Cham, pp. 625–660.
Ke, J., Wang, B., Yoshikuni, Y., 2021. Trends Biotechnol. 39, 244–261.
Khorshed, A.A., Abdelnaeem, F.M., Oraby, M., Nagy, D.M., Derayea, S.M., 2025. Microchem. J. 212, 113407.
Klassen, A., Faccio, A.T., Canuto, G.A.B., da Cruz, P.L.R., Ribeiro, H.C., Tavares, M.F.M., Sussulini, A., 2017. Adv. Exp. Med. Biol. 965, 3–17.
Kohnen-Johannsen, K.L., Kayser, O., 2019. Molecules 24.
Kollist, H., Zandalinas, S.I., Sengupta, S., Nuhkat, M., Kangasjärvi, J., Mittler, R., 2019. Trends Plant Sci. 24, 25–37.
Kolton, M., Graber, E.R., Tsehansky, L., Elad, Y., Cytryn, E., 2017. New Phytol. 213, 1393–1404.
Konarska, A., Grochowska, M., Haratym, W., Tietze, M., Weryszko-Chmielewska, E., Lechowski, L., 2020. Urban For. Urban Green. 56, 126793.
Koprivova, A., Schuck, S., Jacoby, R., Klinkhammer, I., Welter, B., Leson, L., Martyn, A., von Steimker, J., Grabenhorst, N., Mandelkow, J., Zuccaro, A., Zeier, J., Kopriva, S., 2019. Proc. Natl. Acad. Sci. 116.
Kvittingen, L., Sjursnes, B.J., Schmid, R., 2021. J. Chem. Educ. 98, 3600–3607.
Lagunin, A.A., Druzhilovsky, D.S., Rudik, A.V., Filimonov, D.A., Gawande, D., Suresh, K., Goel, R., Poroikov, V.V., 2016. Biochem. Suppl. Ser. B biomed. Chem 10, 43–54.
Lahrmann, U., Strehmel, N., Langen, G., Frerigmann, H., Leson, L., Ding, Y., Scheel, D., Herklotz, S., Hilbert, M., Zuccaro, A., 2015. New Phytol. 207, 841–857.
Laskar, M., Duttachoudhury, M., Chetia, P., 2014. Int. J. Pharm. Pharm. Sci. 7, 345.
Lautié, E., Russo, O., Ducrot, P., Boutin, J.A., 2020. Front. Pharmacol. 11, 397.
Lee, H.-W., Lee, C.G., Rhee, D.-K., Um, S.H., Pyo, S., 2017. Int. Immunopharmacol. 45, 163–173.
Li, B., Li, Y.-Y., Wu, H.-M., Zhang, F.-F., Li, C.-J., Li, X.-X., Lambers, H., Li, L., 2016. Proc. Natl. Acad. Sci. USA. 113, 6496–6501.
Li, Y., Li, R., Ge, J., Nie, S., Chen, R., Yan, X., Qiao, J., 2024. J. Agric. Food Chem. 72, 19071–19080.
Liang, X., Zhang, T., Lu, X., Ellsworth, D.S., BassiriRad, H., You, C., Wang, D., He, P., Deng, Q., Liu, H., Mo, J., Ye, Q., 2020. Glob. Change Biol. 26, 3585–3600.
Liao, P., Liu, D., Xu, T.-R., Yang, Y., Cui, X., 2017. Ind. Crops Prod. 108, 95–105.
Lichman, B.R., 2021. Nat. Prod. Rep. 38, 103–129.
Listos, J., Merska, A., Fidecka, S., 2011. Pharmacol. Rep. 63, 1305–1309.
Liu, C., Ahmad, N., Tao, Y., Hussain, H., Chang, Y., Umar, A.W., Liu, X., 2025. Reprogramming hairy root cultures: a synthetic biology framework for precision metabolite biosynthesis. Plants 14, 1928.
Liu, J., Yin, X., Kou, C., Thimmappa, R., Hua, X., Xue, Z., 2024. Plant Commun. 5, 100845.
Loub, W.D., Farnsworth, N.R., Soejarto, D.D., Quinn, M.L., 1985. J. Chem. Inf. Comput. Sci. 25, 99–103.
López-Solís, R., Castro-Barquero, S., Donat-Vargas, C., Corrado, M., Arancibia-Riveros, C., Martínez-González, M.Á., Salas-Salvadó, J., Sorlí, J.V., Serra-Majem, L., Fitó, M., Pintó, X., Fiol, M., Lapetra, J., Gómez-Gracia, E., Toledo, E., Ramírez-Sabio, J.B., Babio, N., Estruch, R., Ros, E., Lamuela-Raventós, R.M., 2025. BMC Med. 23, 627.
Lv, J., Yang, S., Zhou, W., Liu, Z., Tan, J., Wei, M., 2024. Microbiol. Res. 283, 127688.
Madkour, M., Sebastian, A., Ramadan, W., Menon, V., Lozon, L., Srikanth, G., Tarazi, H., El-Gamal, M., Al-Tel, T., El-Awady, R., 2024. J. Mol. Struct. 1328, 141271.
Majumdar, M., Singh, V., Misra, T.K., Roy, D.N., 2022. Biologia (Bratisl) 77, 1373–1389.
Majumder, R., Mandal, M., 2022. J. Biomol. Struct. Dyn. 40, 696–711.

Makino, T., Izumi, K., Hiratsuka, K., Kano, H., Shimada, T., Nakano, T., Kadomoto, S., Naito, R., Iwamoto, H., Yaegashi, H., Shigehara, K., Kadono, Y., Nakata, H., Saito, Y., Nakagawa-Goto, K., Sakai, N., Iwata, Y., Wada, T., Mizokami, A., 2021. Sci. Rep. 11, 675.
Marchese, A., Orhan, I.E., Daglia, M., Barbieri, R., Di Lorenzo, A., Nabavi, S.F., Gortzi, O., Izadi, M., Nabavi, S.M., 2016. Food Chem. 210, 402–414.
Maurer, H.H., Muddiman, D.C., 2012. Anal. Bioanal. Chem. 403, 1201–1202.
Mehmood, A., Hussain, A., Irshad, M., Hamayun, M., Iqbal, A., Tawab, A., Khan, N., 2020. Symbiosis 81.
Menaga, P., Sakkanan, I., Mariselvam, L., Geetha, P., Nadar, A., College, J., 2021. Int. J. Pharm. Sci. Res. 12, 1300–1309.
Meshram, V., Kapoor, N., Dwibedi, V., Srivastava, A., Saxena, S., 2022. South Afr. J. Bot. 146, 409–416.
Mipeshwaree Devi, A., Khedashwori Devi, K., Premi Devi, P., Lakshmipriyari Devi, M., Das, S., 2023. Front. Plant Sci. 14, 1171154.
Mishra, A.K., Baek, K.-H., 2021. Biomolecules 11.
Mönchgesang, S., Strehmel, N., Schmidt, S., Westphal, L., Taruttis, F., Müller, E., Herklotz, S., Neumann, S., Scheel, D., 2016. Sci. Rep. 6, 29033.
Mohammadi, F., Farjam, M., Gholampour, Y., Tehranineshat, B., Oshvandi, K., Bijani, M., 2020. Risk Manag. Healthc. Policy 13, 785–794.
Mohammed, A.E., Alghamdi, S.S., Shami, A., Suliman, R.S., Aabed, K., Alotaibi, M.O., Rahman, I., 2023. Int. J. Nanomed. 18, 2141–2162.
Mohiuddin, M., Blyth, F.M., Degenhardt, L., Di Forti, M., Eccleston, C., Haroutounian, S., Moore, A., Rice, A.S.C., Wallace, M., Park, R., Gilron, I., 2021. Pain 162, S80–S96.
Monson, R.K., Trowbridge, A.M., Lindroth, R.L., Lerdau, M.T., 2022. New Phytol. 233, 1051–1066.
Mucha, S., Heinzlmeir, S., Kriechbaumer, V., Strickland, B., Kirchhelle, C., Choudhary, M., Kowalski, N., Eichmann, R., Hückelhoven, R., Grill, E., Kuster, B., Glawischnig, E., 2019. Plant Cell 31, 2697–2710.
Mudau, F.N., Soundy, P., du Toit, E.S., Olivier, J., 2006. South Afr. J. Bot. 72, 398–402.
Muhammad, A., Khan, M.H.U., Kong, X., Zheng, S., Bai, N., Li, L., Zhang, N., Muhammad, S., Li, Z., Zhang, X., Miao, C., Zhang, Z., 2025. Plant Sci. 354, 112431.
Mukherjee, D., Wagh, G., Mokalled, M.H., Kontarakis, Z., Dickson, A.L., Rayrikar, A., Günther, S., Poss, K.D., Stainier, D.Y.R., Patra, C., 2021. Development, 148.
Murad, H.A.S., Alqurashi, T.M.A., Hussien, M.A., 2022. BMC Complement. Med. Ther. 22, 35.
Murthy, H.N., Hahn, E.J., Paek, K.Y., 2008. Chin. J. Biotechnol. 24, 711–716.
Murthy, H.N., Joseph, K.S., Hahn, J.-E., Lee, H.-S., Paek, K.Y., Park, S.Y., 2023. Physiol. Mol. Biol. plants an Int. J. Funct. plant Biol. 29, 1153–1177.
Myśkow, E., Sokołowska, K., Słupianek, A., Gryc, V., 2021. Description of Intra-Annual Changes in Cambial Activity and Differentiation of Secondary Conductive Tissues of Aesculus hippocastanum Trees Affected by the Leaf Miner Cameraria Ohridella. Forests.
Nalawade, S.M., Tsay, H.-S., 2004. Vitr. Cell. Dev. Biol. - Plant 40, 143–154.
Nasim, N., Sandeep, I.S., Mohanty, S., 2022. Nucl. an Int. J. Cytol. Allied Top., 65, pp. 399–411.
Newman, D.J., Cragg, G.M., 2016. J. Nat. Prod. 79, 629–661.
Ngbolua, K.-T.-N., Kilembe, J., Matondo, A., Ashande, C., Mukiza, J., Mudogo, C., Ruphin, F., Baholy, R., Mpiana, P.T., Mudogo, V., 2022. Bull. Natl. Res. Cent. 46, 101–112.
Nguyen, D.T.P., Lu, N., Kagawa, N., Kitayama, M., Takagaki, M., 2020a. Short-term root-zone temperature treatment enhanced the accumulation of secondary metabolites of hydroponic coriander (Coriandrum sativum L.) grown in a plant factory. Agronomy 10, 413.
Nguyen, D.T.P., Lu, N., Kagawa, N., Takagaki, M., 2019. Optimization of photosynthetic photon flux density and root-zone temperature for enhancing secondary metabolite accumulation and production of coriander in plant factory. Agronomy 9, 224.
Nguyen, V.P.T., Stewart, J., Lopez, M., Ioannou, I., Allais, F., 2020b. Molecules 25.
Nguyen-Vo, T.-H., Trần, N., Dat, N.T., Le, L., 2016. SpringerPlus 5.
Nielsen, E., Temporiti, M.E.E., Cella, R., 2019. Plant Cell Rep. 38, 1199–1215.
Niinemets, Ü., 2016. Tree Physiol. 36, 129–132.
Nourozi, E., Hedayati, A., Madani, H., Hosseini, B., Hemmaty, S., 2025. Sci. Rep. 15, 6563.
Nunes, D., Loureiro, J.A., Pereira, M.C., 2022. Pharmaceutics 14.
Odhiambo, D., Omosa, L., Njagi, E., Kithure, J., Wekesa, E., 2025. Sci. Afr. 29, e02796.
Ogbourne, S.M., Parsons, P.G., 2014. Fitoterapia 98, 36–44.
Osbourn, A.E., Qi, X., Townsend, B., Qin, B., 2003. New Phytol. 159, 101–108.
Ossowicki, A., Jafra, S., Garbeva, P., 2017. PLoS One 12, e0174362.
Pagadala, N.S., Syed, K., Tuszynski, J., 2017. Biophys. Rev. 9, 91–102.
Pandith, S.A., Dar, R.A., Lattoo, S.K., Shah, M.A., Reshi, Z.A., 2018. Phytochem. Rev. 17, 573–609.
Pasierski, M., Szulczyk, B., 2022. Molecules 27.
Paterska, M., Bandurska, H., Wysłouch, J., Molińska-Glura, M., Moliński, K., 2017. Acta Physiol. Plant. 39.
Pavan, F., Paola, B., Bernardinelli, I., Gambon, N., Zandigiacomo, P., 2003. J. Arboric. 29, 253–258.
Percival, G.C., Barrow, I., Noviss, K., Keary, I., Pennington, P., 2011. Urban For. Urban Green. 10, 11–17.
Perna, F., Vitale, P., Capriati, V., 2019. Curr. Opin. Green Sustain. Chem. 21.
Perović, A.B., Karabegović, I.T., Krstić, M.S., Veličković, A.V., Avramović, J.M., Danilović, B.R., Veljković, V.B., 2024. Ind. Crops Prod. 211.
Pheko, T., Makhzoum, A., 2024. J. Essent. Oil Res. 36, 1–11.
Prachayasittikul, Veda, Worachartcheewan, A., Shoombuatong, W., Songtawee, N., Simeon, S., Prachayasittikul, Virapong, Nantasenamat, C., 2015. Curr. Top. Med. Chem. 15, 1780–1800.
Prasanth, D.S.N.B.K., Murahari, M., Chandramohan, V., Panda, S.P., Atmakuri, L.R., Guntupalli, C., 2021. J. Biomol. Struct. Dyn. 39, 4618–4632.
Prescott, C.E., Grayston, S.J., Helmisaari, H.-S., Kaštovská, E., Körner, C., Lambers, H., Meier, I.C., Millard, P., Ostonen, I., 2020. Trends Ecol. Evol. 35, 1110–1118.
Purbaya, S., Harneti, D., Safriansyah, W., Rahmawati, Wulandari, A.P., Mulyani, Y., Supratman, U., 2023. Toxins 15.
Putra, N., Yustisia, Y., Heryanto, R., Asmaliyah, A., Miswarti, M., Rizkiyah, D., Yunus, M., Irianto, I., Qomariyah, L., Nur Rohman, G.A., 2023. South Afr. J. Chem. Eng. 46.
Puttaswamy, H., Gowtham, H.G., Ojha, M.D., Yadav, A., Choudhir, G., Raguraman, V., Kongkham, B., Selvaraju, K., Shareef, S., Gehlot, P., Ahamed, F., Chauhan, L., 2020. Sci. Rep. 10, 20584.
Qaddir, I., Hussain, W., Mahmood, S., Rasool, N., 2020. Brazilian J. Pharm. Sci. 56.
Qin, K., Liu, F., Zhang, C., Deng, R., Fernie, A.R., Zhang, Y., 2025. Cell Rep. 44, 115715.
Quimque, M.T., Notarte, K.I., Adviento, X.A., Cabunoc, M.H., de Leon, V.N., Delos Reyes, F.S.L., Lugtu, E.J., Manzano, J.A., Monton, S.N., Muñoz, J.E., Ong, K.D., Pilapil, D.Y., Roque, V., Tan, S.M., Lim, J.A., Macabeo, A.P., 2023. Comb. Chem. High Throughput Screen. 26, 459–488.
Quinn, R.J., Carroll, A.R., Pham, N.B., Baron, P., Palframan, M.E., Suraweera, L., Pierens, G.K., Muresan, S., 2008. J. Nat. Prod. 71, 464–468.
Rabadiya, V., Shah, N., Akabari, A., 2025. Green Anal. Chem. 13, 100237.
Rao, S., Aqeel, U., Aftab, T., Gill, R., Gill, S., Naeem, M., 2025. J. Plant Growth Regul. 44, 4203–4226.
Rao, M.M.V., Hariprasad, T.P.N., 2021. Silico Pharmacol, 9, p. 5.
Raza, W., Ling, N., Yang, L., Huang, Q., Shen, Q., 2016. Sci. Rep. 6, 24856.
Razzaq, A., Rather, B.A., Zahra, L.T., Cong, H., Bai, Y., Qadir, F., Khan, M.N., Zafar, M. M., Jiang, X., Qiao, F., 2025. Biotechnol. J. 20, e70122.
Rehman, S. ur, Xu, S., Xu, H., Tao, T., Li, Y., Yu, Z., Ma, K., Xu, W., Wang, J., 2022. Mater. Today Adv. 16, 100287.
Ricigliano, V., Kumar, S., Kinison, S., Brooks, C., Nybo, S.E., Chappell, J., Howarth, D.G., 2016. Phytochemistry 125, 43–53.
Robinson, M.M., Zhang, X., 2011. The World Medicines Situation 2011 Traditional Medicines: Global Situation, Issues and Challenges.
Rodrigues, R.R., Rodgers, N.C., Wu, X., Williams, M.A., 2018. PeerJ 6, e4395.
Romano, J.D., Tatonetti, N.P., 2019. Front. Genet. 10, 368.
Rowe, H.C., Kliebenstein, D.J., 2008. Genetics 180, 2237–2250.
Royer, M., Larbat, R., Le Bot, J., Adamowicz, S., Robin, C., 2013. Phytochemistry 88, 25–33.
Rumberger, A., Marschner, P., 2004. Funct. Plant Biol. 31, 623–631.
Ryu, C.-M., Farag, M.A., Hu, C.-H., Reddy, M.S., Kloepper, J.W., Paré, P.W., 2004. Plant Physiol. 134, 1017–1026.
Samuni-Blank, M., Izhaki, I., Dearing, M.D., Gerchman, Y., Trabelcy, B., Lotan, A., Karasov, W.H., Arad, Z., 2012. Curr. Biol. 22, 1218–1220.
Sarkar, B., Ullah, M.A., Islam, S.S., Rahman, M.H., Araf, Y., 2021. J. Recept. Signal Transduct. Res. 41, 217–233.
Sati, P., Sharma, E., Dhyani, P., Attri, D.C., Rana, R., Kiyekbayeva, L., Büsselberg, D., Samuel, S.M., Sharifi-Rad, J., 2024. Eur. J. Med. Res. 29, 90.
Schlaeppi, K., Abou-Mansour, E., Buchala, A., Mauch, F., 2010. Plant J. 62, 840–851.
Scholz, R., Molohon, K.J., Nachtigall, J., Vater, J., Markley, A.L., Süssmuth, R.D., Mitchell, D.A., Borriss, R., 2011. J. Bacteriol. 193, 215–224.
Scholz, R., Vater, J., Budiharjo, A., Wang, Z., He, Y., Dietel, K., Schwecke, T., Herfort, S., Lasch, P., Borriss, R., 2014. J. Bacteriol. 196, 1842–1852.
Schultz, J.C., 1983. 37–54.
Sehgal, D., Khan, T., 2020. Plant Tissue Culture: Beyond being a Tool for Genetic Engineering, pp. 175–200.
Seidel, V., 2020. Plant-derived chemicals: a source of inspiration for new drugs. Plants 9, 1562. Basel, Switzerland.
Selmar, D., 2014. Agron. Sustain. Dev. 35, 121–131.
Selwal, N., Supriadi, K., Rahayu, F., Mudiana, D., Khamidah, A., Budiaarto, K., Antarlina, S.S., Tripatmasari, M., Wani, A.K., 2025. Elicitation strategies for enhanced secondary metabolite synthesis in plant cell cultures and its role in plant defense mechanism. Plant Gene 41, 100485.
Senizza, B., Zhang, L., Miras-Moreno, B., Righetti, L., Zengin, G., Ak, G., Bruni, R., Lucini, L., Sifola, M.I., El-Nakhel, C., Corrado, G., Rouphael, Y., 2020. Foods (Basel, Switzerland) 9.
Shankar, E., Goel, A., Gupta, K., Gupta, S., 2017. Curr. Pharmacol. reports 3, 423–446.
Sharma, A., Shahzad, B., Rehman, A., Bhardwaj, R., Landi, M., Zheng, B., 2019. Molecules 24.
Sharma, P., Shanavas, A., 2021. J. Biomol. Struct. Dyn. 39, 5819–5830.
Shawky, E., Nada, A.A., Ibrahim, R.S., 2020. RSC Adv. 10, 27961–27983.
Shree, P., Mishra, P., Selvaraj, C., Singh, S.K., Chaube, R., Garg, N., Tripathi, Y.B., 2022. J. Biomol. Struct. Dyn. 40, 190–203.
Singh, V., Dhar, P K (Eds.), 2020. Genome Engineering via CRISPR-Cas9 System. Academic Press, ISBN 9780128181416.
Sivanandhan, G., Selvaraj, N., Ganapathi, A., Lim, Y., 2020. Ind. Crops Prod. 154, 112706.
Sánchez-Sánchez, M.A., Zepeda-Morales, A.S.M., Carrera-Quintanar, L., Viveros-Paredes, J.M., Franco-Arroyo, N.N., Godínez-Rubí, M., Ortuño-Sahagun, D., López-Roa, R.I., 2020. Nutrients, 12.
Sofowora, A., Ogunbodede, E., Onayade, A., 2013. African J. Tradit. Complement. Altern. Med. AJTCAM 10, 210–229.
Song, C., Zhang, Yang, Zhao, Q., Chen, M., Zhang, Yu, Gao, C., Jia, Z., Song, S., Guan, J., Shang, Z., 2024. Microbiol. Res. 278, 127531.
Soni, S., Chahar, M., Priyanka, Chugh, P., Sharma, A., Narasimhan, B., Mohan, H., 2023. Microb. Pathog., 106398

Soni, S., Priyanka, Chugh, P., Ghanghas, N., Chahar, M., Kumar, S., Gupta, M.K., Mohan, H., 2025. Comput. Biol. Chem. 119, 108567.
Sorokina, M., Steinbeck, C., 2020. J. Cheminf. 12, 20.
Srivastava, N., Singh, A., Kumari, P., Nishad, J., Gautam, V., Yadav, M., Bharti, R., Kumar, D., Kharwar, R., 2021. Advances in Extraction Technologies: Isolation and Purification of Bioactive Compounds from Biological Materials, pp. 409–433.
Stermitz, F.R., Rapoport, H., 1961. J. Am. Chem. Soc. 83, 4045–4050.
Stocks, M., 2015. ChemMedChem 10.
Stringlis, I.A., Yu, K., Feussner, K., de Jonge, R., Van Bentum, S., Van Verk, M.C., Berendsen, R.L., Bakker, P.A.H.M., Feussner, I., Pieterse, C.M.J., 2018. Proc. Natl. Acad. Sci. U. S. A. 115, E5213–E5222.
Subramanya, S.B., Venkataraman, B., Meeran, M.F.N., Goyal, S.N., Patil, C.R., Ojha, S., 2018. Int. J. Mol. Sci. 19.
Tallei, T., Tumilaar, S., Niode, N., Umar, F., Kepel, B., Idroes, R., Effendi, Y., 2020. Potential of Plant Bioactive Compounds as SARS-CoV-2 Main Protease (Mpro) and Spike (S) Glycoprotein Inhibitors: a Molecular Docking Study.
Tawfeeq, A., Faisal, M., Abaas, I., Alwan, A., 2018. Asian J. Pharm. Clin. Res. 11, 126.
Teli, D.M., Shah, M.B., Chhabria, M.T., 2020. Front. Mol. Biosci. 7, 599079.
Tena, N., Asuero, A.G., 2022. Antioxidants 11.
Thirumurugan, D., Alagappan, C., Raja, S., Vijayakumar, R., 2018. An Introductory Chapter: Secondary Metabolites.
Thompson, M., Gamage, D., Hirotsu, N., Martin, A., Seneweera, S., 2017. Front. Physiol. 8, 578.
Théroux-Rancourt, G., Roddy, A.B., Earles, J.M., Gilbert, M.E., Zwieniecki, M.A., Boyce, C.K., Tholen, D., McElrone, A.J., Simonin, K.A., Brodersen, C.R., 2021. Proceedings. Biol. Sci. 288, 20203145.
Torres-Ortiz, D., García-Alcocer, G., Berumen-Segura, L.C., Estévez, M., 2024. Sustain. Chem. Environ. 8, 100157.
Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T., Singh, B.K., 2020. Nat. Rev. Microbiol. 18, 607–621.
Trombetta, D., Castelli, F., Sarpietro, M.G., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G., Bisignano, G., 2005. Antimicrob. Agents Chemother. 49, 2474–2478.
Tsavkelova, E.A., Volynchikova, E.A., Potekhina, N.V., Lavrov, K.V., Avtukh, A.N., 2024. Front. Plant Sci. 15, 1360828.
Tshivhandekano, I., Ngezimana, W., Tshikalange, E., Makunga, N., Mudau, F., 2017. Acta Agric. Scand. Sect. B Soil Plant Sci 68, 1–13.
Twaij, B.M., Hasan, M.N., 2022. Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses. Int. J. Plant Biol. 13, 4.
Tyagi, G., Jangir, D.K., Singh, P., Mehrotra, R., 2010. DNA Cell Biol. 29, 693–699.
Tyapkina, A.P., Silaeva, Z.G., Kiseleva, L.L., Shiryaeva, N.A., Parakhina, E., 2022. BIO Web Conf., 47, 11001.
Upadhyay, R., Saini, R., Shukla, P., Tiwari, K., 2024. Phytochem. Rev. 24, 953–983.
Urquiza-López, A., Bazaldúa, C., Cardoso-Taketa, A.T., Villarreal, M.L., 2025. PeerJ 13, e19967.
Valdés-Jiménez, A., Peña-Varas, C., Borrego-Muñoz, P., Arrue, L., Alegría-Arcos, M., Nour-Eldin, H., Dreyer, I., Nuñez-Vivanco, G., Ramírez, D., 2021. Molecules, 26.
Vallish, B.N., Dang, D., Dang, A., 2022. World J. Methodol. 12, 132–147.
Van Nuland, M., Wooliver, R., Pfennigwerth, A., Read, Q., Ware, I., Mueller, L., Fordyce, J., Schweitzer, J., Bailey, J., 2016. Funct. Ecol. 30.
van Santen, J.A., Kautsar, S.A., Medema, M.H., Linington, R.G., 2021. Nat. Prod. Rep. 38, 264–278.
Vaou, N., Voidarou, C.(., Rozos, G., Saldari, C., Stavropoulou, E., Vrioni, G., Tsakris, A., 2025. Unraveling nature's pharmacy: transforming medicinal plants into modern therapeutic agents. Pharmaceutics 17, 754.
Vasconsuelo, A., Boland, R., 2007. Plant Sci. 172, 861–875.
Veer, V., Gopalakrishnan, R., 2016. Herbal insecticides, repellents and biomedicines: effectiveness and commercialization, herbal insecticides. Repellents and Biomedicines: Effectiveness and Commercialization.
Verma, N., Shukla, S., 2015. J. Appl. Res. Med. Aromat. Plants 2, 105–113.
Vickers, N.J., 2017. Curr. Biol. 27, R713–R715.
Voges, M.J.E.E.E., Bai, Y., Schulze-Lefert, P., Sattely, E.S., 2019. Proc. Natl. Acad. Sci. 116, 12558–12565. U. S. A.
Vorholt, J.A., Vogel, C., Carlström, C.I., Müller, D.B., 2017. Cell Host Microbe 22, 142–155.
Vázquez, A., Tabanca, N., 2025. Development of a combined 2D-MGD TLC/HPTLC method for the separation of Terpinen-4-ol and α-Terpineol from tea tree, Melaleuca alternifolia. Essent. Oil. Biomol. 5, 147.
Wang, J., Li, Jian-li, Li, Jing, Li, Jin-xin, Liu, S., Huang, L., Gao, W., 2017. Chinese Herb. Med 9, 115–125.
Wang, J., Xu, C., Wong, Y.K., Li, Y., Liao, F., Jiang, T., Tu, Y., 2019a. Engineering 5, 32–39.
Wang, M., Zhang, Z., Liu, X., Liu, Z., Liu, R., 2025. Biosynthesis of edible terpenoids: hosts and applications. Foods 14, 673.
Wang, S., Liang, W., Yao, L., Wang, J., Gao, W., 2019b. J. Food Biochem. 43, e12794.
Watanabe, Y., Hinata, K., Qu, L., Kitaoka, S., Watanabe, M., Kitao, M., Koike, T., 2021. Effects of elevated CO2 and nitrogen loading on the defensive traits of three successional deciduous broad-leaved tree seedlings. Forests 12, 939.
Wawrosch, C., Zotchev, S.B., 2021. Appl. Microbiol. Biotechnol. 105, 6649–6668.
Weryszko-Chmielewska, E., Haratym, W., 2012a. Acta Agrobot. 64, 11–22.
Weryszko-Chmielewska, E., Haratym, W., 2012b. Acta Agrobot, 65.
White, P., Joshi, A., Rassam, P., Housden, N.G., Kaminska, R., Goult, J.D., Redfield, C., McCaughey, L.C., Walker, D., Mohammed, S., Kleanthous, C., 2017. Proc. Natl. Acad. Sci. 114, 12051–12056. U. S. A.
Wu, L., Wu, H.-J., Qiao, J., Gao, X., Borriss, R., 2015. Front. Microbiol. 6, 1395.
Wulandari, P., 2021. Eureka Herba Indones, 2, pp. 73–79.
Xi, D.-D., Gao, L., Miao, L.-M., Ge, L.-A., Zhang, D.-Y., Zhang, Z.-H., Li, X.-F., Zhu, Y.-Y., Shen, H.-B., Zhu, H.-F., 2023. Changes in diversity and composition of rhizosphere bacterial and fungal community between resistant and susceptible pakchoi under Plasmodiophora brassicae. Int. J. Mol. Sci. 24, 16779.
Xu, Z., Ullah, N., Duan, Y., Hou, Z., Liu, A., Xu, L., 2023. Editorial: plant secondary metabolites and their effects on environmental adaptation based on functional genomics. Front. Genet. 14, 1211639.
Yi, F., Li, L., Xu, L.-J., Meng, H., Dong, Y.-M., Liu, H.-B., Xiao, P.-G., 2018. Chin. Med. 13, 33.
Yin, Y.-C., Hou, J.-M., Tian, S.-K., Yang, L., Zhang, Z.-X., Li, W.-D., Liu, Y., 2019. Bot. Lett 167, 1–13.
Younkin, G.C., Alani, M.L., Páez-Capador, A., Fischer, H.D., Mirzaei, M., Hastings, A.P., Agrawal, A.A., Jander, G., 2024. New Phytol. 242, 2719–2733.
Yousf, S., Hussain, N., Sharma, S., Chugh, J., 2017. Identification & Characterization of Secondary Metabolites in the Biological Soup by NMR Spectroscopy, pp. 47–96.
Yuan, D., Guo, Y., Pu, F., Yang, C., Xiao, X., Du, H., He, J., Lu, S., 2024. Food Chem. 430, 137115.
Yuan, J., Raza, W., Shen, Q., Huang, Q., 2012. Appl. Environ. Microbiol. 78, 5942–5944.
Zaman, M.A., Oparil, S., Calhoun, D.A., 2002. Nat. Rev. Drug Discov. 1, 621–636.
Zamioudis, C., Hanson, J., Pieterse, C.M.J., 2014. New Phytol. 204, 368–379.
Zamorano Miranda, J., Bartomeus, I., Grez, A.A., Garibaldi, L., 2020. Insect Conserv. Divers.
Zarrouk, E., Lenski, M., Bruno, C., Thibert, V., Contreras, P., Privat, K., Ameline, A., Fabresse, N., 2021. Toxicol. Anal. Clin. 34.
Zaynab, M., Khan, J., Al-Yahyai, R., Sadder, M., Li, S., 2024. Toxicon 250, 108118.
Zhalnina, K., Louie, K.B., Hao, Z., Mansoori, N., da Rocha, U.N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B.P., Firestone, M.K., Northen, T.R., Brodie, E.L., 2018. Nat. Microbiol. 3, 470–480.
Zhang, Q.-W., Lin, L.-G., Ye, W.-C., 2018. Chin. Med. 13, 20.
Zhao, J., Davis, L.C., Verpoorte, R., 2005. Biotechnol. Adv. 23, 283–333.
Zhou, X., Pan, Y., Wang, Y., Wang, B., Yan, Y., Qu, Y., Ke, X., 2021. Sci. Rep. 11, 12217.
Zhou, Y., Guo, Y., Sun, X., Ding, R., Wang, Y., Niu, X., Wang, J., Deng, X., 2020. ACS Omega 5, 11424–11438.
Zhu, J., Song, L., Shen, S., Fu, W., Zhu, Y., Liu, L., 2023. Bioactive alkaloids as secondary metabolites from plant endophytic aspergillus genus. Molecules 28, 7789.
Zielińska-Błajet, M., Feder-Kubis, J., 2020. Int. J. Mol. Sci. 21.