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Triterpenoids in food and medicine homology substances: Structural diversity, bioactivities, biosynthesis, and strategies for efficient production

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ABSTRACT: Food and medicine homology (FMH) substances possess the dual capability of being used as both food and medicine. In recent years, the concept of FMH has been emphasized and developed in unprecedented ways. Triterpenoids, as key bioactive constituents within FMH substances, exhibit remarkable structural diversity and multi-target pharmacological activities, including anti-tumor, anti-inflammatory, antioxidant, hypoglycemic, and immunomodulatory effects. This review systematically summarizes the structural classification, spatiotemporal distribution patterns, and pharmacological mechanisms of FMH triterpenoids, with a particular focus on their dual role in disease prevention and functional foods. Addressing the challenge of low extraction efficiency from plants, synthetic biology approaches have elucidated the biosynthetic pathways regulated by rate-limiting enzymes, including oxidosqualene cyclases, cytochrome P450 monooxygenases, and UDP-glycosyltransferases. Synthetic biology strategies for the heterologous production of these triterpenoids in plant and microbial cell factories are critically evaluated. Despite advances in pathway reconstruction and enzyme engineering, challenges such as low yield, poor catalytic specificity, and scalability for industrial production persist. Emerging technologies, such as artificial intelligence-driven protein design and biosensor-based high-throughput screening, offer transformative solutions for optimizing triterpenoid biosynthesis. This work lays the groundwork for harnessing FMH triterpenoids as sustainable resources for pharmaceuticals and functional foods.

Keywords: Triterpenoids; Food and medicine homology; Pharmacological activities; Synthetic biology; High-throughput screening; Artificial intelligence

1. Introduction

The concept of food and medicine homology (FMH) has its roots in ancient Chinese medical philosophy, which recognizes the intrinsic connection between diet and therapy and highlights the dual role of edible substances in disease prevention and treatment. This item was first systematically recorded in *Huangdi*

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Neijing and further refined through centuries of clinical practice in Traditional Chinese Medicine (TCM) (Xue et al., 2022). In recent decades, the FMH framework has been redefined under modern scientific and regulatory systems, particularly following the official designation of FMH substances by the Chinese National Health Commission (NHC). Globally, the FMH concept aligns with the World Health Organization's promotion of functional foods and preventive nutrition, bridging traditional medicine, modern nutrition, and precision health care. In recent decades, FMH substances have attracted renewed attention due to their safety, mild pharmacological effects, and potential for chronic disease management. By August 2024, the number of officially recognized FMH substances has expanded from the initial 87 to the current 114 types (Wang et al., 2025)(Fig. 1A). Citations of research on FMH substances also show an increasing trend, reflecting a continuous expansion of the research and application scope (Fig. 1B). The principal bioactive constituents of FMH substances include flavonoids, polysaccharides, alkaloids, and terpenoids, among which triterpenoids and their saponins represent particularly noteworthy compounds due to their structural diversity and broad spectrum of bioactivities. Recent investigations indicate that triterpenoids are present in more than one-third of officially recognized FMH species and are widely distributed among over forty taxa spanning families such as Araliaceae, Fabaceae, Lamiaceae, Cucurbitaceae, and Rhamnaceae, as well as in edible fungi such as *Ganoderma lucidum* and *Poria cocos* (Lu et al., 2022). Structurally, triterpenoids share a C_{30} skeleton isoprenoid backbone that undergoes extensive oxidation and glycosylation, giving rise to hundreds of derivatives such as oleanane-, ursane-, dammarane-, and lanostane-type compounds. This extraordinary structural diversity underpins their multi-target pharmacological properties, encompassing anti-tumor, anti-inflammatory, antioxidant, hypoglycemic, hepatoprotective, and immunomodulatory effects. Consequently, FMH triterpenoids serve as pivotal molecular mediators linking the nutritional and therapeutic dimensions of food and medicine (Wang et al., 2022). Given their safety, efficacy, and multifunctionality, considerable research has specifically focused on FMH triterpenoids, particularly concerning their active components, pharmacological activities, and applications, and biosynthesis pathways (Fig. 1C). For example, glycyrrhizic acid and glycyrrhetinic acid from *Glycyrrhiza uralensis*, along with mogrosides from *Siraitia grosvenorii*, are high-potency natural sweeteners with therapeutic potential for diabetes and its complications (Chen et al., 2024; Xiang et al., 2025). Ginsenoside CK and Rh2 from ginseng exhibit anti-cancer activity (Fan et al., 2024). Ganoderic acids from *Ganoderma lucidum* serve as immunomodulatory functional food supplements for metabolic syndrome (Radwan et al., 2015). Jujuboside from *Ziziphi Spinosae Semen* (ZSS) shows promising application prospects in functional foods for sedation and sleep improvement (Ruan et al., 2024).

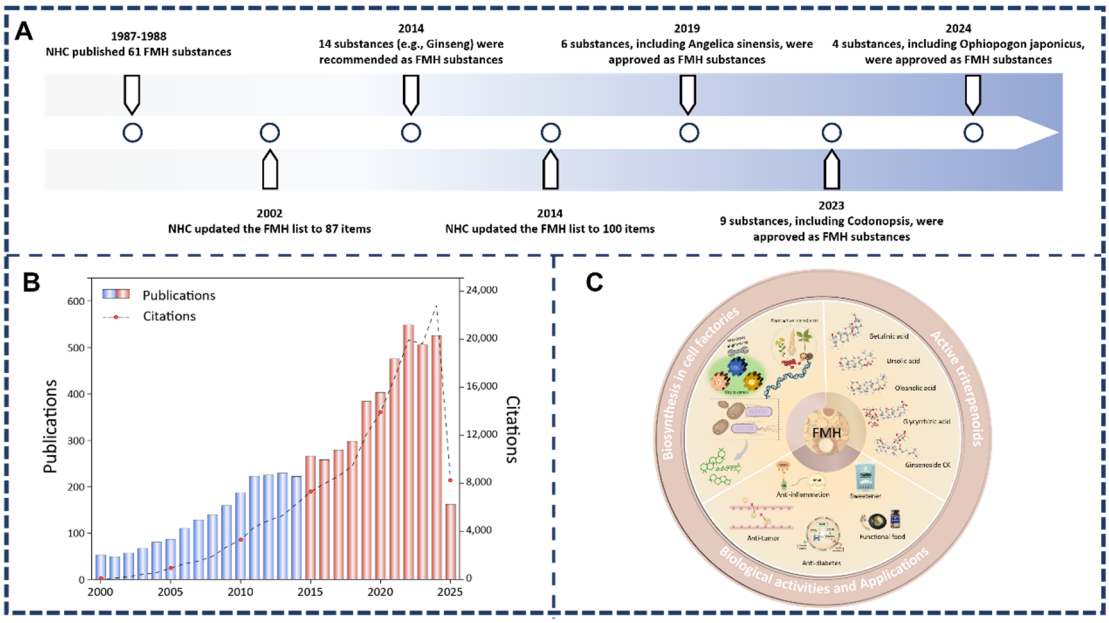

Fig. 1. Analysis framework and research landscape of medicine-food homology (FMH) substances. (A) Regulatory timeline for FMH substance designation by China's National Health Commission (NHC). (B) Publication and citation trends (2000-2025) from Web of Science core collection. (C) Thematic focus distribution: (i) Active triterpenoid discovery, (ii) Bioactivity and applications, (iii) Microbial cell factory biosynthesis.

However, triterpenoids are generally present at extremely low levels in their natural hosts (e.g., saponin content is less than 1% in 50-year-old ginseng roots). Furthermore, conventional extraction processes face challenges such as slow plant growth, high resource consumption, and low efficiency. While chemical synthesis allows for structural modifications, it is hindered by bottlenecks, including complex multi-step procedures, difficulties in chiral control, and environmental pollution, severely limiting its large-scale application. In recent years, breakthrough advances in multi-omics technologies have provided novel perspectives for deciphering the biosynthetic mechanisms of FMH triterpenoids. Genomic and transcriptomic analyses have revealed the evolutionary divergence and functional specificity of key rate-limiting enzymes, including oxidosqualene cyclases (OSCs), cytochrome P450 monooxygenases (CYP450s), and uridine diphosphate-dependent glycosyltransferases (UGTs) (Lu et al., 2023). Concurrently, metabolomics and mass spectrometry imaging (MSI) techniques have precisely mapped the spatiotemporal distribution patterns of triterpenoids within plant organs and even at the cellular micro-region level (Wang et al., 2016). These discoveries not only deepen our understanding of the mechanisms underlying triterpenoid structural diversity but also lay the molecular groundwork for synthetic biology-driven heterologous production. Cell factories utilizing microbial chassis (e.g., *Saccharomyces cerevisiae*, *Escherichia coli*) have successfully synthesized several high-value triterpenoids, such as ginsenoside CK and glycyrrhetinic acid, achieving yields at the gram-per-liter (g/L) level (Sun et al., 2024; Wang et al., 2021). Nevertheless, the heterologous production levels for the majority of FMH triterpenoids remain low, precluding their engineered application. These findings underscore both the promise and the urgent need for strategic enhancements in synthetic biology approaches for triterpenoid production.

This review highlights the structural diversity and spatial-temporal distribution of FMH triterpenoids, analyzing the configurational differences of key compounds. It explores their potential in activities such as

anti-tumor, anti-inflammatory, and hypoglycemic effects, along with food applications, offering theoretical support for the targeted development of these resources. The catalytic mechanisms and engineering strategies for OSC, CYP450, and UGT enzyme systems within FMH substances are systematically summarized. The advancements and challenges in the heterologous synthesis of FMH triterpenoids in microbial and plant systems are critically examined. Finally, emerging cutting-edge technologies, including innovative artificial intelligence (AI)-driven rational enzyme design, directed evolution, and biosensor-guided high-throughput screening, are discussed for improving heterologous production of triterpenoid compounds to increase both yield and catalytic specificity. This review provides a comprehensive framework for advancing FMH triterpenoids from basic research to industrial application, supporting the global scientific effort in preventative treatment.

2. Structure, classification, and sources of FMH triterpenoids

2.1 Structural diversity and classification of FMH triterpenoids

Triterpenoids are composed of six isoprene units, resulting in a core skeleton containing 30 carbon atoms. Structurally, triterpenoids can be classified into acyclic and cyclic types based on the connectivity of their carbon skeletons. Cyclic triterpenoids are further subdivided according to the number of rings in their core structure, including tetracyclic and pentacyclic triterpenoids. Cyclic triterpenoids are abundantly present in FMH substances; 40 have been reported to contain triterpenoids among the 114 recognized FMH substances (Table S1). These compounds exhibit significant structural diversity, stemming from configurational heterogeneity in their pentacyclic or tetracyclic skeletons and dynamic modifications by various substituents (Fig. 2).

Pentacyclic triterpenoids are primarily categorized into four major types, including oleanane, ursane, lupane, and friedelane. Additionally, other skeleton types, such as ceanothane, have been identified. Oleanane and ursane, as core pentacyclic configurations, feature *trans* fusion between the A/B, B/C, and C/D rings, with the D/E ring typically being *cis*. They are isomers of each other, distinguished by methyl group substitutions at C-20 and C-19, respectively. The oleanane-type includes oleanolic acid, glycyrrhetinic acid, maslinic acid, macranthoidin B, and hederagenin, widely distributed in *Glycyrrhiza glabra*, *Lonicera japonica*, *Lonicera macranthoides*, *Platycodon grandiflorus*, and *Prunella vulgaris*. The ursane-type is represented by ursolic acid, primarily found in *Perilla frutescens*, *Crataegus pinnatifida*, *Chaenomeles speciosa*, and *Rubus chingii* Hu. The lupane-type possesses a five-membered E ring with an α-isopropyl group at C19; its A/B, B/C, C/D, and D/E rings are all fused in a *trans* configuration. Lupeol, betulinic acid, betulin, and betulinic acid are the most studied bioactive representatives, mainly discovered in *Taraxacum mongolicum* and *Zanthoxylum bungeanum*. Furthermore, the ceanothane-type, derived from lupane, has garnered significant scientific interest due to its unusual structural features. Key characteristics distinguishing it from the lupane-type include a five-membered A ring and a carboxylic acid substitution on the E ring. This configuration is specific to the Rhamnaceae family (e.g., *Ziziphus jujuba* var. *spinosa* and *Z. jujuba* Mill.). Studies have found that natural derivatives, such as epiceanothic acid, exhibit significant anti-tumor pharmacological activity (Ruan et

al., 2024). Friedelane is derived from oleanane through methyl translocation. To date, friedelane-type compounds like friedelin and friedelanol have only been identified in *Codonopsis pilosula*.

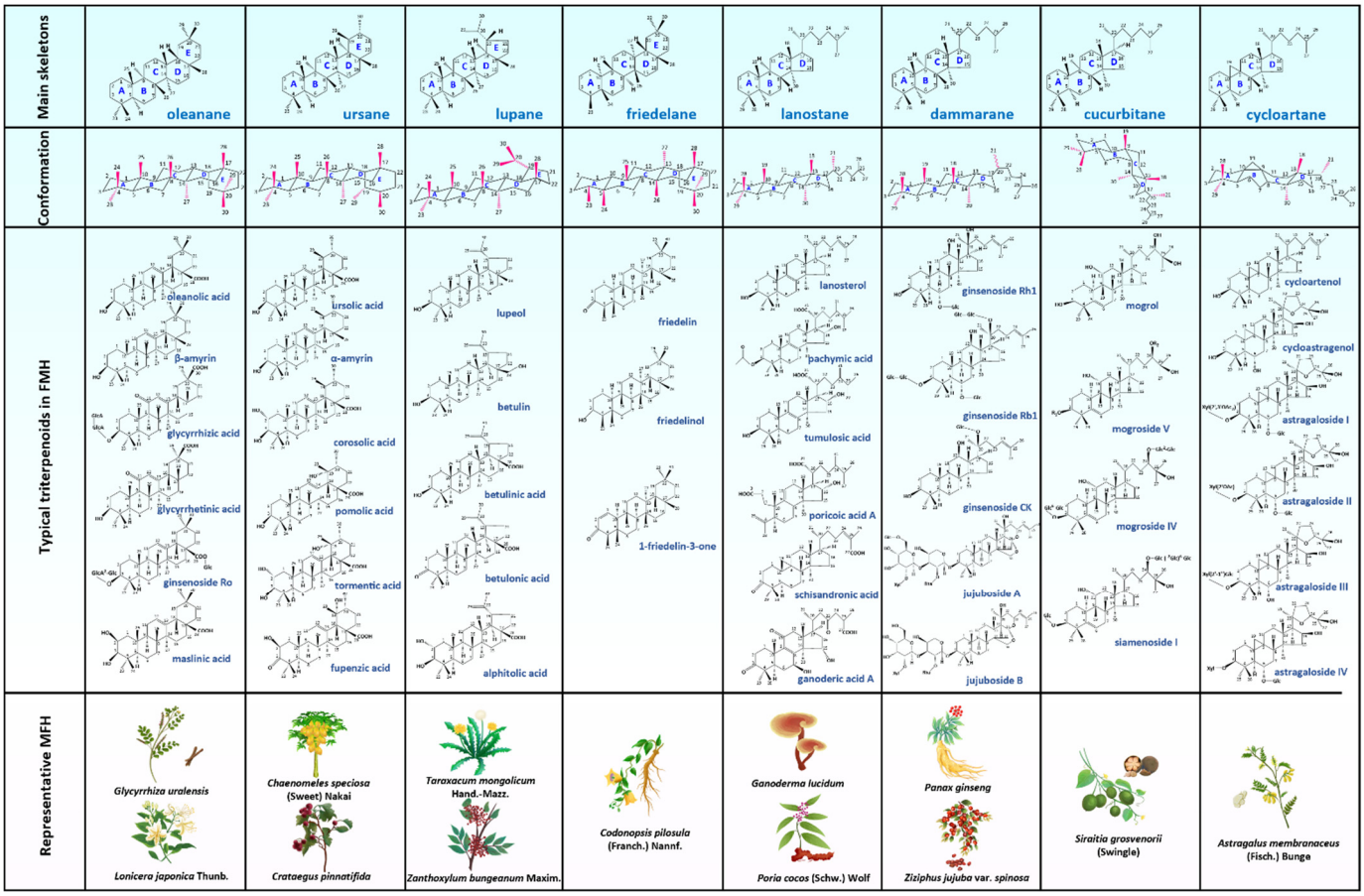

Fig. 2 Main classification and structures of FMH triterpenoids.

Tetracyclic triterpenoids are predominantly found in dicotyledonous plants and edible fungi species. They are mainly classified into lanostane, dammarane, cucurbitane, and cycloartane types. All share a common core structure featuring three six-membered rings and one five-membered D ring, with five angular methyl groups. Four methyl groups are attached at C4 (two), C10, and C14, while the fifth methyl group (C18) is attached at either C8 or C13. A β-side chain is present at C17. The lanostane type features *trans* fusion between the A/B, B/C, and C/D rings. Representative compounds include ganoderic acids, pachymic acid A, and tumulosic acid A, present in edible fungi such as *G. lucidum* and *Poria cocos*. The dammarane type is derived from lanostane via migration of the C-13 methyl group to C-8. It constitutes a major bioactive component in plants of the Araliaceae and Rhamnaceae families. Hundreds of ginsenosides, such as Rb1, Rg3, and the rare ginsenosides Rh1 and CK, have been identified in *Panax ginseng* and *P. quinquefolius*. Compounds like jujuboside A and B, and jujubasaponin I/II/III have been identified in ZSS and jujube fruit. Notably, ocotillol-type ginsenosides retain the dammarane core skeleton but are characterized by a unique oxygen-containing five-membered side-chain ring. First identified in *P. quinquefolius*, they serve as hallmark constituents differentiating this species from *P. ginseng* (Tian et al., 2024). Cucurbitane differs from lanostane by the migration of the C-19 methyl group to C-9 and features *trans* fusion of the A/B and C/D rings, but *cis* fusion of the B/C ring. Mogrosides from *S. grosvenorii* are representative compounds. The cycloartane type

resembles lanostane but is characterized by a cyclopropane ring formed between C9, C10, and C19. This type is exemplified by astragaloside IV, abundantly enriched in *Astragalus membranaceus*.

Besides natural cyclic triterpenoids, highly oxidized complex triterpenoid derivatives known as tetranortriterpenoids have been reported in some FMH substances, primarily within the Rutaceae family. Examples include limonoids in *Citrus medica*, as well as limonin, nomilin, and limonexic acid in orange peel.

2.2 Distribution patterns of FMH triterpenoids

Triterpenoids in FMH plants are primarily distributed in the Araliaceae, Fabaceae, and Rhamnaceae families, and edible fungi. Their structural diversity is closely linked to spatiotemporal distribution patterns, with accumulation exhibiting significant specificity across two dimensions: time (developmental stage, seasonal cycle) and space (geographical environment, organ or tissue). This synergy demonstrates both the ecological adaptability of plants and the scientific foundation for the precise development of medicinal-food resources.

Triterpenoid synthesis and accumulation vary throughout plant growth and development. For instance, dammarane-type triterpenoid content (e.g., ginsenoside Rg1) in *P. ginseng* roots increases markedly during years 4-6 (Szczuka et al., 2019). Similarly, *S. grosvenorii* fruits accumulate predominantly cucurbitane-type mogroside IIE within 30 days post-pollination, while mogroside V achieves maximal sweetness at 85 days (Wu et al., 2022). Fruit triterpenoids in *Z. jujuba* var. *spinosa* and *Z. jujuba* Mill. exhibit biphasic accumulation patterns, characterized by an initial increase followed by a decline, reaching peak concentrations at the white mature stage with 5.3-fold and 2.9-fold higher content than immature fruit, respectively (Wen et al., 2022). Genotype and phenological stage significantly influence the dynamic accumulation of total terpenes in raspberry, highlighting the importance of genotype-environment interactions in the metabolic regulation of FMH plants.

The spatial distribution of triterpenoids encompasses three levels: geographical environment, organ function, and tissue micro-regions. Natural factors such as climate, light, soil, and altitude contribute to distribution differences. The glycyrrhizic acid content in *Glycyrrhiza* spp. from the genuine producing region in Inner Mongolia is higher than in other regions, potentially related to stress responses driven by the local arid climate and alkaline soil (Zhang et al., 2021). Zhu et al. (2022) found that the quality of mountain forest-cultivated ginseng is closely associated with topographic, climatic, and soil factors, with warm, relatively humid, and shady environments favoring ginsenoside accumulation. FMH triterpenoids accumulate locally in specific plant organs, such as roots, leaves, flowers, fruits, seeds, fruiting bodies, and sclerotia, highly consistent with traditional medicinal parts (Fig. 3). Dammarane-type ginsenosides and cycloartane-type astragaloside IV are enriched in roots; cucurbitane-type mogrosides are distributed in the pulp of *S. grosvenorii*, while lanostane-type ganoderic acids specifically accumulate in the fruiting bodies of *G. lucidum*. Notably, non-traditional medicinal parts also hold development potential. For instance, 13 types of malonyl saponins undetected in roots were identified in ginseng leaves and flowers (Piao et al., 2020); the oleanolic acid content in the fruit shell of *Alpinia oxyphylla* is 10 times higher than in its seeds (Xiang et al.,

2025); and saponins present in the roots of *P. grandiflorus* also exist at certain concentrations in stems and leaves (Zhang et al., 2022). This provides a molecular basis for utilizing waste resources and expands the source of raw materials for functional food development.

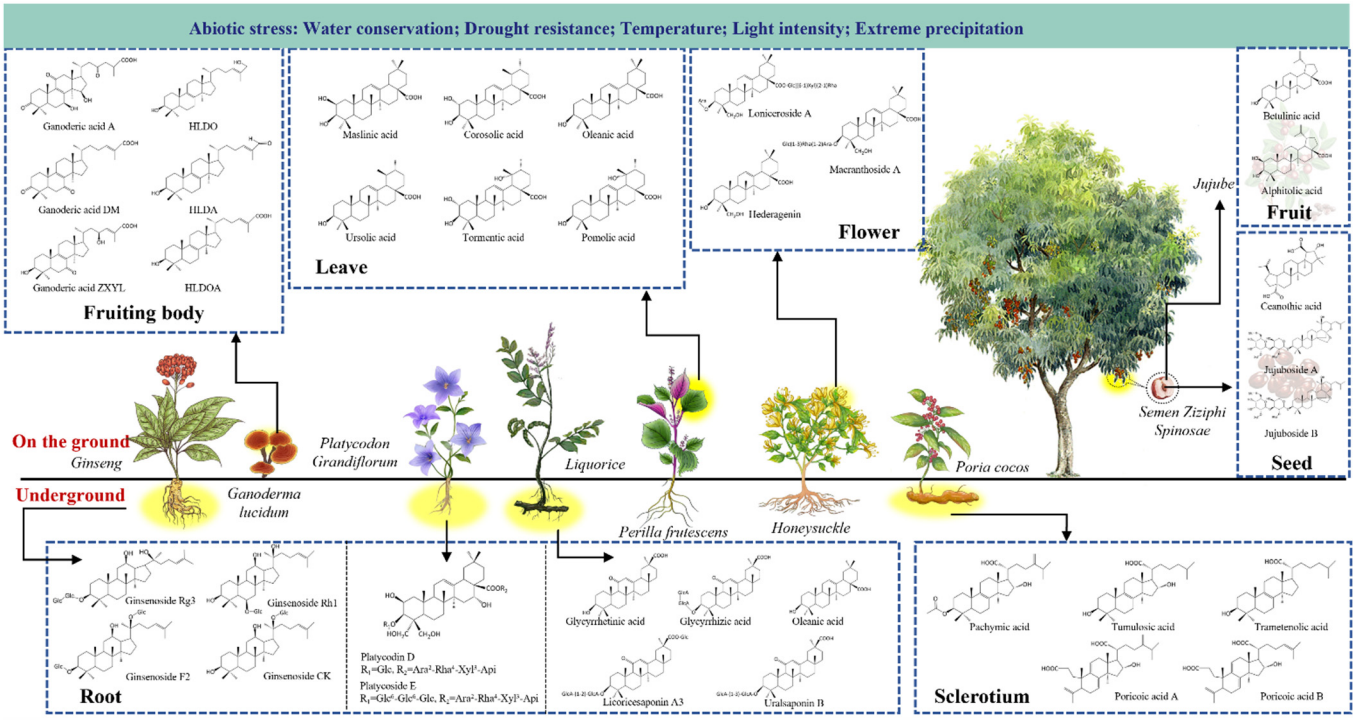

Fig. 3. Organ-specific accumulation of characteristic triterpenoids in FMH plants.

Recently, breakthroughs in omics and MSI technologies have enabled the visualization of triterpenoid spatial distribution at the tissue and even cell cluster level. Li et al. (2014) applied high spatial resolution AP-MALDI MSI to analyze cross-sections of *G. glabra* rhizomes, revealing that saponins like glycyrrhizic acid and licoricesaponin A3 are primarily distributed in the cortex, phloem fibers, and xylem fibers. Xia et al. (2024) combined non-targeted metabolomics with MSI to study the chemical and spatial information of *G. lucidum* at different growth stages and in different tissues. Metabolomics identified 142 metabolites, while MSI revealed the spatial distribution information of ganoderic acids in the fruiting body. Wang et al. (2016) utilized MSI for rapid localization of saponins in *P. ginseng* and *P. quinquefolius*, finding a preference for saponin distribution in the cork cambium of the roots. MALDI-MSI technology has been applied to study the tissue distribution of triterpenoids in various FMH materials such as ginseng, licorice, *G. lucidum*, and *P. grandiflorum*, enabling precise localization and tracking of the spatial distribution of triterpenoid components, especially trace components. This provides crucial scientific evidence for the continuous elucidation of their biosynthetic pathways and regulatory mechanisms

3. Pharmacological activities and food application potential of FMH triterpenoids

The structural diversity of FMH triterpenoids (e.g., differences in carbon skeletons and functional group modifications such as hydroxylation and glycosylation) directly determines their diverse biological activities and pharmacological properties, including anti-inflammatory, immunomodulatory, blood glucose regulation, and sleep improvement effects. This aligns with the healthcare concept of “preventative treatment”. The

inherent structure-function relationship endows them with significant potential in dietary supplementation and disease prevention, making them a focal point in natural product research. In recent years, the continuous discovery of new pharmacological actions and the in-depth elucidation of molecular mechanisms have further expanded the application value of FMH triterpenoids (Fig. 4 and Table 1).

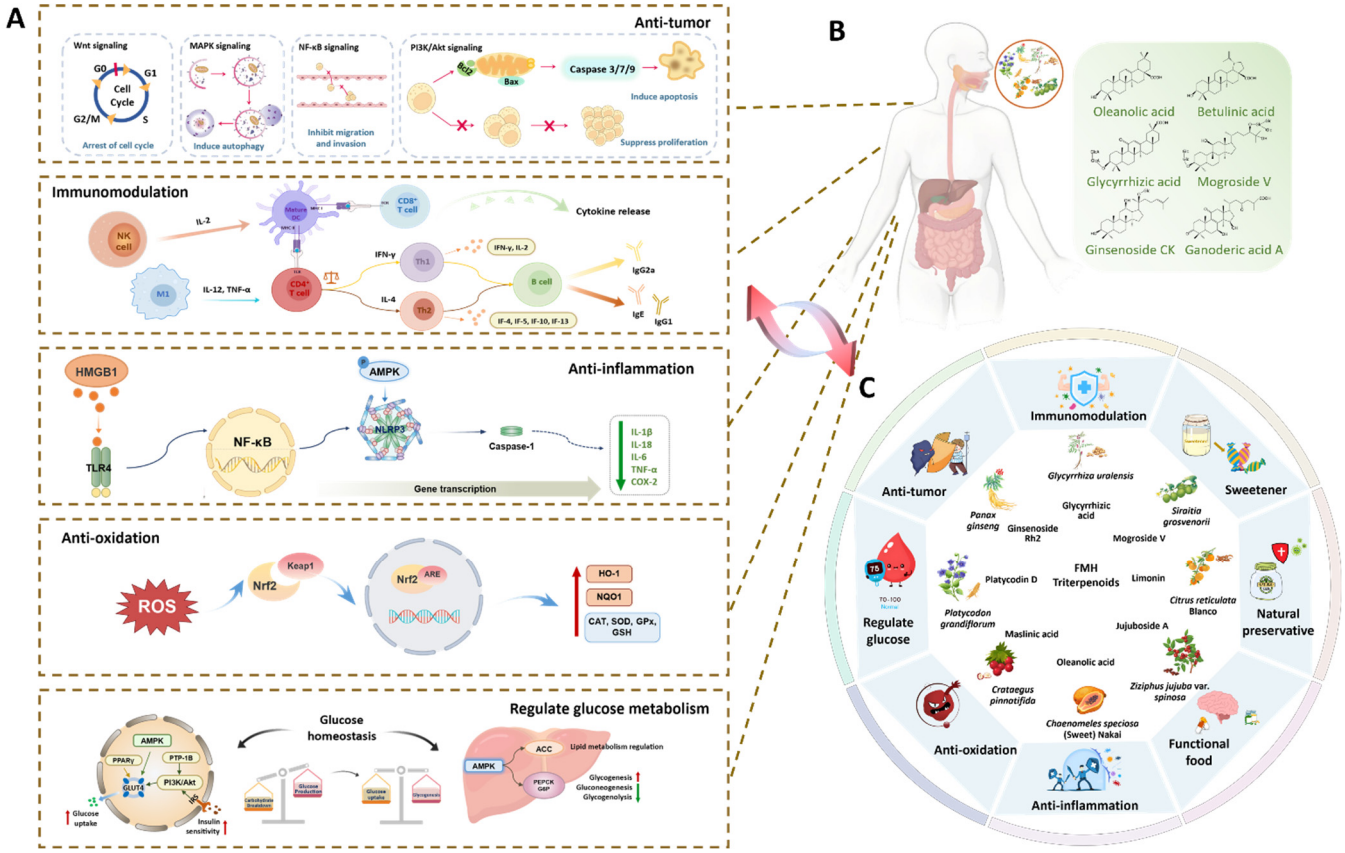

Fig. 4. Bioactivities and applications of FMH triterpenoids. (A) Key pharmacological mechanisms of triterpenoid compounds. (B) Representative FMH-derived triterpenoids. (C) Applications of FMH-derived triterpenoids in pharmacology and nutraceuticals.

Table 1 Representative FMH triterpenes and their pharmacological effects

No	Representative triterpenes	Source	Pharmacological effect	Reference
1	Glycyrrhizic acid	*Glycyrrhiza uralensis*	Anti-inflammatory, immunomodulatory, antidiabetic, antiviral	(Thakur et al., 2020)
2	18β-Glycyrrhetinic acid	*Glycyrrhiza uralensis*	Anti-inflammatory, immunomodulatory	(Shi et al., 2020)
3	Betulinic acid	*Prunella vulgaris*	Antitumor, anti-inflammatory, antidiabetic, antiviral	(Luis Rios & Manez, 2018)
4	Maslinic acid	*Crataegus pinnatifida*	Antitumor, antibacterial, antioxidant	(Liu et al., 2020)
5	Ganoderic acid A	*Ganoderma lucidum*	Antitumor, kidney protection, immunomodulatory	(Das et al., 2020)
6	Poricoic acid GM	*Poria cocos*	Anti-inflammatory, antioxidant, antidiabetic	(Bao et al., 2022)
7	pachymic acid	*Poria cocos*	Antitumor, anti-inflammatory	(Wen et al., 2018)
8	Astragaloside IV	*Astragalus membranaceus*	Anti-inflammatory, immunomodulatory, antioxidant, antiviral, antitumor	(Graziani et al., 2019; Huang et al., 2024)
9	Mogroside V	*Siraitia grosvenorii*	Antidiabetic, antioxidant	(Chen et al., 2011)
10	Platycodin D	*Platycodon grandiflorus*	Antidiabetic, cardiovascular protection	(Wang et al., 2024)
11	Jujuboside A/B	*Ziziphus jujuba* Mill.	Neuroprotective, sedative, and calming	(Ruan et al., 2024)
12	Limonin	*Citrus medica*	Antibacterial, antioxidant	(Liu et al., 2021)
13	Ginsenoside Rg3/Rh2/CK	*Panax ginseng*	Antitumor, immunomodulatory, anti-fatigue	(Zhang et al., 2022)

14	Ceanothic acid	*Ziziphus jujuba* var. *spinosa*	Antitumor	(Guo et al., 2011)
15	Pseudo-ginsenoside F11	*Panax quinquefolium*	Central system protection, cardioprotective effect, anti-tumor	(Tian et al., 2024)
16	Lupeol	*Taraxacum mongolicum*	Differentiation-inducing on melanoma cells	(Hata et al., 2000)
17	Oleanolic acid	*Rubus chingii* Hu	Anti-viral, anti-inflammatory, anti-bacterial, hepatoprotective, antidiabetic	(Castellano et al., 2022)
18	Ursolic acid	*Rubus chingii* Hu	Antitumor, antioxidant	(Hussain et al., 2017; Sun et al., 2020)
19	Taraxasterol	*Taraxacum mongolicum*	Antiallergic, antioxidant, anti-inflammatory, antitumor	(Schuetz et al., 2006)

3.1 Biological activities and therapeutic potential of FMH triterpenoids

3.1.1 Anti-tumor activity

Anti-tumor activity is a major biological effect of FMH triterpenoids. These compounds act through multiple pathways, including inducing tumor cell apoptosis, arresting the cell cycle, inhibiting invasion and metastasis, and suppressing angiogenesis. Their mechanisms involve modulating signaling pathways such as PI3K/Akt, Wnt, NF-κB, and MAPK. Well-studied pentacyclic triterpenoids with documented clinical anti-tumor activity include oleanolic acid, ursolic acid, and betulinic acid. Oleanolic acid dose-dependently inhibits viability and proliferation while promoting apoptosis and inducing G0/G1 phase arrest in prostate cancer cells (Li et al., 2016). Ursolic acid hinders the malignant phenotype of colorectal cancer and disrupts the cell cycle by attenuating Wnt/β-catenin signaling (Zhao et al., 2023). Betulinic acid induces apoptosis or autophagy via mechanisms like activating the mitochondrial apoptotic pathway and inhibiting NF-κB (Kumar et al., 2018). Maslinic acid inhibits MAPK/ERK signaling pathway proteins, hindering migration and invasion (Liu et al., 2020). Glycyrrhizic acid and glycyrrhetinic acid target and inhibit NF-κB and HMGB1, blocking inflammation-driven tumor progression (Tang et al., 2015).

Fungal-derived triterpenoids exhibit broad-spectrum anti-tumor potential. Ganoderic acid A and ganoderic acid DM promote apoptosis and inhibit the proliferation of anaplastic meningioma cells by inhibiting N-myc downstream-regulated genes (e.g., Bax, MMP-9), downregulating Wnt-2 protein expression, and suppressing the activation of the PI3K/AKT/mTOR pathway (Cheng & Xie, 2019; Das et al., 2020). *Poria* triterpenoids, such as pachymic acid, induce apoptosis in osteosarcoma cells by upregulating PTEN expression, reducing AKT phosphorylation, and activating Caspase 3/7 (Wen et al., 2018). Furthermore, ginsenosides Rg3, Rh2, and CK also possess potent anti-tumor activity (Zhang et al., 2022). Characteristic Rhamnaceae configuration compounds, such as ceanothic acid derived from sour jujube fruit, also demonstrate significant anti-tumor activity (Guo et al., 2011). These findings highlight the potential of FMH triterpenoids as multi-target anti-tumor lead compounds. Beyond mechanism-specific examples, recent perspective-style studies articulate a research roadmap for translating FMH triterpenoids into oncology. Li et al. (2025a) clarify the theoretical rationale for applying FMH principles to cancer and argue that building a rigorous evidence base is imperative; they explicitly call for coordinated use of mass-spectrometry-based analytics, network pharmacology, AI-driven modeling, and, where appropriate, multi-omics profiling to dissect multi-target actions and guide study design. Complementarily, they discuss FMH triterpenoids such as

ginsenosides and glycyrrhizic acid as multi-target candidates for cancer prevention and adjuvant therapy, emphasizing standardized quality control, pathway-level mechanistic validation, and the need for rigorous clinical evaluation (Li et al. 2025b). These position papers outline a concrete agenda encompassing the methodological approaches to employ, the types of evidence to generate, and the translational checkpoints required to move from traditional use to scientifically grounded applications in oncology. Further investigation into their molecular mechanisms, safety profiles, and optimized delivery strategies will pave the way for their development into novel anticancer agents.

3.1.2 Anti-inflammatory activity

Inflammation is a defense mechanism against injury or infection; however, excessive or persistent inflammation can trigger chronic diseases (colitis, atherosclerosis, Alzheimer's disease, etc.), leading to irreversible tissue damage. Triterpenoids from FMH plants exert anti-inflammatory effects through multi-target regulation, including suppressing the release of inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β), blocking the activation of the NF-κB signaling pathway, and activating AMPK.

Glycyrrhizic acid significantly reduces NLRP3 inflammasome activity and CXCR4 expression in diabetic neuropathy models by targeting the HMGB1-TLR4 signaling axis, improving pain thresholds (Thakur et al., 2020). Its derivative, 18β-glycyrrhetinic acid, alleviates liver inflammation damage in viral hepatitis by inhibiting HMGB1-mediated secretion of IL-17 and IL-22 (Shi et al., 2020). Furthermore, oleanolic acid, a classic AMPK activator, regulates inflammatory responses in hepatocytes by phosphorylating the AMPKα subunit and is clinically used for hepatitis treatment (Liu et al., 2021). Betulinic acid reduces lipopolysaccharide (LPS)-induced inflammation by downregulating the expression of NF-κB and MAPK/JNK signaling molecules, thereby inhibiting atherosclerotic plaque formation (Zhang et al., 2021). Poricoic acid GM blocks NF-κB nuclear translocation by inhibiting IκBα phosphorylation, reducing COX-2-mediated PGE2 generation (Bao et al., 2022).

Structure-activity relationship (SAR) studies indicate that the triterpenoid skeleton and its substituents are crucial for anti-inflammatory efficacy. Specific structural features in lanostane-type triterpenoids (e.g., ganoderic acids), such as a double bond at C-24 and a methyl group at C-4, may confer potent anti-inflammatory capabilities (Yang et al., 2020). Glycosylation modifications, as seen in ginsenosides Rh1 and Rg2, inhibit inflammatory signaling by promoting mitophagy and suppressing NLRP3 inflammasome activity (Wang et al., 2021). Astragaloside IV inhibits the progression of osteoarthritis by suppressing NOD-like receptor protein inflammasome and Caspase-1 protein expression, thereby inhibiting IL-1β and TNF-α production. Its activity depends on glycosylation modifications at the C-3 and C-6 positions (Huang et al., 2024). SAR studies summarizing the anti-inflammatory effects of glycyrrhizic acid and its derivatives reveal that the 18α-epimer is superior to the 18β-epimer, and the number of glucuronic acid groups at the C-3 position influences anti-inflammatory activity(Vergoten & Bailly, 2020). These findings provide a theoretical basis for developing functional foods and drugs targeting chronic inflammatory diseases.

3.1.3 Antioxidant activity

Excessive accumulation of reactive oxygen species (ROS) induced by oxidative stress is closely associated with biological aging and various pathological processes, including neurodegenerative diseases, diabetes, and cancer. Triterpenoids from FMH substances exhibit significant antioxidant activity by directly scavenging free radicals or activating endogenous antioxidant systems. This effect is likely mediated through activation of the Nrf2-HO-1/NQO-1 signaling pathway. For instance, triterpenic acids (such as maslinic acid, ursolic acid, and oleanolic acid) isolated from jujube fruit show a positive correlation with their antioxidant capacity, suggesting their potential value as natural antioxidants (Sakna et al., 2023). Ursolic acid from raspberry can activate endogenous antioxidant enzymes and scavenge DPPH free radicals, exhibiting even higher binding affinity to SOD than the positive control quercetin (Ali et al., 2007). It also alleviates neurodegeneration in isolated rat brain tissue by inhibiting $FeSO_4$-induced oxidative damage (Salau et al., 2021). Betulinic acid from ZSS reduces blood pressure and enhances endothelial function in hypertensive rats by inhibiting ROS, increasing NO/SOD/eNOS activity, and improving vasodilation (Fu et al., 2011). *Poria* triterpenoids can control inflammatory factors and regulate inflammatory pathways, indirectly mitigating oxidative stress responses. Poricoic acid GM protects against peroxidative damage by promoting the dissociation of Nrf2 from Keap1, activating Nrf2 nuclear translocation, and upregulating the expression of antioxidant genes such as heme oxygenase-1 (HO-1) (Bao et al., 2022). Astragaloside IV ameliorates oxidative stress by activating the Nrf2/HO-1 pathway and enhances autophagy levels in an H1N1 infection model to reduce IL-1β secretion (Yang et al., 2020; Zhang et al., 2020). Furthermore, hydroxyl or carboxyl groups at the C-28 position of ursane-type triterpenoids derived from sour jujube may enhance free radical scavenging capacity through an electron-donating effect, indicating that their antioxidant activity is closely linked to structural modifications (Qiao et al., 2014). In fact, triterpenoids can participate in improving and treating various chronic metabolic diseases through their antioxidant actions. This aligns with the "preventative treatment" of FMH substances, holds significant importance for regulating chronic diseases, and provides a scientific basis for functional food development and disease treatment strategies.

3.1.4 Hypoglycemic activity

FMH triterpenoids regulate glucose metabolic homeostasis through multiple targets, improving insulin resistance and promoting glucose uptake, making them promising therapeutic agents for managing diabetes and its complications.

Mogroside sweeteners have been identified as potent AMPK activators in HepG2 cell lines. AMPK plays a crucial role in maintaining energy homeostasis and preventing metabolic diseases, including diabetes (Chen et al., 2011). Poricoic acid significantly enhances the expression and membrane translocation efficiency of the glucose transporter GLUT4 in 3T3-L1 adipocytes by activating the AMPK signaling pathway. It concurrently increases the phosphorylation levels of insulin receptor substrate-1 (IRS-1) and Akt, thereby boosting cellular glucose uptake capacity (Huang et al., 2010). Platycodin D effectively inhibits type 2 diabetes induced by cardiac injury in mice and enhances energy metabolism in HG-PA-stimulated H9c2 cells by activating the AMPK signaling pathway (Wang et al., 2024). Oleanolic acid enhances insulin signaling by inhibiting protein

tyrosine phosphatase 1B (PTP1B) activity, thereby blocking its dephosphorylation of the insulin receptor (IR) and IRS-1. Experimental studies show that oral gavage of oleanolic acid (25 mg/kg) for 10 weeks significantly reduces serum insulin levels in insulin-resistant model rats and ameliorates glucose metabolic disorders by upregulating key genes in the IRS-1/PI3K/Akt pathway (Li et al., 2014). Betulinic acid regulates glucose and lipid metabolism through a dual mechanism. On one hand, it acts as a PPARγ antagonist, inhibiting adipogenesis and promoting osteogenic differentiation to improve diabetes-related metabolic imbalances. On the other hand, by activating the AMPK pathway, it downregulates the expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, while upregulating GLUT-1/GLUT-2 expression to promote glucose uptake and glycogen synthesis in peripheral tissues (Luis Rios et al., 2018). Glycyrrhizic acid improves glucose tolerance in type 2 diabetes models by modulating lipid metabolism and insulin secretion (Tan et al., 2022). Additionally, triterpenoid constituents (such as ursolic acid and oleanonic acid) in jujube extracts significantly increase glucose consumption in L6 myotubes by inducing GLUT translocation (Kawabata et al., 2017). These findings position FMH triterpenoids as promising candidates for novel anti-diabetic therapies targeting diabetes and its complications

3.1.5 Immunomodulatory activity

FMH triterpenoids achieve dynamic equilibrium of immune homeostasis by regulating immune cell function and the inflammatory cytokine network through multiple targets. These cytokines regulate both immune responses and participate in inflammatory reactions, representing an intersection for exerting anti-inflammatory and immunomodulatory effects. Notably, TNF-α can directly kill tumor cells and is an important anti-tumor active factor. This demonstrates that the immunostimulatory effects of FMH triterpenoids are inextricably linked to their anti-inflammatory and anti-tumor activities.

Glycyrrhizic acid exhibits T-cell activation regulatory effects, interferon (IFN) modulation, Natural killer (NK) cell activation, and enhancement of extrathymic T lymphocyte differentiation. It activates the expression of CD40, CD80, CD83, and CD86 on dendritic cells (DCs), promotes DC maturation and antigen-presenting capacity, and stimulates processed DCs to secrete IL-12, thereby strongly inducing T-cell proliferation and differentiation (Bordbar et al., 2012). Regarding humoral immunity, glycyrrhizic acid significantly elevates serum IgA, IgG, and IgM levels while suppressing B-cells producing allergen-specific IgE and IgG1 (Han et al., 2017). Lanostane triterpenoids of *P. cocos* enhance innate cellular immunity by activating NK cells and promoting IFN-γ secretion by T helper type 1 (Th1) cells, concurrently reducing the secretion of IL-4 and IL-5 by T helper type 2 (Th2) cells (Chao et al., 2021). Ganoderic acid A selectively induces lymphoma cell apoptosis while enhancing antitumor immunity through attenuating myeloid suppression and improving antigen presentation with T-cell recognition, thereby delaying lymphoma formation (Radwan et al., 2015). Total saponins from ZSS regulate macrophage polarization (M1 to M2) by antagonizing LPS-induced TNF-α release and its receptor binding (Fu et al., 2016).

In summary, FMH triterpenoid components act by promoting antigen-presenting cell function, the mononuclear phagocyte system, humoral and cellular immunity, and modulating the inflammatory cytokine

network. This provides natural molecular tools for the prevention and treatment of immune-related diseases, such as autoimmune disorders and chronic infections

3.1.6 Other activities

Beyond the aforementioned activities, triterpenoids also possess diverse pharmacological potentials, including antibacterial, antiviral, renoprotective, neuroprotective, and metabolic regulatory effects.

Maslinic acid exhibits dose-dependent inhibitory activity against pathogenic fungi such as *Aspergillus flavus* and *A. niger*. The mechanism may involve disrupting fungal cell membrane integrity and interfering with oxidative stress defense (Jamkhande et al., 2016). Licoricesaponin A3 displays inhibitory activity against the cellular entry of the SARS-CoV-2 ancestral strain *in vitro* (Yi et al., 2022). Ganoderic acid A, an active triterpenoid from *Ganoderma*, retards renal cyst growth and impedes fibrosis in polycystic kidney disease by suppressing TGF-β/Smad and MAPK signaling (Meng et al., 2020). Jujubosides A/B exert sedative, tranquilizing, and neuroprotective functions by modulating the GABA receptor signaling pathway, ameliorating oxidative stress-induced neuronal apoptosis (Ruan et al., 2024).

Despite the extensive validation of triterpenoid pharmacological diversity *in vitro* and in animal models, clinical translation remains challenging. Currently, only a few compounds, such as glycyrrhizic acid and ginsenosides, have advanced to clinical trial stages. The mechanisms of action for most triterpenoids are still largely confined to basic research, meaning the majority of phytogenic drug candidates remain in the developmental phase. This outcome is disappointing. Future efforts should systematically advance the pharmacokinetics, safety evaluation, and multicenter clinical trials of triterpenoids to fully unlock their value as FMH agents.

3.2 Role of FMH triterpenoids as functional food ingredients

Owing to their diverse biological activities, FMH triterpenoids play key roles in functional food development, offering integrated functions such as preservation, flavor enhancement, nutritional fortification, and health promotion.

As natural preservatives, triterpenoids extend food shelf life by inhibiting the proliferation of foodborne pathogens and scavenging free radicals to delay lipid oxidation. Ursolic acid and oleanolic acid exhibit particularly significant efficacy in controlling microbial contamination (Spaggiari et al., 2024). Liquid beverages formulated with *G. lucidum* extracts demonstrate potent inhibitory effects against spoilage bacteria such as *Bacillus spizizenii* and *Staphylococcus epidermidis* (Sharma et al., 2019). Limonoids, leveraging their antibacterial and antioxidant activities, offer a natural alternative to chemical preservatives like sodium benzoate (Liu et al., 2021). As bioactive natural sweeteners, glycyrrhizic acid and its derivative glycyrrhetinic acid, along with mogrosides (possessing sweetness intensities 50, 250, and 300 times that of sucrose, respectively), replace synthetic additives. Their zero-calorie properties make them ideal choices for diabetes-friendly beverages and healthy snacks (Suri et al., 2020).

Beyond food processing applications, triterpenoids from specific sources confer health-promoting value to foods. They enhance the prebiotic value of foods by modulating gut microbiota composition (e.g.,

promoting *Lactobacillus* proliferation) and strengthening intestinal barrier integrity. *Ganoderma* triterpenoids endow functional foods with adjunctive therapeutic potential for metabolic syndrome through their anti-inflammatory and immunomodulatory activities. Oleanolic acid, ursolic acid, and lupeol show the highest potential for alleviating food allergy reactions (Hu et al., 2024). Saponins from ZSS demonstrate promising application prospects in functional foods for sedation and sleep improvement (Ruan et al., 2024).

However, inherent challenges such as triterpenoid instability, poor water solubility, and low oral bioavailability often prevent them from reaching their intended bioactive levels *in vivo*. Their performance in food systems is determined not only by their intrinsic bioactivity but also by their physicochemical interactions with other food components. Due to their amphiphilic nature, comprising a hydrophobic triterpene skeleton and hydrophilic sugar chains, triterpenoid saponins can interact with macromolecules such as proteins, lipids, and polysaccharides during food processing, thereby influencing the structural stability and nutritional bioaccessibility of food systems. For instance, ginsenoside Rg1 can form noncovalent complexes with whey proteins, and plasma pretreatment enhances this binding strength, leading to improved rheological behavior and oxidative stability of emulsions (Chen et al., 2023). Meanwhile, glycyrrhizic acid, as a surface-active triterpenoid saponin, can cooperatively stabilize edible emulsions or gels when combined with soy protein isolate–pectin composite nanoparticles, showing controllable interactions and potential for active compound delivery (Li et al., 2020; Taarji et al., 2021). During thermal processing, natural triterpenoid sweeteners such as mogrosides can suppress protein and lipid oxidation, thereby enhancing antioxidant capacity and product stability (He et al., 2022). Beyond protein-based systems, composite nanoparticles based on zein (a corn-derived prolamin) have been shown to significantly improve the aqueous dispersibility and sustained-release behavior of triterpenoids such as betulinic acid, supporting their feasibility as food-grade delivery vehicles (Peng et al., 2022). Similarly, cyclodextrin inclusion complexes can increase the solubility and thermal stability of oleanolic and ursolic acids, enhancing their compatibility with food processing and fortification systems(Huang et al., 2017). Collectively, these studies underscore that elucidating the physicochemical interactions between triterpenoids and food matrix components, particularly proteins, polysaccharides, and hydrophobic carriers, is fundamental to improving their stability, sensory performance, and health-promoting efficacy in functional foods.

Future advancements utilizing delivery technologies like microencapsulation and nanoemulsification offer solutions to optimize the stability of functional triterpenoids within food matrices and enhance their bioavailability and bioactivity. Examples include *Coix* seed oil loaded with *Ganoderma* triterpenoids (Qu et al., 2014); lactoferrin-ginsenoside Rg3 nanoemulsions (Yan et al., 2021); polyvinyl alcohol/ethyl acrylate-grafted lignin polymer nanocarriers loaded with betulinic acid (Zhang et al., 2021); and zein-alginate nanoencapsulated limonin (Bautista et al., 2019). In parallel with formulation advances, Zhang et al. (2025) propose a precision-application framework that links triterpenoid chemistry with targetable network signatures and biomimetic delivery design, offering an operational blueprint to align carrier selection, dosing, and personalization with molecular readouts. Although conceptual, this framework is directly actionable for

functional-food contexts. It motivates using network-informed criteria (e.g., inflammatory and metabolic signatures) to choose emulsions, protein–polysaccharide complexes, or lipid systems that enhance stability and bioavailability while matching intended physiological endpoints. This integrated "bioactivity-delivery optimization-health enhancement" strategy significantly contributes to maximizing the therapeutic efficiency of FMH triterpenoids and advancing food valorization and industrialization.

4. Synthetic biology-driven production of FMH triterpenoids

As previously mentioned, FMH triterpenoids possess broad biological activities and are thus considered natural dietary supplements and food additives with significant market potential. However, the content of highly active components in FMH plants is extremely low (e.g., bioactive saponins constitute less than 1% in 50-year-old ginseng roots). Furthermore, traditional chemical synthesis faces bottlenecks such as complex multi-step procedures, low yields, and environmental pollution, limiting their widespread application. Several physicochemical and enzymatic processes have been developed to enhance the conversion and utilization efficiency of FMH triterpenoids. Physicochemical methods, including hydrothermal, high-temperature steaming, and subcritical-water treatments, can hydrolyze or structurally triterpenoid saponins into more bioavailable aglycones. For instance, ginsenosides can be transformed into less-polar, more bioavailable derivatives such as 20(S)-Rg3, Rk1, and Rg5, within minutes at ≥140°C. These processes often outperform conventional steaming in both speed and yield (Ryu et al., 2017; Ryu et al., 2018). For licorice triterpenoids, steam-explosion processing induces auto-hydrolysis of glycyrrhizic acid to glycyrrhetinic acid 3-O-mono-β-D-glucuronide (GAMG) and 18β-glycyrrhetinic acid, increasing the formation of these more lipophilic products; high-temperature honey-roasting likewise promotes glycyrrhizic acid deglycosylation to18β-glycyrrhetinic acid (Sui et al., 2020; Wu et al., 2024). In addition, enzymatic modification provides a milder and more selective route. β-Glucuronidase preparations have been shown to convert glycyrrhizic acid to glycyrrhetinic acid efficiently in batch or continuous processes; microbial β-glucuronidases can also generate GAMG with high specificity, illustrating controllable routes to tailor solubility and activity (Xu et al., 2018). Lipase-assisted esterification affords triterpenoid fatty-acid esters (e.g., 3-O-acyl betulinic-acid derivatives) under mild conditions using immobilized Candida antarctica lipase; related work demonstrates lipase-catalyzed ester formation for triterpene alcohols as well (Moghaddam et al., 2010). Although these physicochemical and enzymatic processes offer high efficiency and operational flexibility, they are still constrained by substrate specificity, energy input, and product selectivity. To overcome these limitations, microbial or plant-based biosynthesis has been proposed as a sustainable and highly selective alternative. Synthetic biology offers a revolutionary strategy for the efficient and green production of triterpenoids by reconstructing natural biosynthetic pathways. Through screening and optimizing microbial chassis, directionally regulating metabolic flux in the mevalonate (MVA) or methylerythritol phosphate (MEP) pathways, and utilizing gene editing technologies to block competing branches, targeted accumulation of specific triterpenoids can be achieved. Concurrently, the discovery and engineering of key enzymes further enhance triterpenoid structural diversity and bioactivity. This approach overcomes the resource limitations

and high costs of plant extraction while enabling large-scale production of high-purity FMH triterpenoids, advancing their application in pharmaceuticals, functional foods, and other fields.

4.1 Biosynthetic pathways of FMH triterpenoids

The biosynthesis of FMH triterpenoids follows the classical pathway of plant secondary metabolism, with the MVA pathway and MEP pathway serving as the core precursor supply routes, generating isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP). Although IPP biosynthesis pathways independent of MVA exist, the synthesis of many biologically important isoprenoids still relies predominantly on the MVA pathway. The MVA pathway plays a dominant role in the biosynthesis of triterpenoid saponins. Through the sequential action of farnesyl pyrophosphate synthase (FPS), squalene synthase (SS), and squalene epoxidase (SE), IPP and DMAPP are converted into farnesyl pyrophosphate (FPP), squalene, and 2,3-oxidosqualene. Subsequently, OSCs catalyze the formation of the fundamental triterpenoid skeleton. Following this, CYP450s mediate oxidative modifications such as hydroxylation and epoxidation, while UGTs are responsible for glycosylation, ultimately yielding structurally diverse and functionally specific bioactive triterpenoids. The primary biosynthetic pathways for major FMH triterpenoids are illustrated in Fig. 5.

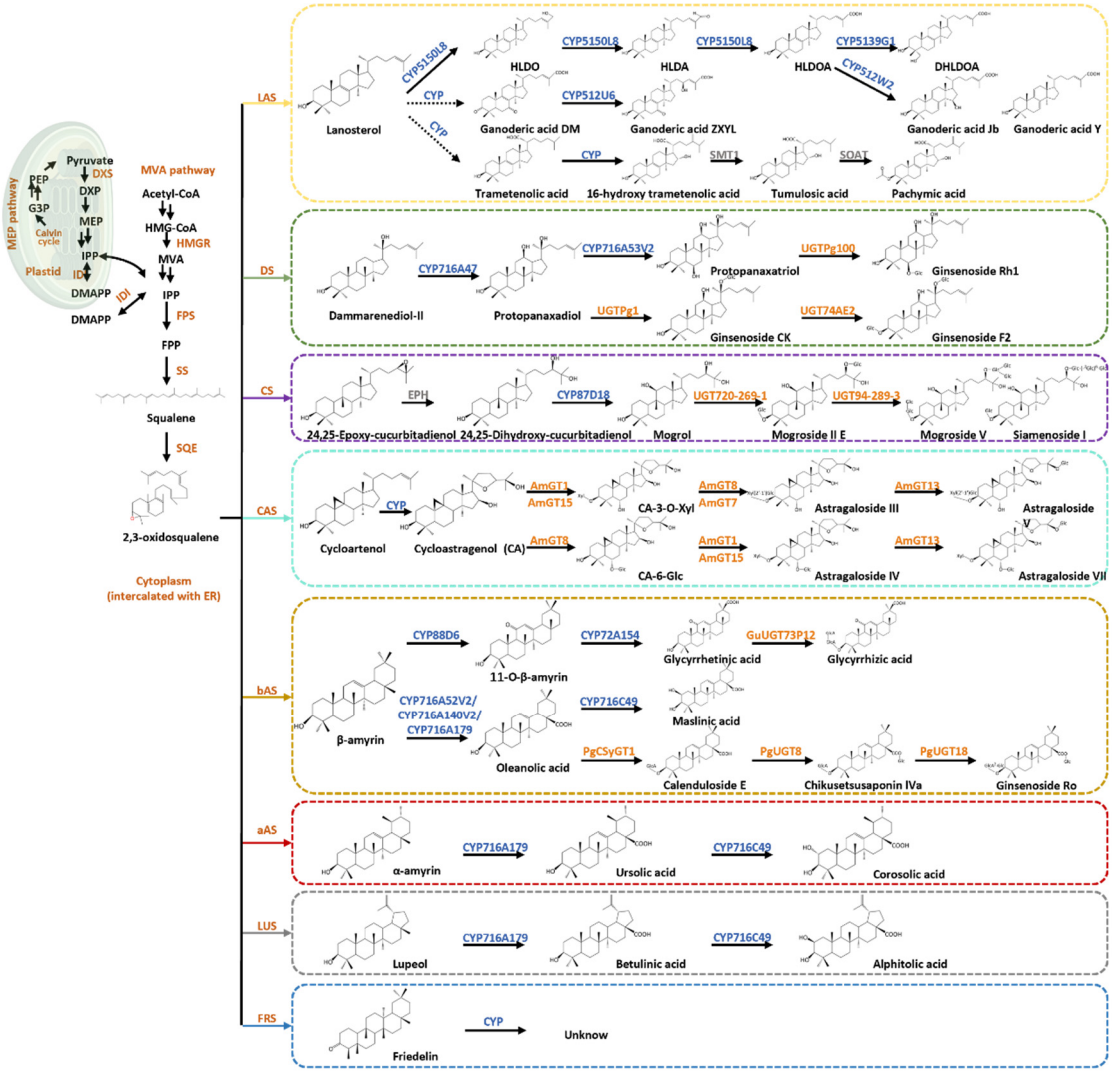

Fig. 5. The primary biosynthetic pathways for major FMH triterpenoids

4.2 Key enzymes for FMH triterpenoid synthesis

4.2.1 Oxidosqualene cyclases

As mentioned earlier, OSCs are rate-limiting enzymes in the downstream synthesis stage of triterpenoids, catalyzing the cyclization of 2,3-oxidosqualene and its analogs into different triterpenoid skeletons. OSCs generate over 100 triterpenoid skeletons, thereby enriching the terpene family (Wang et al., 2022). This catalytic process involves substrate recognition and conformational changes, epoxide ring opening initiated by protonation, cyclization coupled with carbocation rearrangements, and reaction termination through deprotonation or nucleophile addition (Chen et al., 2021). This article summarizes the OSCs identified in FMH substances, along with their GenBank IDs, source species, and products (Table S2). Their protein sequences were used to build a phylogenetic tree, and catalytic outputs were analyzed (Fig. S1). Current research confirms that representative FMH plants such as *P. ginseng*, *G. glabra*, *S. grosvenorii*, and *Taraxacum coreanum* have evolved highly efficient and specific OSCs. Among these OSCs, β-amyrin synthases are the most common, followed by cycloartenol synthases and dammarenediol-II synthases. Functionally distinct OSC types, such as α-amyrin, β-amyrin, lupeol, and friedelin synthases, cluster on separate phylogenetic branches, which underscores the high sequence conservation observed among OSCs sharing the same catalytic function.

Interestingly, OSCs display highly conserved motifs or key residues that play a critical role in determining their products. The structural diversity of triterpenoid skeletons may be related to the variability and mutable core sites in the associated enzymes, where a single amino acid change in an OSC can lead to distinct product formation. For example, GgbAS1 from *G. glabra* efficiently produces β-amyrin, providing the core precursor for oleanane-type glycyrrhizic acid. (Hayashi et al., 2001).PfOSC4 and PfOSC9 from *P. frutescens* are responsible for lupeol and β-amyrin biosynthesis, respectively; molecular docking and site-directed mutagenesis reveal that Leu253 and Trp257 constitute their key active-site residues (Huang et al., 2023). SgCbQ of *S. grosvenorii* specifically catalyzes cucurbitadienol formation, establishing the structural basis for cucurbitane-type mogroside V. Among missense mutation-derived single-nucleotide polymorphism variants of wild-type cucurbitadienol synthase, the 50R573L mutant exhibits the highest activity. Further site-directed mutagenesis demonstrates that the 50K573L variant enhances catalytic efficiency by 33% compared to wild-type 50R573L (Qiao et al., 2019). The characterized AmCAS1 in *A. membranaceus* drives cycloartenol synthesis, facilitating the biosynthesis of cycloartane-type astragaloside IV (Duan et al., 2023).

Advancements in synthetic biology have enabled substantial elucidation of molecular mechanisms governing bioactive metabolite synthesis in FMH substances, notably ginseng. Functional characterization of several ginseng OSC genes reveals their specific triterpenoid products. For example, PgPNX, PgPNY1, PgPNZ1, and PgDS catalyze the biosynthesis of cycloartenol, β-amyrin, lanosterol, and dammarenediol-II, respectively. The protein sequences of these four enzymes belong to distinct clusters, potentially representing different evolutionary lineages or specialized functions within the species.

Recent research has comprehensively investigated the uneven evolutionary patterns of triterpenoid saponin biosynthetic genes in ginseng (Song et al., 2024). Divergent evolution within these pathways underpins the vast structural variety of natural triterpenoids. The expansion and functional diversification of OSC genes through duplication events enable the generation of distinct triterpenoid scaffolds. Notably, the dammarenediol-II synthase gene family arose following an ancient Araliaceae-specific whole-genome duplication event in *Panax* (Wang et al., 2022; Yang et al., 2023).

Additionally, some OSCs exhibit promiscuity, producing multiple products instead of a single one. For example, TcOSC1 from *T. coreanum* can simultaneously generate the lupane-type marker taraxasterol, δ-amyrin, and dammarenediol-II. TcOSC2 can produce bauerenol, lupeol, α-amyrin, and taraxasterol (Han et al., 2019). This explains, at the enzymatic level, the cause of the complex triterpenoid profile in dandelion.

4.2.2 Cytochrome P450 monooxygenases

CYP450s account for approximately 1% of protein-coding genes in plant genomes, representing the largest enzyme family involved in plant metabolism. CYP450s are a superfamily of heme-containing monooxygenases. Most CYP450s activate molecular oxygen and catalyze monooxygenase reactions, relying on the redox partner cytochrome P450 oxidoreductase (CPR), which transfers two electrons from NAD(P)H to the heme center (Germann et al., 2024). CYP450s significantly increase the structural diversity and complexity of plant triterpenoids by hydroxylating, carboxylating, and epoxidizing triterpenoid skeletons. The abundance of CYP450 isoforms, coupled with their stringent substrate specificity, high sequence divergence, and near-identical chromatographic behavior, hinders purification and complicates analysis of their biotransformation pathways. Modern integration of metabolomic, transcriptomic, and genomic approaches has enabled comprehensive identification of plant CYPs. Currently, 127 CYP families are documented in plants, with biosynthesis of plant triterpenoid saponins primarily involving members of the CYP51, CYP71, CYP72, and CYP85 families. Among these, CYP716 subfamily enzymes in the CYP85 clan are major contributors to biosynthetic diversity in dicotyledonous plants. Twenty-six CYPs associated with triterpenoid synthesis in FMH substances were identified, with a phylogenetic tree constructed (Table S3 and Fig. S2). The clustering results confirm the classification characteristics of the aforementioned CYP families. Their catalytic products include oleanolic acid, ursolic acid, betulinic acid, 24-hydroxy-β-amyrin, maslinic acid, and corosolic acid. Furthermore, the major reactions catalyzed by CYPs include C-28 oxidation, C-24 oxidation, C-24 hydroxylation, C-23 hydroxylation, C-11 oxidation, C-11 hydroxylation, C-22 oxidation, and C-16 β-hydroxylation.

Among the identified CYP450s, the CYP716 subfamily is the most common, with C-28 oxidases and C-16 oxidases being the most prevalent. In *P. grandiflorus*, PgCYP716A140v2 catalyzes the C-28 three-step oxidation of β-amyrin to oleanolic acid, while PgCYP716A141 oxidizes β-amyrin at the C-16 position. Additionally, PgCYP716S5 catalyzes C12-C13α epoxidation, expanding the functional diversity of CYP716 enzymes (Miettinen et al., 2017). In *G. uralensis*, GuCYP716A179 catalyzes the oxidation of β-amyrin, α-amyrin, and lupeol to produce oleanolic acid, ursolic acid, and betulinic acid, respectively (Tamura et al.,

2017). Hydroxylation by CYPs further converts these into structurally and functionally diverse ginsenosides. Specifically, three CYP716 enzymes have been identified: CYP716A47 (protopanaxadiol synthase, PPDS) (Han et al., 2011), CYP716A53v2 (protopanaxatriol synthase, PPTS) (Han et al., 2012), and CYP716A52v2 (oleanolic acid synthase, OAS) (Han et al., 2013), which promote the production of protopanaxadiol (PPD)-, protopanaxatriol (PPT)-, and oleanane-type ginsenosides, respectively.

Members of the CYP93E subfamily are primarily involved in triterpenoid synthesis in leguminous plants. In *Glycyrrhiza* species, the C24-hydroxylases GuCYP93E3 and GgCYP93E6 catalyze the 24-hydroxylation of β-amyrin to form 24-hydroxy-β-amyrin. The CYP72A subfamily also participates in the biosynthesis of certain triterpenoids. For example, GuCYP72A154 catalyzes the oxidation of oleanane-type pentacyclic triterpene lactones (Xiang et al., 2025). GuCYP88D6 is responsible for glycyrrhizic acid biosynthesis, catalyzing the C-11 two-step oxidation of β-amyrin to 11-oxo-β-amyrin (Seki et al., 2008). GuCYP72A154 catalyzes the final C-30 oxidation involved in glycyrrhizic acid biosynthesis, and GuCYP72A566 catalyzes C-22 oxidation of 24-hydroxy-β-amyrin (Xiang et al., 2025).

4.2.3 Glycosyltransferases

UGTs catalyze triterpenoid glycosylation as the terminal saponin biosynthetic step, underpinning their structural and bioactive diversification. UGTs drive saponin biosynthesis by glycosylating triterpenoid hydroxyl/carboxyl groups. thereby increasing their diversity. The typical glycosylation pattern for triterpenoids involves attaching oligosaccharide chains of 2 to 5 monosaccharides to the C-3 and/or C-28 positions. Structural diversity in triterpenoid glycosylation stems from variations in sugar chain number, monosaccharide composition, and linkage positions on the aglycone scaffold. This modification substantially increases molecular polarity, thereby modifying physicochemical properties and bioactivity. The predominant UDP-sugar donors, including glucose, arabinose, galactose, rhamnose, and xylose, support oligosaccharide assembly through sequential sugar addition rather than en bloc transfer (Zhang et al., 2022). Unlike CYP450s, most UGTs can be expressed in the model strain *E. coli*, which also expands the scope for UGT discovery. Table S4 and Fig. S3 list 71 UGTs identified in triterpenoid biosynthetic pathways, primarily characterized in *P. ginseng*, *P. quinquefolius*, *G.* spp., *S. grosvenorii*, and *A. membranaceus*. They mainly belong to the UGT73, UGT74, and UGT94 subfamilies, with major reactions involving C3 glucosylation, C6 glucosylation, C-20 glycosylation, C-28 glycosylation, and C3 glucuronosylation.

UGTs often exhibit positional specificity. UGTPg1 exclusively glycosylates C20 to produce ginsenosides CK, Rd, and F1 (Yan et al., 2014), while PgUGT74AE2 transfers glucose from UDP-Glc to C3 of PPD and CK, yielding Rh2 and F2 (Jung et al., 2014). GuUGT73F17 specifically glucosylates C-30 of glycyrrhetinic acid or glycyrrhizic acid with 83.6% and 82.0% efficiency (He et al., 2018). PgUGT71A29 targets C20-OH of Rh1 and Rd to form PPT- and PPD-type ginsenosides. Meanwhile, Pq3-O-UGT1 mediates C3-glucosylation of both PPD and PPT substrates. This suggests certain UGTs exhibit biosynthetic promiscuity toward PPD- and PPT-type aglycones (Lu et al., 2017). UGTPg101 displays promiscuity in catalyzing C-6/C-20 bis-glycosylation of PPT (Wei et al., 2015). Utilizing regioselectively promiscuous

UGTs may be an effective strategy for obtaining glycosides with multi-site glycosylation. Although UDP-Glc predominates, transfers of rhamnose, xylose, arabinose, and glucuronic acid occur. PgUGT8 and PgUGT18 enable glucuronic acid transfer for ginsenoside Ro biosynthesis. (Zhang et al., 2022). GuUGT73P12 preferentially uses UDP-GlcA to convert glycyrrhetinic acid monoglucuronide to glycyrrhizic acid, while GuRhaGT adds Rha/Xyl to licorice saponins. Engineered PgURT94 utilizes UDP-Xyl for C6-O-Glc rhamnosylation, converting Rh1/Rg1 to Rg2/Re (Li et al., 2022).While past research prioritized cloning OH/COOH-glycosylating UGTs, future efforts should focus on characterizing sugar-sugar transferases.

The unprecedented diversity of UGTs may be caused by species-specific whole-genome duplications and tandem duplications. Recently, Yan et al. (2014) successfully obtained 46 diverse PgUGT94s from ginseng, primarily responsible for glucosylating the C-3 position of PPD. Additionally, a subset of these enzymes can transfer xylose to the C6-O-Glc position, converting ginsenoside Rg1 into saponin R1. UGTs from the same subfamily are tightly clustered within tandem repeat arrays in the genome. UGT94-289-1, UGT94-289-2, and UGT94-289-3 from *S. grosvenorii* are localized within a tandem repeat array (Yang et al., 2020). Tandem repeat organization suggests gene duplication drives subfunctionalization and neofunctionalization. Conversely, evolutionarily distant UGTs may develop identical biochemical functions via convergent evolution, as exemplified by GuUGT84F6 and GuUGT73F24, which catalyze equivalent C3-glucosylation of glycyrrhizic acid despite their phylogenetic divergence.

Mining candidate UGTs through genomic, transcriptomic, and bioinformatic analyses is a classical approach. Over 500 glycosyltransferase genes potentially involved in ginsenoside biosynthesis have been identified in ginseng's transcriptome and genome, though few are functionally validated. Furthermore, by constructing regulatory networks integrating transcriptome and metabolome data, key UGT genes correlated with differential metabolites can be identified. For example, in jujube, candidate CYP93D1 and UGT71A16 were identified in the correlation matrix, potentially associated with the specific glycosylation of jujuboside B1; however, their functions remain to be validated (Bai et al., 2024). A large proportion of UGTs still require functional validation. Despite conserved regioselectivity across UGT families and functional convergence at equivalent sites on diverse substrates, plant UGTs demonstrate substantial substrate promiscuity. Resolving their sugar donor/acceptor specificity and catalytic activity remains highly complex. Consequently, predicting biological functions through conventional characterization methods like phylogenetics and in vitro assays yields limited accuracy.

4.3 Heterologous biosynthesis of FMH triterpenoids

4.3.1 Strategies to promote heterologous biosynthesis of FMH triterpenoids

Synthetic biology enables efficient, green triterpenoid production by reconstructing FMH biosynthetic pathways for heterologous expression. The core of heterologous expression lies in cross-species gene transfer, utilizing host systems encompassing prokaryotes (e.g., *E. coli*, *Bacillus* spp., *Streptomyces*) and eukaryotes (e.g., yeast, filamentous fungi, *Nicotiana benthamiana*) (Li et al., 2023). Although plants themselves are natural factories for triterpenoid synthesis, their genetic engineering still faces bottlenecks such as long cycles

and low efficiency, making them less competitive compared to microbial systems. Existing studies (Table S5) have successfully utilized the aforementioned platforms to synthesize high-value FMH triterpenoids, but the two types of hosts exhibit significant performance differences. Prokaryotic platforms, particularly *E. coli*, often achieve superior yields in pathways independent of membrane proteins, exemplified by their utilization of endogenous MEP pathways for terpene precursor production. However, MEP pathway is under complex regulation and has high demands for NADPH and ATP. In contrast, eukaryotic platforms (e.g., *S. cerevisiae*) are widely used to construct cell factories for natural product biosynthesis due to advantages like rapid and stable growth, high homologous recombination efficiency, and a mature genetic system. Their key advantage is better compatibility with the functional expression of plant-derived membrane proteins (such as CYP450s), benefiting from eukaryotic cellular features like endoplasmic reticulum localization (Germann et al., 2024). Yeast primarily utilizes the MVA pathway, whose metabolism is relatively simpler, making it the preferred choice in certain cases for synthesizing FMH triterpenoids.

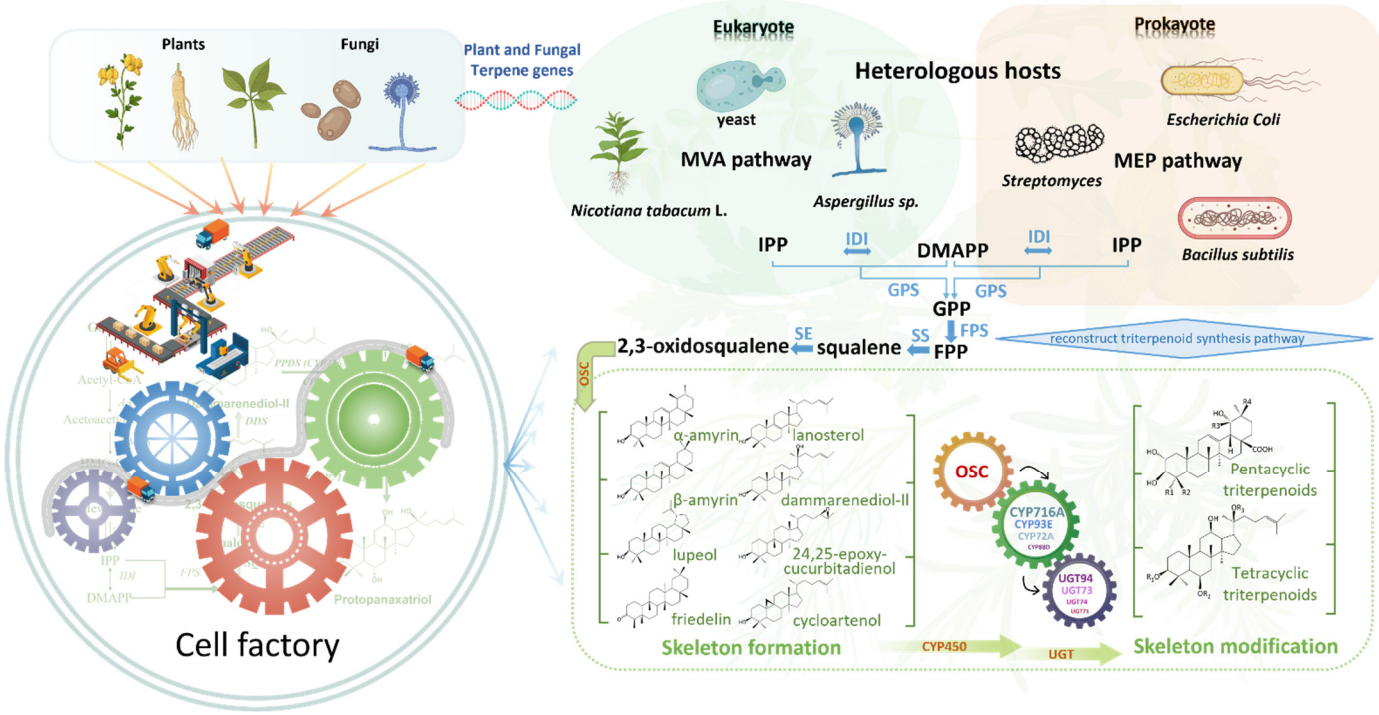

Fig. 6. Heterologous biosynthetic pathways of FMH triterpenoids in cell factories

FMH triterpenoid biosynthesis in cell factories progresses sequentially through pathway reconstruction, heterologous enzyme enhancement, and host optimization (Fig. 6). Increasing productivity is the primary goal for the biomanufacturing of rare FMH triterpenoids. To achieve this, the expression efficiency and stability of heterologous enzymes are critical factors for efficient product synthesis within the host, particularly for P450/CPR and UGTs. Heterologous enzymes like plant P450s consistently exhibit low activity in microbes, representing a major bottleneck in the process. Mining efficient natural and non-natural enzyme orthologs is the most direct approach to overcome this bottleneck. Therefore, the enzymes used in reconstructing organisms and pathways originate from a wide range of sources (Fig. 7A). Besides enzyme components from FMH plants themselves, enzymes from other species such as *Arabidopsis thaliana*, *Glycine max*, and

Medicago truncatula are frequently utilized to achieve more efficient biosynthesis. For example, by mining the licorice transcriptome database, the more active Unigene25647 was identified to replace CYP88D6, which had been consistently used in yeast for glycyrrhetinic acid biosynthesis. Pairing with CPRs from different plant sources revealed that fusion expression with GuCPR1 had the best compatibility, leading to a 1.94-fold increase in 11-oxo-β-amyrin production (Zhu et al., 2018). Ren et al. (2022) identified 7 high-efficiency enzymes from 5 species through cross-species screening of 29 candidates from 15 organisms. These were engineered into an artificial diglycosylation pathway in S. cerevisiae, achieving de novo ginsenoside Ro production at (528.0 ± 18.0) mg/L, a 374,000-fold increase over the PgUGT8/PgUGT18 system (1.41 μg/L). This engineering integration of non-FMH enzymes validates the synthetic biology principle of "function over species origin", providing a paradigm for unexploited FMH resources.

Given that P450 and glycosylated terpenoid production in yeast is often inefficient, optimization methods such as increasing copy number, enhancing promoter strength, codon optimization, and rational/semi-rational design are employed to improve the expression efficiency and selectivity of target enzymes (Fig. 7C). MuCYP72A63 catalyzes the oxidation of 11-oxo-β-amyrin at the C-29/C-30 positions, producing mixed products. Its T338S mutation brings the C-30 hydroxyl group of glycyrrhetinic acid closer to the heme iron via hydrophilic interactions while repelling the C-29 position, thereby enhancing C-30 regioselectivity and increasing glycyrrhetinic acid yield by 7.1-fold (Xue et al., 2020). By engineering ginseng to overexpress URT94, UDP-rhamnose could be used instead of UDP-glucose as the sugar donor in yeast, producing the diglycosylated and triglycosylated ginsenosides Rg2 and Re, respectively (Li et al., 2022). To promote the production of GAMG in yeast cell factories, after rationally designed computer-aided site-directed mutagenesis of GuUGT73F24, the mutant UGT73F24-I23G/L84N enabled the highest GAMG titer of 26.31 $mg \cdot L^{-1}$ in engineered yeast (Zhang et al., 2020). Through successive rounds of random mutagenesis and sequence consensus analysis, a variant of the triterpenoid glycosyltransferase SgUGT74AC1 exhibited a catalytic efficiency for mogrol increased by 10^2-10^4 fold, attributed to hydrophobic interactions between the enzyme and the substrate anchoring the triterpenoid in the active center (Li et al., 2020). Related enzyme mining and engineering will provide components for heterologous expression, and the heterologous expression of enzymes will lay the foundation for functional studies.

Redirecting metabolic flux towards the heterologous pathway to improve FMH triterpenoid production is also an effective measure. By directionally regulating metabolic flux in the MVA or MEP pathways and utilizing gene editing technologies (e.g., CRISPR/Cas9) to block competing branches, targeted accumulation of specific triterpenoids can be achieved (Fig. 7B). For instance, genes in mogroside biosynthesis, such as *SgSQS* and *SgCAS*, can be overexpressed to increase mogroside yield (Dai et al., 2015). Besides targeted regulation, overexpression of the global transcription factor UPC2-1 from yeast ergosterol synthesis upregulates the transcription of MVA genes. Further combined with targeted regulation strategies, ginsenoside CK production increased 5-fold (Yan et al., 2014). This compound has been used for arthritis prevention and treatment and has completed all Phase I clinical trials (CDEL20130379) (Chen et al., 2018).

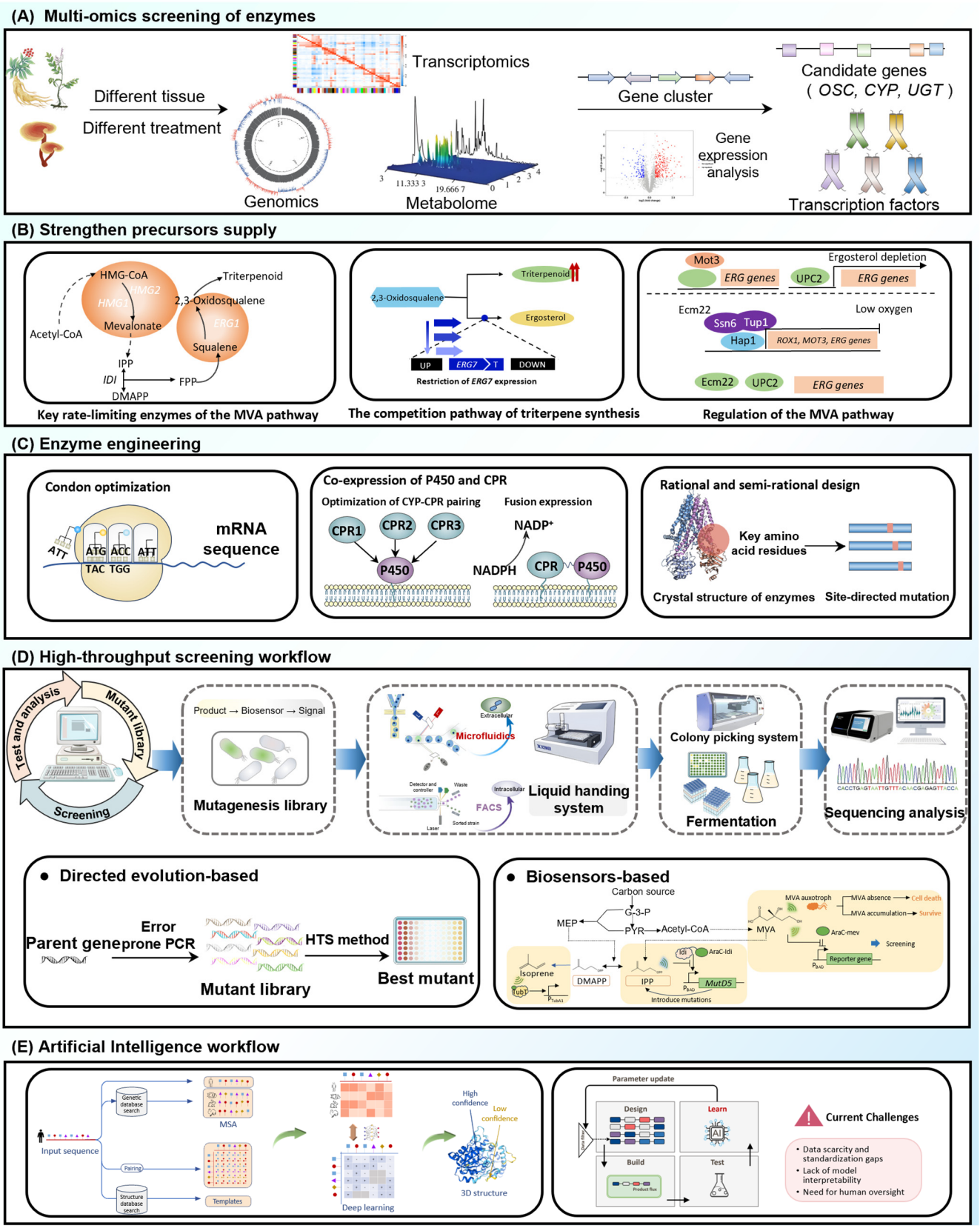

Fig. 7. Engineering strategies for enhancing triterpenoid production in FMH synthetic biology. (A) Multi-omics-driven enzyme discovery. (B) Precursor supply enhancement. (C) Enzyme engineering and optimization. (D) High-throughput screening platform. (E) AI-guided design and prediction.

4.3.2 Current status of heterologous biosynthesis of FMH triterpenoids

Recent breakthroughs in heterologous FMH triterpenoid biosynthesis have provided preliminary solutions to challenges like low pathway flux and inefficient synthesis. However, limitations still persist.

Current systems predominantly produce oleanane-, dammarane-, and cucurbitane-type triterpenoids using primarily *S. cerevisiae*, with scarce application of the plant host *N. benthamiana*. Microbial and plant platforms differ fundamentally in production metrics; meaningful comparison requires evaluating material costs and labor inputs rather than direct output measures. While microbial systems consume organic feedstocks, heterologous plants utilize light energy for biosynthesis.

Substantial progress has been made in the heterologous biosynthesis of oleanane-type triterpenoids, with successful synthesis of oleanolic acid, glycyrrhetinic acid, and maslinic acid achieved in heterologous hosts (Table S5). Nevertheless, the origins of common functional group structures found in some rare oleananes (e.g., licoricesaponin A3, 22β-acetoxyl-glycyrrhizic acid), such as monooxygenation at C-16, C-21, and C-23 positions, remain unclear. Further identification of endogenous UGT enzymes capable of catalyzing the formation of rare glycosides (e.g., Api, Ara, Rha, Xyl) is required. Representative ursane-type pentacyclic triterpenoids, including ursolic acid and taraxasterol, have been heterologously produced. However, the mechanisms underlying the actions of related CYP450 enzymes and glycosyl modifications are not fully elucidated, hindering research into ursane-type heterologous biosynthesis. Given the structural similarity between ursane and oleanane skeletons, some enzymes in heterologous pathways can be utilized interchangeably, offering significant potential for pathway development through cross-referencing of natural biosynthetic routes. Heterologous biosynthesis of lupane-type FMH triterpenoids, including lupeol, betulinic acid, and betulin, has been reported, but yields remain low. Most current methods suffer from issues like low enzyme activity, poor specificity, excessive byproduct formation, and low yield, necessitating stepwise optimization of chassis cells and metabolic flux. Friedelane-type triterpenoids are exceptionally scarce, detected only in a few plants like *C. pilosula*, though not as major or characteristic active components. Due to their extremely low natural abundance and high research costs, key synthases (e.g., friedelin synthase) remain unidentified, resulting in slow progress and limited exploration of their biosynthetic pathways.

Dammarane-type research has focused extensively on ginsenosides like Rb1 and Rh2, with ongoing studies on rare ginsenosides (e.g., CK, Ro) yielding preliminary results. The heterologous synthesis pathways for specific stereoisomers, such as 20(*R*)-ginsenosides, require further in-depth exploration. Studies on cucurbitane-type compounds, particularly mogrosides, have primarily utilized plant host cells. The recent *de novo* synthesis of mogroside V in *S. cerevisiae* represents a breakthrough (Qu et al., 2025), yet the mogrol-to-mogroside pathway remains poorly characterized. This demands identification of enzymes with high catalytic efficiency and product specificity. Consequently, developing integrated pathways for complete *de novo* mogroside production remains unexplored. Representative lanostane-type triterpenoids include ganoderic acid A and pachymic acid A. Key progress was recently made in the heterologous synthesis of ganoderic acids in *S. cerevisiae*, identifying several CYP450s crucial for catalyzing the lanostane skeleton—a longstanding bottleneck. However, current *de novo* synthesis yields remain low. With the refinement of the *G. lucidum* genome and transcriptome data, combined with modern biotechnology, the discovery and optimization of more efficient and compatible enzymes holds promise for yield improvement (Li et al., 2025).

Heterologous synthesis of *P. cocos* triterpenoids (e.g., pachymic acid) has not yet been realized, hindered by pathway complexity and the lack of cloned key CYP450s. Recent comprehensive genome and transcriptome sequencing of *P. cocos* revealed three candidate CYP450 genes potentially involved in pachymic acid biosynthesis (Liu et al., 2024); their functional validation is needed to elucidate the complete pathway. Cycloartane-type triterpenoids are represented by astragaloside IV. The UGTs required for synthesizing various astragalosides have been identified, including a UGT responsible for specific O-xylosylation. However, insufficient activity of some UGTs limits the flux towards final products (Zhang et al., 2024). Although the production of astragaloside IV has been reported in tobacco, its yield has not been thoroughly investigated. Overall, heterologous synthesis research in this area is limited and urgently needs strengthening.

Despite significant improvements in the biosynthesis levels of specific triterpenoids (e.g., ginsenosides, glycyrrhizic acid), with many ginsenosides reaching g/L levels and even achieving commercial production in *S. cerevisiae*, the heterologous biosynthesis of FMH triterpenoids collectively faces severe challenges. On one hand, the discovery of novel triterpenoids is surging, yet redundant discovery is prevalent, and the vast majority of newly identified terpenoid products exhibit extremely low yields (often remaining at $mg \cdot L^{-1}$ or even $\mu g \cdot L^{-1}$ levels). This not only restricts their large-scale application to meet low-cost demands but also severely impedes the comprehensive evaluation of their bioactivities. On the other hand, the limited compound library scale accessible to individual research groups, coupled with the scarcity of truly novel terpenoid products, makes efficient, multi-functional, and multi-target activity screening exceptionally difficult. While the development of diverse metabolic engineering strategies in recent years has yielded initial success in boosting yields, the enhancement effect remains limited for most FMH triterpenoids; the achievable variety and total output fall far short of requirements. Therefore, technological innovation is imperative to effectively overcome the core obstacles of redundant discovery, low product yield, low concentration, and insufficient research throughput. These persistent challenges collectively indicate that current heterologous biosynthesis of FMH triterpenoids has reached a critical stage where conventional metabolic engineering alone can no longer deliver the required leap in yield, diversity, or efficiency. The discovery of structurally novel triterpenoids contrasts sharply with the limited capacity for scalable production and functional assessment, exposing a growing gap between molecular innovation and manufacturing realization. Moreover, the intricate multi-enzyme cascades, extensive oxidation–reduction reactions, and strict regioselective modifications characteristic of FMH triterpenoid biosynthesis impose substantial burdens on heterologous hosts, often leading to unpredictable metabolic crosstalk and system instability. Overcoming these barriers requires transformative strategies that transcend traditional pathway optimization. Therefore, emerging technologies that couple AI-assisted enzyme and pathway design, high-throughput Design-Build-Test-Learn (DBTL) cycles, and adaptive dynamic control have become indispensable to accelerate discovery-to-production translation. These frontier approaches are discussed in detail in the following section, focusing on how intelligent design and data-driven biomanufacturing can enhance the efficiency and predictability of FMH triterpenoid biosynthesis.

5. Emerging strategies for the efficient synthesis of FMH triterpenoids in cell factories

5.1 High-throughput screening and directed optimization of key elements

Interdisciplinary advances, notably AI and machine learning (ML)-driven paradigm shifts, structural biology, and HTS platforms, are revolutionizing natural product research, including FMH triterpenoid studies. Research on FMH triterpenoids is evolving from the stages of heterologous expression and metabolic engineering towards a new phase of directed evolution and intelligent design. This integrated strategy continuously optimizes biosynthetic efficiency and target product properties, establishing a robust, innovative framework for the engineered application of FMH triterpenoids.

Multi-omics approaches, encompassing genomics, metabolomics, and transcriptomics, represent key methods for mining novel biosynthetic elements of natural products. Effectively utilizing vast genomic information to efficiently uncover the "dark matter" of natural products has become a major research focus and challenge. Furthermore, researchers traditionally rely on labor-intensive methods such as liquid chromatography (LC)- or gas chromatography (GC)-based analysis and enzyme activity assays to evaluate the performance of each enzyme variant. These time-consuming and costly approaches often hinder efficient product screening and synthesis. Therefore, developing faster, more sensitive, and higher-throughput screening and detection methods is a critical pathway to advance the discovery and efficient synthetic modification of natural products.

When mechanistic understanding is limited, directed evolution remains a primary strategy for enhancing the efficiency and selectivity of rate-limiting enzymes in triterpenoid biosynthesis (Li et al., 2018). Although independent of crystal structures, the process can incur significant labor and time costs. HTS methods are crucial for accelerating directed evolution (Kozono et al., 2017) (Fig. 7D). For instance, Wang et al. (2019) screened an error-prone PCR library (over 1500 transformants) in 96-well plates, identifying the variant UGTPg45-HV with two missense mutations. This variant showed 70% higher ginsenoside Rh2 production than wild-type UGTPg45, accompanied by a near doubling of V_{max} and a > 40% increase in catalytic efficiency (k_{cat}/K_m). This screening effort required over 125 h of HPLC analysis time. Yuan et al. (2022) developed a yeast host and HTS platform to iteratively screen up to 158 P450 enzymes from *G. lucidum*, successfully identifying those capable of converting ganoderic acids from Type I to Type II.

Biosensor-based designs are also recognized as an ideal HTS method. Biosensors offer advantages such as high sensitivity, rapid response, and diverse signal transduction mechanisms, converting target compound concentrations into detectable signals to easily identify enzyme mutants with desired properties during directed evolution. For example, Aharoni et al. (2006) developed a fluorescence-activated cell sorting (FACS)-based HTS method capable of screening > 10^6 glycosyltransferase gene mutants within 2 hours, establishing a link between genotype and phenotype for rapid and efficient screening of specific enzymes. Guo et al. (2023) constructed a UDP-aptamer-based biosensor for screening active glycosyltransferases. Using a novel method called Capture and In Vitro Transcription System Evolution of Ligands by Exponential Enrichment (CIVT-SELEX), they identified a transcription switch responsive to UDP-Glc conversion to

UDP, demonstrating the immense potential of aptamer-based UDP biosensors to simplify the massive task of LC-MS testing for all samples.

MVA, a key MVA pathway intermediate, indicates pathway expression and terpenoid production capacity. MVA biosensor development, pursued for decades, typically involves engineering MVA response via auxotrophic strains or altering transcription factor specificity. Rugbjerg et al. (2018) developed a synthetic addiction system for high-titer MVA producers. By using the product-responsive transcription factor $AraC^{MEV}$ to dynamically control essential genes via its cognate promoter, they enabled effective coupling of MVA production and *E. coli* cell growth. This system significantly aids robust industrial-scale bioproduction by maintaining cellular productivity during long-term cultivation. Tang and Cirino (2011) utilized the AraC-P_{BAD} regulatory system as a screening tool for engineering *E. coli* to improve MVA production. They applied site-saturation mutagenesis to AraC, creating variants whose response to MVA was linear within the 10-100 mmol/L range, and applied this to microbial strains expressing HMG-CoA reductase (tHMGR). Pfleger et al. (2007) constructed a MVA biosensor strain by creating an *E. coli* MVA auxotroph.

Monitoring IPP/DMAPP levels offers insights into isoprenoid precursor pool capacity. Whole-cell biosensors provide significant potential for identifying active enzymes via HTS to explore isoprene-producing genotypes. For instance, Chou and Keasling (2013) engineered an IPP biosensor by fusing AraC to the Id regulator. This synthetic transcription factor undergoes a conformational change in the presence of IPP, preventing PBAD promoter activation. Kim et al. (2018) developed a biosensor using the XylR-type regulator TbuT from *Ralstonia pickettii*. It monitors intracellular isoprene (0.05-8 mmol/L linear range) and activates the PTbuA1 promoter in both *E. coli* and *Pseudomonas putida*.

However, currently established biosensor-based HTS methods primarily target compounds like amino acids, flavonoids, and organic acids. Research on triterpenoids is largely limited to precursors and ligands. Therefore, establishing universal HTS systems tailored to specific triterpenoid products remains a critical need.

5.2 AI-based protein and metabolic engineering

ML and deep learning (DL) offer another promising approach within the DBTL framework of synthetic biology (Fig. 7E). ML/DL algorithms can identify patterns within large datasets, assisting in the processing and analysis of large-scale biological omics data, and predict the impact of genetic modifications on triterpenoid production. This predictive capability facilitates the creation of efficient microbial cell factories through iterative optimization cycles.

AI and neural networks tailor protein structure and sequence optimization for specific functions, even without detailed structural information. Paradigm-shifting examples include the application of platforms like AlphaFold (Kortemme, 2024), trRosetta (Yang et al., 2020), and RoseTTAFold (Baek et al., 2021). AI prediction of protein structures, exemplified by AlphaFold2, generates high-accuracy structures comparable to experimentally determined crystal structures, breaking the limitation of unavailable 3D structures. AI technologies like AlphaFold aid in the homology modeling and active pocket analysis of wild-type UGTs and

CYPs, enabling rational design and structural modifications to enhance their substrate specificity and catalytic activity (Jumper et al., 2021).

For instance, to address the insufficient robustness of UGTs for ginsenoside Rh1 production, Deng et al. (2025) employed a dual-strategy framework starting from UGTPg100, integrating AlphaFold3 structure guidance and computer-aided variant prediction via the Consensus Finder platform. They investigated the mechanism behind the enhanced catalytic efficiency of mutants using molecular docking simulations, ultimately creating a thermostable UDP-dependent glycosyltransferase (UGT227). Hong et al. (2024) utilized AlphaFold2 combined with molecular docking to explore, for the first time, the 3-O-glycosylation by the promiscuous glycosyltransferase OsSGT2 for 20(R)-dammarane ginsenosides. This demonstrated the feasibility of synergistic action between structural simulation and docking-assisted reactivity prediction, providing new insights for exploring and exploiting novel enzyme activities. Sun et al. (2025) developed CMDmpnn, combining comparative molecular dynamics with ProteinMPNN. This virtual screening approach rapidly identified glycosyltransferase variants with broadened phenolic substrate spectra and retained thermostability within two weeks, demonstrating its efficiency.

Unlike structure-guided engineering, co-evolution-guided enzyme engineering leverages Rosetta/GREMLIN-computed amino acid correlations. This approach enables functional optimization of enzymes lacking crystallographic data, such as CYP450s, and has successfully generated high-resolution protein structures (Ma et al., 2016). Through three rounds of iterative design and evaluation of 96 protein variants, Li et al. (2019) converted the multifunctional P450 enzyme CYP87D20 into a monooxygenase that specifically catalyzes the hydroxylation of cucurbitadienol at the C-11 position to produce 11-hydroxycucurbitadienol. This enzyme can be further applied to create *de novo* pathways for mogrol production.

Beyond protein design, several AI-based frameworks have demonstrated tangible success in pathway-level and process-level engineering of triterpenoid biosynthesis. For instance, Mukherjee et al. (2022) applied a machine-learning-guided strategy to dissect flux control points in the MVA pathway of *S. cerevisiae*, identifying non-rate-limiting enzymes whose overexpression most effectively increased triterpene precursor pools. The optimized yeast chassis showed markedly enhanced squalene and downstream triterpenoid titers, establishing a reusable ML-driven platform for terpenoid biosynthesis. Similarly, van Lent et al. (2023) integrated AI simulations into DBTL loops to iteratively refine metabolic-pathway architectures. Their AI-DBTL system predicted the most informative design variables, reduced experimental iterations, and consistently improved biosynthetic output across production rounds. Complementary reviews have also emphasized that data-driven automation and AI-enhanced predictive modeling will become indispensable for optimizing heterologous terpenoid production in microbial systems (Bureau et al., 2023). In parallel, emerging AI-knowledge platforms such as TeroSeek are beginning to curate terpenoid-specific datasets and retrieval pipelines, further accelerating the integration of AI tools into metabolic-engineering workflows (Kang et al., 2025). Collectively, these studies demonstrate that AI now extends beyond structure prediction to enable

quantitative flux control, process consistency, and intelligent experimental design, thereby offering a practical pathway toward predictive, automated triterpenoid biomanufacturing.

Despite significant advances in AI-driven exploration and optimization of triterpenoid biosynthesis, mature application still faces challenges, particularly the interpretability limitations of complex "black box" algorithms. The mechanistic opacity and limited interpretability of complex models, coupled with the scarcity of standardized, high-resolution data and inherent biases, represent key constraints on AI-driven biosynthesis progress and predictive model development. Furthermore, ensuring sufficient human oversight to identify and mitigate risks, avoiding over-reliance on algorithms, is an important consideration (Groff-Vindman et al., 2025). In the future, ML/DL-mediated strategies could be further applied to optimize pathway design, rapidly predict and resolve bottlenecks and constraints in chassis cell metabolic pathways, or integrate multi-omics data to promote a holistic approach to metabolic engineering. They could also facilitate the development of scalable production processes by predicting optimal conditions for large-scale synthesis.

6. Conclusion and future perspectives

FMH triterpenoids are essential functional components in medicine and food systems, where their structural diversity is directly linked to activities like anti-tumor, anti-inflammatory, and metabolic regulation, providing unique value for functional foods and disease prevention. However, their low natural abundance in plants and incomplete characterization of bioactivities significantly restrict their applications. Growing industrial demand urgently calls for deeper research into biosynthetic mechanisms, biological functions, and commercialization strategies. While large-scale production is still in early stages, understanding and engineering pathways involving CYP450s and UGTs lay the groundwork for efficient heterologous production through synthetic biology. Advances in microbial synthesis for ginsenosides and natural sweeteners, along with lab-scale production of other FMH triterpenoids, demonstrate sustainable manufacturing potential. Major obstacles remain, including low catalytic efficiency, byproduct buildup, insufficient yields, and poor bioavailability—primarily due to solubility issues—which collectively hinder industrial application. Beyond biosynthesis, their incorporation into complex food systems presents additional hurdles. The instability of triterpenoids during processing, their limited solubility, and strong interactions with proteins and lipids complicate formulation control and product uniformity. Moreover, their inherent bitterness and astringency reduce sensory acceptability, while the absence of standardized analytical protocols and safety frameworks continues to challenge regulatory approval. These technological and perceptual barriers collectively constrain the broader utilization of FMH triterpenoids in the food industry.

Emerging technologies now offer a path toward integration rather than incremental adjustment. The combination of synthetic biology with AI- and HTS-driven DBTL cycles can accelerate enzyme discovery, pathway optimization, and metabolic balancing, ultimately achieving higher titers and reproducible production at an industrial scale. Meanwhile, insights from matrix-aware formulation science, together with standardized analytical fingerprints and harmonized quality frameworks, will promote stability, safety, and traceability throughout the manufacturing chain. At the same time, advances in flavor modulation and

controlled-release encapsulation provide feasible routes to mitigate bitterness and improve consumer experience without sacrificing bioactivity. Looking ahead, the convergence of AI-assisted biomanufacturing, intelligent formulation, and sensory innovation is expected to bridge the long-standing gap between molecular functionality and practical application. Such integration will transform FMH triterpenoids from underutilized natural resources into standardized, evidence-based, and consumer-trusted bioactive ingredients for the next generation of functional foods and therapeutics.

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Declaration of competing interest

The authors declare that there are no conflicts of interest.

Data availability

Data will be made available on request.

Reference

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