Review Articles

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A comprehensive review of azadirachtin: physicochemical properties, bioactivities, production, and biosynthesis

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Abstract

Azadirachtin, a complex tetratriterpenoid limonin with potent insecticidal properties, is the most widely used biological pesticide worldwide. Its versatile pharmacological applications include the inhibition of tumor growth and anti-malarial, anti-bacterial, and anti-inflammatory properties. Azadirachtin plays a pivotal role in pest control and novel drug development. The primary source of azadirachtin is the neem tree (*Azadirachta indica* A. Juss), with an azadirachtin content ranging from 0.3% to 0.5%. Despite the market demand for botanical pesticides reaching approximately 100,000 tons per year, the annual neem production in China is only 1.14 tons. Although azadirachtin can be obtained through plant extraction or chemical synthesis, the quantity obtained does not meet the market demand in China. The sluggish pace of azadirachtin biosynthesis results from the limited availability of genetic information and the complexity of the synthetic pathway. Recent advancements in azadirachtin biosynthesis hold promise as an efficient collection method. In this study, we explored the physicochemical properties, biological activities, mechanisms of action, and acquisition methods of azadirachtin. We also delved into recent progress in azadirachtin biosynthesis and assessed potential future usage challenges. This study aims to establish a theoretical foundation for the scientific application and efficient synthesis of azadirachtin, offering valuable reference information to the industry.

Keywords: Azadirachtin, Biosynthesis, Mechanism of action, Neem

Graphical abstract: http://links.lww.com/AHM/A79.

Introduction

Azadirachtin, a complex tetranortriterpenoid limonoid renowned for its insecticidal properties, offers distinct advantages over chemical insecticides. It has remarkable persistence and resistance resilience. With a broad spectrum of actions, it has effectively prevented and controlled over 550 pest species. Notably, the decomposition products of this pesticide can enrich soil as natural fertilizers. Azadirachtin also exerts potent anti-feeding, repellent, and growth-inhibiting effects on pests, while enhancing plant conduction. Importantly, they pose no threat to humans, livestock, birds, or natural enemies of pests[1–2]. Azadirachtin is an important commercial biopesticide used in China. Currently, products containing azadirachtin compounds are registered for use in more than 40 countries, including China, the United States, India, and Austria[3]. In China, provisional registration of azadirachtin was initiated in 1997. Currently, 39 azadirachtin insecticides are registered in China. As the world's largest agricultural producer, China experiences a substantial demand for botanical pesticides, with a market demand of 100,000 tons annually, of which azadirachtin holds a significant share. However, China's annual neem agricultural output is only 1.14 tons, highlighting a substantial gap between market supply and demand. Notably, the agricultural sector commands the lion's share (40%) of the total neem extract market revenue. This market is poised to reach a staggering $1.8 billion US by 2022, with an annual growth rate of 16.3%. In addition to its prowess as an insecticide, azadirachtin has numerous medicinal properties, including tumor growth inhibition and anti-malarial, anti-rheumatism, anti-bacterial, anti-inflammatory, anti-pyretic, and diuretic properties[4–5].

Azadirachtin predominantly resides in the seeds of neem plants and its content ranges from 0.3% to 0.5% of the dry weight. Conversely, azadirachtin content in the branches and leaves is exceedingly low. Notably, Ley achieved total chemical synthesis of azadirachtin in 2007, a feat requiring a daunting 71-step reaction process with a meager total yield of only 0.00015%[6]. By leveraging synthetic biology principles, we may potentially harness effective components or critical intermediates that are either present in minute concentrations or are challenging to obtain through traditional medicinal plant production (Figure 1).

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Progress in azadirachtin biosynthesis has languished for years, primarily because of the scarcity of genetic information and complexity of the synthetic pathway. Recently, significant breakthroughs have been made in azadirachtin biosynthesis. Many studies have examined the azadirachtin genome and completed transcriptome analyses, including complementary DNA (cDNA) library[7], draft genome[8–9], expressed sequence tag (EST) libraries[10–11], transcriptome data[8,12–14], and chromosome-level genome[15–16]. Through systematic transcriptome and genome exploration, coupled with co-expression, phylogenetic, and comparative analysis, Osbourn et al. achieved a transformative breakthrough by successfully converting the intermediate skeletal compound from 2,3-oxidized squalene to tirucalla-7,24-dien-3β-ol[17]. This complex process involves ring-opening rearrangements, C4 bond cleavage, furan ring formation, and oxidative modifications common to limonoid compounds, ultimately culminating in the formation of a core C26 scaffold and completing the synthesis of azadirone and kihadalactone A[17–18]. Bioinformatics related to azadirachta and the azadirachtin metabolic pathway have advanced significantly, providing greater clarity.

This study aims to comprehensively explore the physicochemical properties, biological activities, mechanisms of action, and methods of acquisition of azadirachtin. Additionally, we intend to delve into the latest research on azadirachtin biosynthesis and the potential future challenges associated with this compound. Our objective was to establish a robust theoretical foundation for the scientific utilization and efficient synthesis of azadirachtin, while providing valuable reference material to the industry. We aspire to equip researchers engaged in the chemical analysis and biosynthesis of azadirachtin with valuable insights. This review draws on literature sourced from the China National Knowledge Infrastructure, Web of Science, and PubMed search engines. Keyword searches were designed to identify papers featuring the terms "*Azadirachta indica*" "azadirachtin" "neem" "synthetic biology" "genome" or "transcriptome" with a focus on publications up to 2023, particularly those released after the year 2000. A total of 129 studies were included in this review.

Neem: the main source of azadirachtin

Neem (*Azadirachta indica* A. Juss) is the primary source of azadirachtin compounds and belongs to the Meliaceae family. Indigenous to India and Myanmar, this tree thrives in tropical and subtropical regions and its presence now extends across southern Asia, Africa, South America, and more than 70 other countries worldwide[19]. Notably, wild neem does not naturally occur in China; it was initially introduced from

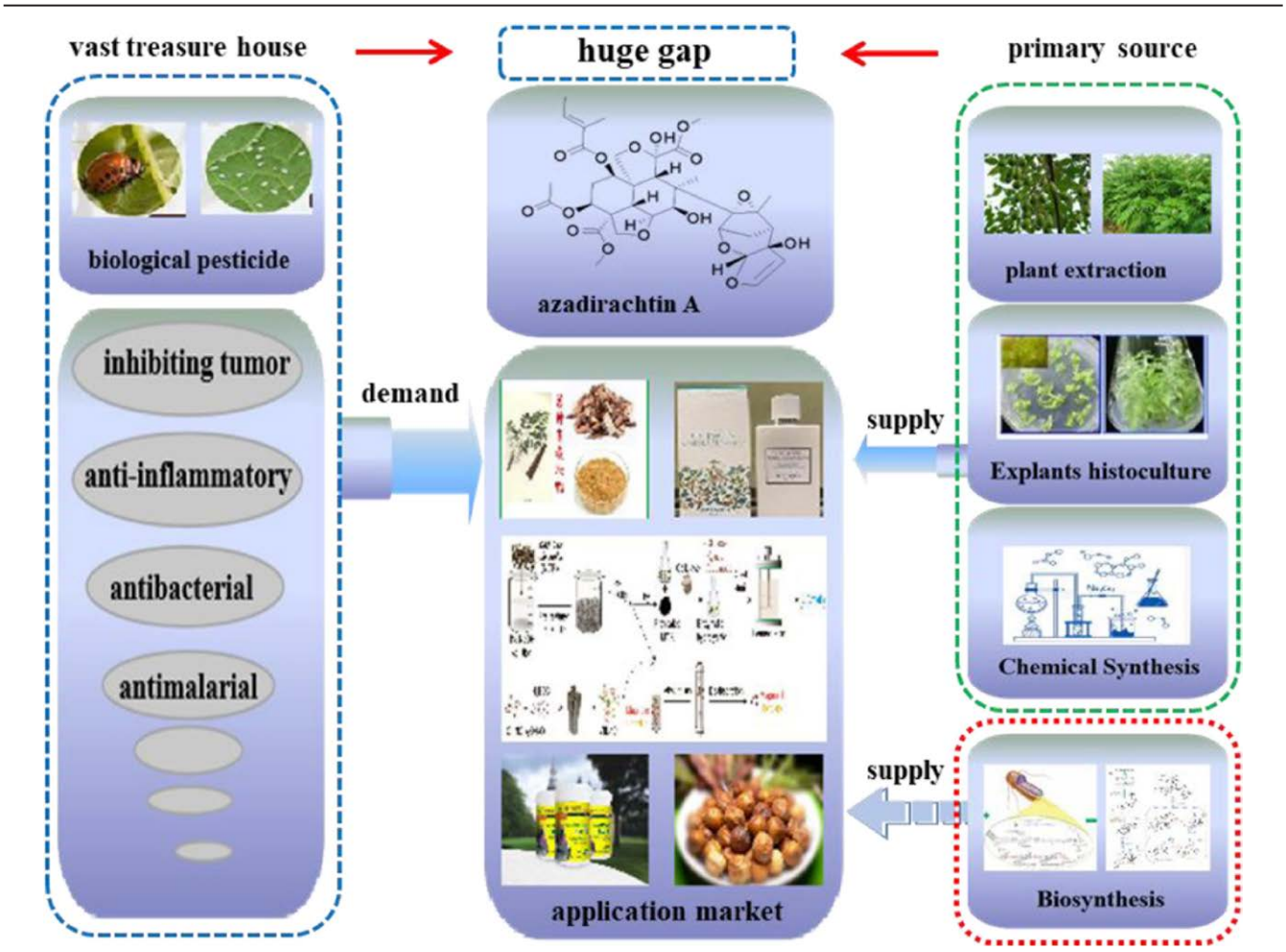

Figure 1. Azadirachtin acquisition method.

Africa by Professor Zhao[20] and successfully cultivated in Wanning County, Hainan Province. Following years of expansive growth and cultivation, the neem-planted area in Yunnan Province has surpassed 400,000 mu, establishing itself as the world's largest neem plantation[21]. Neem has garnered international acclaim for its substantial economic worth, boasting over 20 practical applications, with the most lucrative role as an insecticide[22]. The active insecticidal component within the neem plant, azadirachtin A, is recognized globally because of its exceptional nontoxic properties[5]. Moreover, neem has an extensive array of medicinal applications, including anti-malarial, anti-pyretic, and anti-tumor properties, as well as the capacity to inhibit fungal growth in both animals and humans. The United States Department of Agriculture hails it as "a tree for solving global problems"[23].

The chemical composition of neem trees was studied primarily in the 1950s. The structural diagrams of the main functional components of neem are shown in Figure 2. Common terpenoids found in neem include diterpenoids, triterpenoids, and pentanortriterpenoids. Non-terpenoids include tannins, sulfides, fatty acids, coumarins, polysaccharides, polyphenols, and amino acids. There are 130 terpenoids found in neem[24], including azadirachtin, among which are a series of similar structural analogs, including trichilin, trichilinin, nimbolinin, and meliacarpinin[25]. Table 1 lists the active terpenoid compounds found in the neem tree. Azadirachtin includes the analogs A, B, D, E, F, G, H, I, K, M, N, O, and Q[49]. Azadirachtin A and azadirachtin B comprise 99% of the azadirachtin components in neem[50]. Indian researchers have constructed and integrated these components into a neem metabolite database (Neem Secondary Metabolites Database, Neem DB, http://vmsrfdatabase.org/)[51].

Azadirachtin, a scientific gold mine

Physicochemical property

Azadirachtin primarily refers to azadirachtin A, as identified by the CAS registration number 11141-17-6. Its molecular formula is $C_{35}H_{44}O_{16}$ with a relative molecular mass of 720.71. In 1968, Butterworth and Morgan[52] isolated this chemical compound from neem seeds through a series of fractionations. The resulting colorless or slightly yellowish microcrystalline powder was named azadirachtin. It possesses a melting point of 154–158°C, with its maximum absorption peak occurring at 217 nm. Its photometric rotation registers at −13.1 (c = 1.75, Acetone) or −71.4 (c = 0.21, $CHCl_3$). Azadirachtin readily dissolves in polar organic solvents, such as acetone, ethanol, methanol, or dimethyl sulfoxide. It exhibits hydrophilic properties (log P = 0.13), is photosensitive, and is non-volatile[3,53]. Based on existing chemical, chromatographic, and spectroscopic data, scientists have established that azadirachtin is a blend of tetratriterpenoids that share structural connections with nimbin and salanin present in neem seeds. The chemical structure of azadirachtin can be broadly categorized into ten hydrogen and tricyclic furan rings. It encompasses 16 chiral centers, comprising seven quaternary carbons, nine secondary carbons, and 16 oxygen atoms arranged into four esters, two hydroxyl groups, a half acetal, an epoxide, and a dihydrofuran[54]. Historically, this intricate structure has impeded progress in structural analyses; it was not until 1986 that Broughton et al. provided the first structural analysis. Subsequent nuclear magnetic resonance spectrometer (NMR) and X-ray crystallographic examinations confirmed the chemical structure of azadirachtin, thereby catalyzing further research endeavors[55].

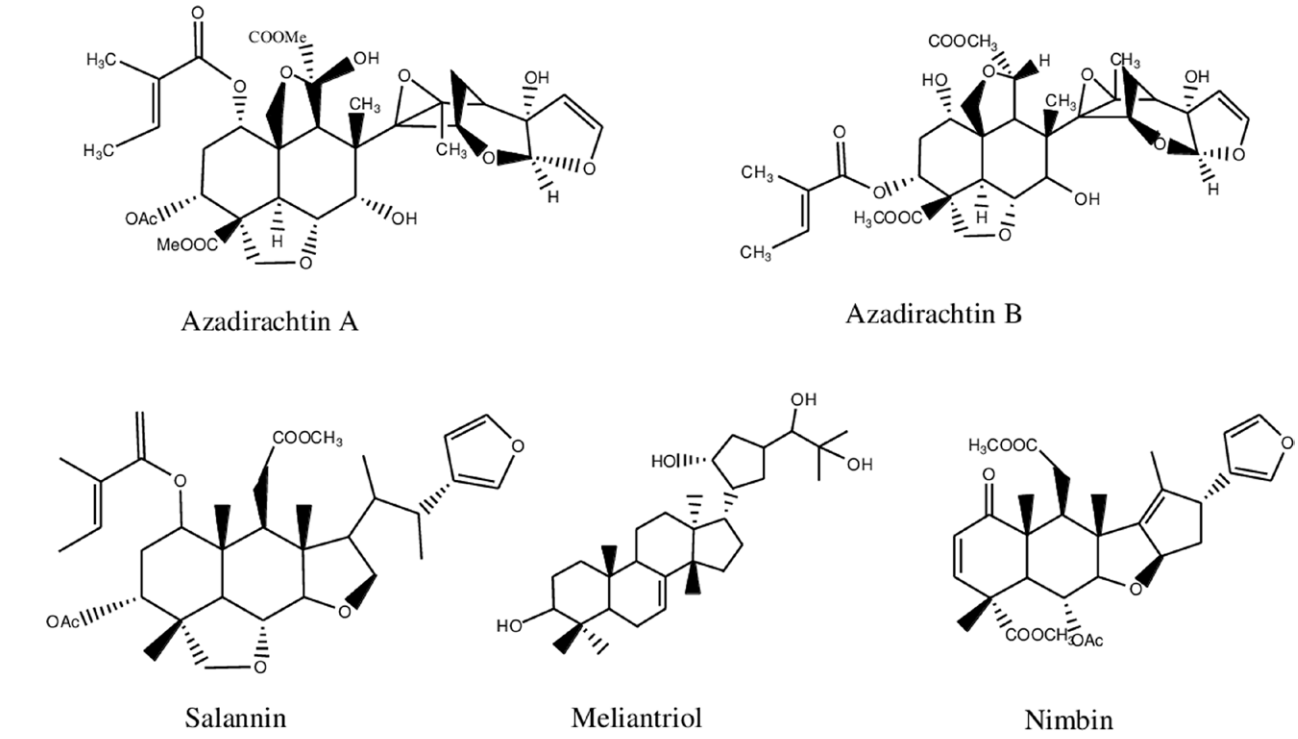

Figure 2. The chemical structures of azadirachtin and its analogs.

Table 1

Active compounds in neem

Class	Compound	Part	Biological activity	Reference
Terpenes	Azadirachtin A	Seeds, bark	Insecticide, anti-tumor, anti-virus, anti-malarial	[26]
	Azapirone	Seeds,bark	Anti-malarial, insecticide	[27–28]
	Nimbin	Seeds, leaves	Anti-fungal	[29]
	Salannin	Seeds	Ant-ifeedant, anti-fungal	[30]
	meliantriol	Seeds	Anti-feedant, insecticide	[26]
	Nimbolide	Leaves	Anti-cancer, insecticide	[26]
	Nimbidin	Seeds	Anti-inflammatory	[31]
	Nimbiol	Seeds, leaves	Anti-viral	[32]
	Nimbocinol	Seeds, leaves	Anti-viral	[32]
	Nimbidol	Seeds, leaves	Anti-inflammatory	[33]
	Nimbandiol	Leaves	Anti-viral	[34]
	Nimbinin	Seeds	Anti-viral	[35]
	Nimolinone	Seeds, leaves	Anti-tumor	[36]
	17-Hydroxyazadiradione	Leaves	Anti-viral, anti-malarial, anti-fungal	[34,37]
	7-Desacetyl-7-benzoylazadiradione	Leaves	Anti-diabetic	[38]
	7-Deacetyl-7-benzoylgedunin	Leaves	Antiviral	[38]
	Gedunin	Seeds, bark	Anti-malarial	[39]
Sterol	β-sitosterol	Seeds, flowers	Anti-hypertensive, anti-cancer	[40]
	Stigmasterol	Leaves	Anti-hypertensive, anti-cancer	[41]
Coumarin	7-hydroxy-6-methoxycoumarin	All	Anti-bacterial	[42]
Tannin	Glycerite	Bark	Anti-inflammatory	[43]
Sulfide	Propyl, propyl trisulfide	Seeds, leaves	Insecticidal, repellent,	[44]
Polysaccharide	Polysaccharide GI	Bark	Anti-cancer	[45]
	Polysaccharide GII, III		Anti-allergic	[45]
Flavone	Prenylated flavanone	Flowers	Anti-mutation	[46]
	Isoprenylated flavanone	Leaves	Hypotensive	[47]
	Quercetin	Flowers, Leaves	Lipid-lowering, hypotensive	[41]
	Kaempferol	Leaves	Lipid-lowering, hypotensive	[41]
Aliphatic acids	Nonacosane	Leaves	Anti-filtering pathogens	[48]

Azadirachtin is a highly oxidized limonin with a multitude of analogous functional groups. Within the fundamental structure of azadirachtin, R1 and R2 are the positions at which diverse groups can be substituted, engendering a spectrum of analogs. This inherent variability contributes to the wide array of mechanisms through which azadirachtin operates[56]. The presence of intra-molecular hydrogen bonds and the profusion of reactive functional groups in close proximity allows azadirachtin to assume an intricate complex structure characterized by a rigid conformation. Moreover, azadirachtin contains a distinct collection of oxygen-containing functional groups, and its enol ether configuration makes it susceptible to the influence of acids, alkalis, and light. Consequently, during the isolation and extraction of azadirachtin, the addition of ultraviolet filters such as p-aminobenzoic acid mitigates the potential loss of azadirachtin and diminishes its biological activity. Notably, azadirachtin is renowned for its proneness to rearrangement, an outcome directly attributed to its chemical structure[3,19,53,57].

Biological activity and mechanism of action

Azadirachtin, a tetracyclic triterpenoid compound structurally similar to steroid hormones, is an effective insecticide. The mechanism of action of azadirachtin emerged in 1993[58]. Several recognized mechanisms of action are attributed to azadirachtin, including: (1) anti-feedant effect: azadirachtin operates by obstructing chemical receptors responsive to phagocytic stimuli or by stimulating "deterrent" cells, thus inducing a potent feeding deterrent response in target insects[58]. This phenomenon of food rejection may be mediated by chemical receptors in insect mouthparts and the modulation of digestive enzyme production within the midgut[59–60]. (2) Inhibition of insect oviposition and post-embryonic development: azadirachtin can disrupt normal development by interfering with ecdysone steroid function, owing to structural similarities[61]. (3) apoptosis: research by Zhao et al. (2019)[62] demonstrated that azadirachtin triggers apoptosis *via* the mitochondrial pathway. Midgut damage resulting from apoptosis can lead to disruptions in digestive enzyme function, subsequently affecting the digestion and absorption of nutrient metabolites and impeding the growth and development of insect larvae. Furthermore, Zhao et al.[63] revealed that azadirachtin can influence the formation of silk glands and fatty acids, as well as affect energy metabolism in *Spodoptera frugiperda*. Additionally, studies have shown that azadirachtin administration leads to the inhibition of cell proliferation and RNA synthesis in protozoa, specifically *Tetrahymena thermophila*, along with the explicit blockade of malarial microtubule formation in a critical stage of the *Plasmodium berghei* parasite[64–65].

Beyond its insecticidal properties, azadirachtin has a spectrum of medicinal applications, including the inhibition of tumor growth and a remarkable array of properties such as anti-malarial, anti-rheumatic, anti-bacterial, anti-inflammatory, anti-pyretic, and diuretic effects[4,66]. In the context of cancer control, azadirachtin activates certain pro-apoptotic factors, while inhibiting anti-apoptotic factors that are crucial for cancer management. This results in a reduction in the *Bcl-2/Bax* ratio and an

elevated expression of *Apaf-1* and *caspase-3*, culminating in the downregulation of proliferating cell nuclear antigens. Consequently, this leads to cell fragmentation, condensation, and ultimately, tumor cell apoptosis. Additionally, *NF-κB* has been identified as a potential target for controlling tumor growth[67]. The anti-malarial mechanism of azadirachtin primarily centers on its capacity to impede microtubule formation in parasites during developmental stages, thereby influencing parasite growth and development[4]. These extraordinary medicinal attributes hold promise for effectively combating infectious diseases, highlighting the remarkable therapeutic potential of this plant.

Access to azadirachtin and the gap between supply and demand

Source plant extraction

Azadirachtin, the most prevalent compound in neem[3], is distributed across various parts of the neem plant, including seeds, calluses, fruits, leaves, branches, flowers, and bark, albeit in varying quantities. Among these, neem seeds have the highest azadirachtin content. Typically, mature neem fruits contain azadirachtin levels ranging from 0.3% to 0.5%, with some fruits registering azadirachtin levels close to 1%[19]. This variability arises because of the multifaceted influences on neem trees, including geographical region, climate, genetic diversity, agronomic conditions, plant physiology, morphology, collection methods, and storage practices[68].

Azadirachtin is typically derived from neem seed powder, and common extraction methods include solvent, supercritical, and microwave extraction[19]. The solvent extraction process typically involves a sequence of steps: initial degreasing by squeezing, crushing (either preceding or following degreasing), polar/water solvent extraction, concentration of the extractant, n-hexane degreasing, re-extraction with an organic reagent, concentration of the extractant, and multiple extraction cycles. Although solvent extraction yields azadirachtin and related limonoids, the seeds contain approximately 40% oil, necessitating early removal during extraction[3]. Solvent extraction agents can be organic reagents (such as methanol, ethanol, hexane, and petroleum ether) or water. Since it has low solubility in water, the extraction efficiency when water is used as the solvent is generally limited. Research has indicated that organic reagents, particularly ethanol, offer extraction rates up to 50 times higher than water-based methods[69]. In fact, most published studies have predominantly employed alcohols, specifically methanol and ethanol, to extract azadirachtin from neem trees.

Supercritical fluid extraction (SCF) emerged as an advanced method in the 1980s. It offers a streamlined approach in which extraction and separation occur in a single step, resulting in improved production efficiency, reduced energy consumption, fluid recycling, and cost savings. This technique is widely regarded as environment-friendly. Given the global shift towards eco-friendly products, supercritical fluid extraction has become the preferred solvent extraction method. Notably, this separation process was conducted in a light-controlled environment with an extraction temperature close to room temperature, rendering it particularly suitable for the extraction of photochemically sensitive azadirachtin[19]. Table 2 provides azadirachtin content data for various plant tissues from different sources, while Table 3 offers insights into the azadirachtin yield using different extraction methods.

Explant histoculture

Obtaining azadirachtin through *in vitro* culture offers numerous advantages over the natural growth yield. It is characterized by speed, controllability, minimal spatial footprint, and immunity to local geography and climate variations[4]. *In vitro* growth can be achieved using three primary methods: callus culture, suspension cell culture, and hairy root culture. In 1999, Lei et al. optimized the callus culture technique to achieve an azadirachtin production level of 3.53 mg/L. In another study, Singh et al.[103] investigated azadirachtin accumulation in callus tissues induced by the dedifferentiation and redifferentiation of diverse neem explants (such as zygotes, leaves, and ovaries). Their findings revealed that the highest azadirachtin content reached 2.33 mg/g dry weight. Srivastava and Srivastava[104] observed that conventional bioreactor designs (stirred tanks and bubble columns) were unsuitable for neem root growth. Nevertheless, an enhanced configuration utilizing a bubble column reactor with polyurethane foam as the root support facilitated dense azadirachtin production. The ultimate yield from hairy roots amounted to 9.2 g/L (dry weight), with an azadirachtin yield of 3.2 mg/g and a total yield of 1.14 mg/L/day. Although techniques such as tissue

Table 2

Azadirachtin contents in various plant tissues of different origins

Sample	Content	Origin	Reference
Seeds	0.19%(w/w)	India	[70]
	210.93 mg/g	India	[71]
	0.08% (w/w)	India	[72]
	3030.8 g/kg	India	[73]
	0.56%–0.30% (w/w)	India	[73]
	142–9,527 μg/g	India	[74]
	0.08%–0.095% (w/w)	India	[75]
	3,862.9–48,521 μg/g	China	[76]
	6.5–8.2 mg/g	China	[77]
	8.2 g/kg	China	[77]
	0.0867% (w/w)	Togo West Africa	[78]
	752 ppm	Germany	[79]
	0.16%–0.27% (w/w)	Brazil	[80]
	0.20%–0.51% (w/w)	Brazil	[81]
	0.431% (w/w)	Brazil	[82]
	0.476–3.09 mg/g	Sudan	[83]
	2.24 g/kg	Senegal	[84]
	5,419.08 pg/μg	Mali	[85]
Callus	0.00005% (w/w)	Togo West Africa	[78]
	0.2470 μg/g	India	[86]
	214.53 mg/g DW	India	[87]
Leaves	182.42 pg/μg	Mali	[85]
	182.42 μg/g	India	[88]
	969.9–5,419.08 μg/g	China	[76]
	86.45 mg/g DW	Iran	[89]

Table 3

Extraction and detection methods of different neem parts

Sample	Extraction method	Time	Dissolvant	Test method	Reference
Seed	Ultrasound	18.41 min	Hexane	UPLC-QTOF	[70]
Seed	Liquid-solid extraction	24 h	N-hexane, Ethyl acetate	HPLC	[90]
Leaves, fruits, flowers	Liquid-solid extraction	–	Acetone, Ethanol, Methanol	UHPLC Q-Orbitrap	[91]
Seed, Leaf	Ultrasonic-assisted method	40 min	acetonitrile	LC–Q-TOF–MS	[76]
Seed	Liquid-solid extraction	48 h	n-Hexane	HPLC-UV	[92]
Seed	Schroeder and Nakanishi Method	30 min	Alcohol	SFC	[93]
Kernels	Soxhlet extraction	2 h	n-Hexane	HPLC	[79]
Leaves, bark, roots	Soxhlet extraction	–	Water	LC–MS	[94]
Fruit	Reflux extraction	48 h	Alcohol	HPLC-UV	[92]
Seed	Thorns extraction	24–72 h	Carbinol	HPLC-UV	[72]
Seed	Screw press	20 min	–	–	[95]
Leaf	Pressurized liquid extraction	–	Carbinol	HPLC-UV	[71]
Bark	Shaking	–	Methanol	Spectrophotometric	[96]
Seed	Supercritical fluid extraction	150 min	Methanol	HPLC	[97]
Kernels	Cold extraction	–	Ethanol	HPLC	[98]
Leaf	Cold extraction	48 h	Petroleum ether	–	[99]
Seeds	Cold-pressing	–	Hexane	HPLC-UV	[100]
Fruits	Ultrasound-assisted extraction	30 min	Ethanol	HPLC-UV	[82]
Seeds	Maceration	3 d	Methanol	HPLC-UV	[71]
Leaf	Percolation, decoction, freeze drying or spray drying	6 h	Water	TLC	[101]
Fruits	Percolation	–	Hexane	HPLC-UV	[102]

HPLC: High performance liquid chromatography; LC–MS: Liquid chromotography with mass spectrometry; LC–Q-TOF–MS: Ultra-high performance liquid chromatography coupled with quadrupole/time of flight mass spectrometry; SFC: Supercritical fluid chromatography; TLC: Thin layer chromatography; UHPLC: Ultra high performance liquid chromatography; UPLC-QTOF: Ultra performance liquid chromatography-quadrupole-time-of-flight mass spectrometry.

culture enable the *in vivo* biosynthesis of azadirachtin, the low yields fall short of meeting the actual demand.

Chemical synthesis

The laboratory-based chemical synthesis of azadirachtin presents a formidable and intricate challenge[6,19]. This complexity stems primarily from the distinctive chemical characteristics of azadirachtin, notably its intricate chirality, susceptibility to alkaline and strong acids, light sensitivity, hydrogen bonds, and intricate internal structure[6,105]. The chemical synthesis of azadirachtin is divided into two core components: decalin and tricyclic dihydrofuran segments (Figure 3). In 1991, significant breakthroughs were achieved by successfully assembling suitable decahydronaphthalene fragments. This involves coupling two quaternary carbon centers and introducing a methyl group at the C8 position to form a highly obstructed bond between C8 and C14. This strategic maneuver improved the feasibility of linking the left and right segments in the subsequent stages. The resultant decahydronaphthalene fragment encompassed all the requisite functional groups, but required 31 chemical steps for synthesis. This phase of synthesis represents one of the most formidable challenges in the artificial production of azadirachtin, serving as a critical foundation for its overall synthesis[6,19,53,106–108]. The tricyclic dihydrofuran segment on the right side of azadirachtin serves as a pivotal intermediate in its chemical synthesis. Shibasaki et al.[109] achieved its initial synthesis in 1989, which was a significant milestone in the artificial synthesis of azadirachtin. Subsequently, numerous methods for the synthesis of tricyclic dihydrofuran fragments have emerged, offering diverse avenues for azadirachtin synthesis[110].After 22 years of persistent research, Ley et al.[6,53] successfully joined decadene and tricyclic dihydrofuran fragments through an O-alkylation reaction involving two intermediates: the decalin fragment and pyran fragment a/b. Subsequently, the previously formed allene participates in another carbon-carbon bond formation reaction involving the cyclization of 5-racemates to create a double-ring system. Finally, a series of additional reactions and processes culminated in the synthesis of azadirachtin and attainment of the final product. The complete synthesis of azadirachtin required 71 steps, resulting in a yield of 0.00015%[6]. Boyer et al.[111] managed to reduce the number of steps to 62 by enhancing the synthesis route but remained far from meeting the fundamental requirements for large-scale azadirachtin production. Currently, plant-derived azadirachtin is the preferred choice for biotesting and field applications.

Biosynthesis to meet demand

Despite considerable efforts by both domestic and international researchers to enhance azadirachtin production, synthetic technology and large-scale production of azadirachtin remain elusive. Whether azadirachtin is pursued through tissue culture or biosynthesis, further research on the biosynthetic pathway and regulatory network of azadirachtin is imperative. The rapid evolution of omics technology has opened new avenues for analyzing the synthetic pathway of azadirachtin through the combined application of diverse multidisciplinary technologies (Figure 4). For a comprehensive perspective on the advantages and drawbacks of the various azadirachtin acquisition methods, please refer to Table 4.

Progress in transcriptome/genome research

The elucidation of the neem genome was a gradual process, culminating in its completion in 2011[7]. Rapid advancements in sequencing technology have propelled comprehensive analysis of the neem genome and

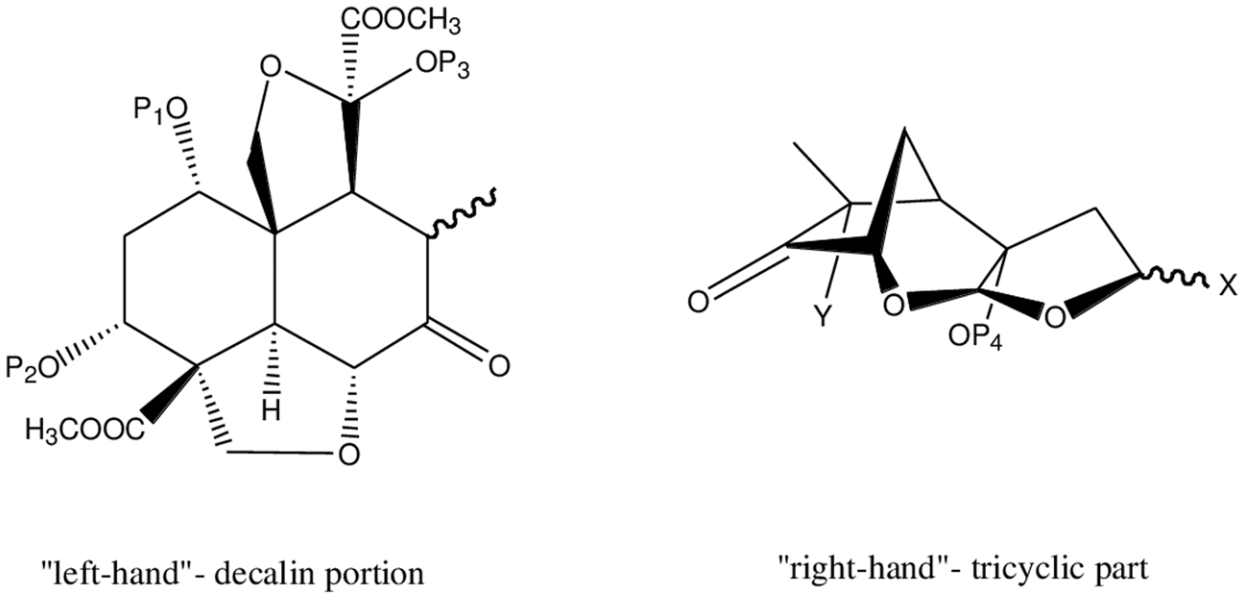

Figure 3. Two parts of the chemical synthesis of azadirachtin.

transcriptome. Building on this foundation, scientists have identified pivotal genes within the azadirachtin synthesis pathway, providing valuable insights into its biosynthetic route. For a compilation of known *Meliaceae* genomes, please refer to Table 5.

Between 2011 and 2016, scientists employed diverse sequencing and assembly techniques to obtain genomic data from various neem plant parts, including the fruits, roots, stems, leaves, and flowers. These efforts yielded genome size estimates ranging from 182.93 to 364.12 Mb, with N50 values averaging 15,948 bp. Although this study enabled the selection of key genes within the azadirachtin synthesis pathway, their functions still require verification. In 2011, Krishnan et al.[7] conducted *de novo* sequencing, assembly, and preliminary transcriptome analysis of mature neem fruit using RNA-seq technology. They compared the annotated transcripts from 24 different species, including citrus, castor, and grape. This study classified neems within the same systematic grouping as citruses and marked the completion of the neem Genome Project, laying the groundwork for deciphering the biosynthetic pathways of citrine compounds. In 2012, researchers used multiple sequencing platforms and libraries to sequence the genomes and transcriptomes of various neem tissues (roots, stems, leaves, and flowers). This yielded a draft map of the neem genome, covering 364 Mb (95% of the published genome). The transcript counts for the root, stem, leaf, and flower tissues were 27,916, 27,369, 34,518, and 31,223, respectively. This comprehensive study expanded on previous efforts, encompassing the entire genome and transcriptome of neems in these tissues. Molecular phylogenetic analysis and sequence similarity confirmed the close relationship between neems and citrus species. Analyses of potential genes involved in the terpenoid biosynthetic pathway indicated a relatively greater abundance of enzymes related to azadirachtin synthesis in neems than in plants like Arabidopsis[8]. In 2014, Rajakani et al. and Narnoliya et al.[10–11] cloned and analyzed small subtraction transcriptomes from neem fruit, peel, and leaves, and constructed relevant inhibitory subtraction hybridization and transcriptome differential expression databases. While this study enriched the transcriptome data of the neem genome and identified several P450 genes, functional validation remained incomplete. In 2015, Kuravadi et al.[112] sequenced and assembled three neem genomes from different regions of southern India, yielding a genome size of 267 Mb (70% of the estimated neem genome) containing 44,495 genes. A comparative analysis revealed an overlap with citrus chromosomes. Weighted correlation network analysis identified potential candidate genes involved in the azadirachtin biosynthetic pathway. In 2016, Krishnan et al.[9] improved the genome assembly (v2.0) by employing Illumina for short reads and the Pacific Biosciences Single Molecular Real-Time (SMRT) sequencer for long reads. These technologies significantly enhanced assembly, with a three-fold increase in N50 and N75, a 2.6-fold reduction in the number of scaffolds, and a 1.25-fold increase in effective transcriptome alignments compared to the earlier assembly (v1.0). Misassemblies were reduced by 13.4-fold and the percentage of repeats increased by 1.85%. However, the low proximity of the neem genome draft limits its application in downstream genome research. In 2022, scientists achieved the first neem chromosome-level assembly. Du et al.[16] performed Illumina sequencing, and the combination of PacBio and Hi-C technologies led to the assembly of a chromosome-level genome with a size of 281 Mb. Comparative genomic analysis revealed that most neem-specific TPS and CYP genes reside in a terpene-associated cluster on chromosome 13, suggesting their crucial role in terpene biosynthesis in neem. Subsequently, Cui et al.[15] assembled a chromosome-level whole-genome sequence spanning 237.16 Mb. Contig N50 improved nearly 400-fold compared with that of the reference genome, offering high base accuracy and continuity. This study traced the evolutionary history of genes, whole-genome duplication events, and the evolution of secondary metabolite clusters and resistance genes. Notably, extended families of terpene synthases,

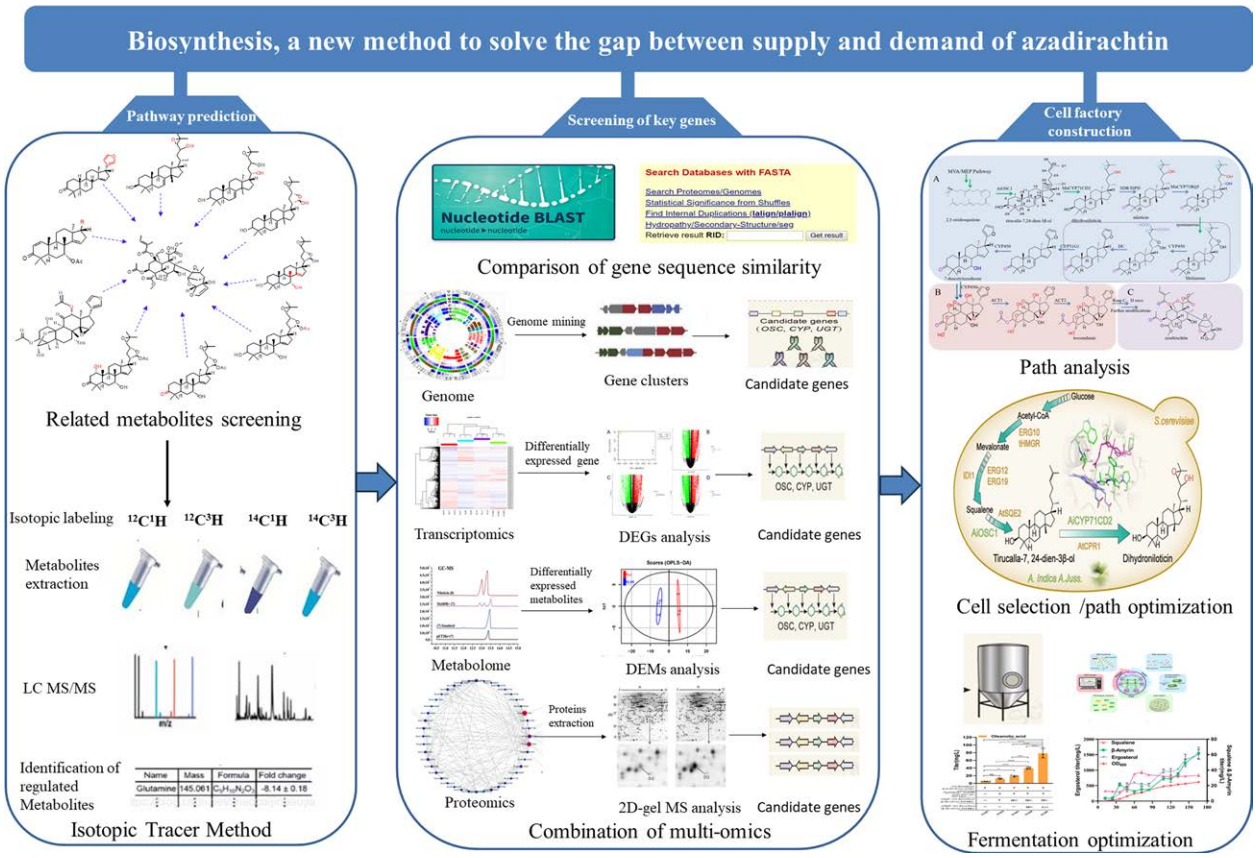

Figure 4. Biosynthesis, a new method to solve the gap between supply and demand of azadirachtin.

O-methyltransferases, and cytochrome P450s primarily arose from tandem duplications, indicating that such duplications play a pivotal role in the interspecies variation in limonoids.

The correlation between gene expression and metabolic profiles across different neem tissues shows promise for identifying gene family members involved in the later stages of neem triterpene biosynthesis[12]. Researchers have made significant strides in exploring the neem transcriptome, leveraging transcriptome lobes alongside metabolome differential expression analysis and other pertinent genetic data to facilitate relevant gene screening and metabolic pathway analysis (Table 6). In 2012, Krishnan et al.[8] conducted transcriptome measurements of neem roots, stems, leaves, and flowers. Subsequently, in 2017, Bhambhani et al.[12] sequenced and assembled the transcriptomes of neem fruit tissues (FS3) with high azadirachtin content, as well as neem leaf tissues with low azadirachtin content. Expression and molecular docking analyses indicated the involvement of specific members of the CYP450 family in secondary modifications of bioactive triterpenes. Three P450 genes (eg, *AiCYP16671*, *AiCYP16365*, and *AiCYP18835*) linked to triterpene biosynthesis were identified by integrating transcriptome data with metabolomic differential expression analysis. Molecular docking further revealed robust binding of azadirachtin A and azadirachtin B to *AiCYP16671* and *AiCYP16365*, forming multiple hydrogen bonds. However, further characterization of these CYP enzymes is required to confirm their role in the azadirachtin biosynthetic pathway. In 2019, Hodgson et al.[17] integrated the comparative information from neem and citrus genomes and transcriptomes. They identified three 2,3-oxygen squalene cyclase (OSC) genes and two genes involved in azadirachtin synthesis, including P450. This investigation unveiled that *AiOSC1* catalyzes the conversion of 2,3-oxygen squalene into tirucalla-7,24-dien-3β-ol, a foundational skeletal compound. Consequently, it was established that the initial scaffold in azadirachtin synthesis is tirucalla-7,24-dien-3β-ol, rather than tirucallol, euphol, or eupha-7,24-dien-3β-ol. This breakthrough in the identification of the core compounds marked a pivotal advancement in deciphering the azadirachtin pathway. Furthermore, in 2020, Lian et al.[13] conducted an analysis of azadirachtin content across different neem tissues. They observed that the highest azadirachtin content was present in the developing fruit and gradually diminished as the fruit matured. Building on this discovery, researchers gathered 346,116 transcripts from combined RNA samples obtained from neem fruits, leaves, and young bark. From these data, they predicted 186,263 genes. By examining gene expression and metabolite profiles, they identified six OSC genes. Among these, *MtOSC1* was found to catalyze the formation of the skeleton compound tirucalla-7,24-dien-3β-ol, while *MtOSC6* acted as a lupinol synthase, a pivotal enzyme in luxol synthesis. In the same year, Wang et al.[14] employed hybrid sequencing [using Illumina HiSeq and Pacific Biosciences SMRT to analyze transcriptomic data from five distinct neem tissues. This analysis aimed to identify crucial genes. Candidate genes were initially screened based on expression level comparisons across

Table 4

Advantages and disadvantages of azadirachtin acquisition method

Methods	Advantages	Disadvantages
Source plant extraction	Rich resources	Low content, occupying a large amount of land resources, difficult to extract and separate, vulnerable to environmental, storage and other conditions
Chemical synthesis	Reaction conditions are controllable; full synthesis has been achieved	The reaction process is complicated, the preparation period is long, the yield is low, the cost is high and the separation is difficult
Endophytic fermentation	Can realize the synthesis of various functional components, and does not need to know the synthesis route.	Difficult to isolate strains and purify fermentation products
In vitro culture (callus, suspension cell, hair root culture)	Fast, controllable, small footprint and unaffected by geography and climate	Low yield
Biosynthesis	Novel, efficient, green, and environment-friendly, with simple components and easy separation	The biosynthetic pathway is difficult to analyze and the metabolic pathway is complex

Table 5

Known *Meliaceae* genomes

Species	*Azadirachta indica*	*A. indica*	*A. indica*	*A. indica*	*A. indica*	*Melia azed*arach
Year	2012[8]	2015[112]	2016[9]	2022[16]	2022[15]	2022[15]
Tissue	Root, leaf, stem, flower	Leaves	Root, leaf, stem, flower	Leaves	Leaves	Leaves
Sequencing platform	Illumina, IonTorrent	Illumina, Roche/454	Illumina, PacBio	Illumina, PacBio, HiC	Illumina, ONT, HiC	Illumina, ONT, HiC
Denovo	SOAP denovo	Velvet	SOAP denovo2	RACON, Pilon	NextDenovo	NextDenovo
Genome size/Mb	364.12	267	182.93–308.83	281	232.68	239.23
Contig N50/bp	740	15,948	3,491	6,039,544	8,907,986	8,068,821
Longest Contig/bp	10,111	241,170	–	15,111,501	19,556,515	18,913,001
Longest Scaffold/bp	3,641,215	–	12,211,325	–	21,501,397	22,810,243
Number of Scaffolds	9,714	–	21,743	70	82	98
Number of genes	20,169	44,495	32,316.77	25,767	23,087	21,983
Average gene length/bp	1,695.95	876 (CDS)	–	25,767	3,101.7	3,308.55
Total repeat element/bp	47,427,034	86.9 Mb	54,375,206	115,181,900	77,049,478	89,633,253
Repeat element ratio/%	13.03	32.44	24.15	40.99	33.11	37.47
Level	Draft	Draft	Draft	Chromosome	Chromosome	Chromosome

the five tissues. Subsequently, 22 potential azadirachtin A-related genes were identified using phylogenetic analysis, domain prediction, and molecular docking. These genes encompass a range of functions including OSC, ethanol dehydrogenase, cytochrome P450 (CYP450), acyltransferase, and esterase. Notably, this set of genes included *MaOSC1*, which exhibited high homology to the previously identified *AiOSC* gene, as well as *MaCYP71CD2* and *MaCYP71BQ5*. This is the first report on the transcriptome analysis of neems utilizing hybrid sequencing. Moreover, Osbourn et al.[18] harnessed systematic transcriptome and genome mining techniques, along with co-expression, phylogenetic, and comparative analyses; they managed to convert an intermediate into a compound with a core C26 scaffold.

Progress in synthetic pathway research

The biosynthesis of medicinal plants relies on the meticulous examination of their intricate metabolic pathways, often in conjunction with the host cell's metabolic routes. This allows for replication or transfer of the desired metabolic pathways. Alternatively, the chemical structures of existing target components or intermediates can serve as a basis for identifying new metabolic pathways that can be integrated into host cells. Given the intricate chemical composition of azadirachtin, a comprehensive understanding of its synthetic pathway requires the understanding of various biosynthetic steps, including oxidation, esterification, demethylation, side-chain degradation, and ring-opening. Presently, the upstream synthesis pathway for plant triterpenes and sterols is well established and involves mevalonate (MVA) in the cytoplasm and 2-C-methyl-D-erythritol-4-phosphate (MEP) originating from 2,3-oxidized squalene in the plastid. This 2,3-oxidized squalene serves as a common precursor for all steroids and triterpenes. Diverse cyclases catalyze the conversion of 2,3-oxidized squalene into various triterpene skeleton compounds. These intermediates undergo oxidation, esterification, demethylation, and other modifications to yield a wide array of triterpene compounds[113–114].

In the context of azadirachtin biosynthesis, researchers have focused on the first step in the downstream pathway, involving 2,3-oxide-squalene cyclase and its triterpene skeleton. Previous research suggested a synthetic

Table 6

Known *Meliaceae* transcriptomes

Species	Sequencing platform	Total reads	Read number	Inchworm contigs	Total transcripts	N50	GC (%)	Screening gene	Year	References
Root Leaf Stem Flower	Illumina, IonTorrent	4,566,554 57,670,643 148,819 27,916	– – – –	5,429,138 67,596,347 172,507 34,518	5,058,312 60,219,435 150,126 31,223	– – – –	– – – –	8 genes: TPS21, lytB/ispH, ispE, GGPS, +neomenthol dehydrogenase, FDPS, FDFT1, and SQLE	2012	[8]
Fruit Leaf	454-GS FLX ROCHE GS Assembler	707,392 849,781	14.56 18.17	563,212 697,725	60,876 66,335	– –	41 41.1	CYP16365, CYP16671, and CYP18835	2017	[12]
Leaf Flower Stem Fruit Root	Illumina, HiC	– – – – –	41.14 41.35 40.60 40.59 40.64	– – – – –	50,394 62,426 65,762 66,668 45,459	2,201 2,335 2,372 2,077 2,100	40.64 40.13 40.39 41.93 41.10	22 genes (1 OSC, 2 ADH, 2 ACT, 5 ESTs, and 12 P450): AiOSC1, MaCYP71CD2, and MaCYP71BQ5 have been verified.	2020	[14]
Fruits, leaves, and barks	Illumina, PacBio	394,754,246	–	–	346,116	2,258	–	6 OSCs: MtOSC1 and MtOSC6 have been verified	2020	[13]

pathway from 2,3-epoxidized squalene to azadirachtin A. It was suggested that 2,3-epoxidized squalene initially forms a *Euphorbia* dienol intermediate under enzymatic catalysis, followed by a series of reactions leading to the production of azadirachtin A. However, this inference was deemed inconclusive at the time[115]. Subsequently, the abundant azadirachtin genome and transcriptome information available to the public was analyzed, including the complementary DNA (cDNA) library[7], draft genome[8–9], EST libraries[10–11], transcriptome data[8,12–14], and chromosome-level genome data[15–16]. This search encompassed genes from the MVA pathway, MEP pathway, 2,3-oxosqualene synthesis, and cytochrome P450 (CYP450s). Nevertheless, none of these isolated genes has been functionally characterized, and dissection of their pathways yielded limited progress until Osbourn et al. successfully identified the key enzyme AiOSC1 from the neem transcriptome. AiOSC1 plays a pivotal role in converting 2,3-oxidized squalene into tirucalla-7,24-dien-3β-ol, marking a significant breakthrough in the analysis of the azadirachtin pathway[17].

In 2019, Osbourn et al. made a significant breakthrough in the identification of *AiOSC1* in azadirachtin synthesis by meticulously mining the existing neem transcriptome data. Their study revealed that *AiOSC1* plays a catalytic role in transforming 2,3-epoxidized squalene into tirucalla-7,24-dien-3β-ol. Notably, their findings challenged prior assumptions by establishing tirucalla-7,24-dien-3β-ol as the initial scaffold for azadirachtin synthesis, debunking earlier notions that tirucallol, euphol, or eupha-7,24-dien-3β-ol served this role[17]. Notably, in 2009, the gene *PEN 3* was identified as another catalyst for 2,3-epoxidized squalene, resulting in the generation of tirucalla-7,24-dien-3β-ol. Furthermore, this process produced small quantities of other compounds such as butyrospermol (6%), tirucallol (6%), isotirucallol (1.5%), and 13β-H-malabarica-14(27),17,21-trien-3β-ol and dammara-20,24-dien-3β-ol (0.5%)[116]. In addition to the identification of 2,3-oxosqualene cyclases, Osbourn's study also pinpointed the involvement of two P450 enzymes, *MaCYP71CD2* and *MaCYP71BQ5*, in the side-chain oxidation modification of tirucalla-7,24-dien-3β-ol. *MaCYP71CD2* catalyzes C23 hydroxylation and oxygenation between the C24 and C25 carbon bonds, establishing this gene as a multisite oxidase. On the other hand, *MaCYP71BQ5* catalyzes the C21 hydroxylation of tirucalla-7,24-dien-3β-ol, resulting in the formation of tirucalla-7,24-dien-21,3β-diol. Two successive genes then act on tirucalla-7,24-dien-3β-ol to produce dihydroniloticin and melianol. This remarkable progress in enzyme identification was further advanced by Lian and Wang in 2020, who identified MtOSC1 from China and *AiOSC1*, *AiCYP71CD2*, and *AiCYP71BQ5* from neem, respectively[13–14]. In 2023, Osbourn et al.[18] and his team achieved another milestone by identifying a set of candidate genes related to citrus biosynthesis. They employed a systematic approach that combined transcriptome and genome mining, co-expression analysis, phylogenetics, and comparative studies across citrus and neem datasets. This exhaustive effort resulted in the discovery of 22 new enzymes spanning 12 catalytic steps. These steps include ring-opening rearrangement, C4 bond cleavage, furan ring formation, and critical oxidative modifications, which are typical of limonoids. Importantly, these enzymes enable the complete synthesis of azadirachtin from the core C26 scaffold compound, encompassing the entire journey from the skeletal structure to azadirone and kihadalactone A. The detailed synthesis steps are illustrated in Figure 5.

Multi-omics techniques facilitate pathway resolution

Research on the biosynthesis of terpenoid active ingredients hinges on meticulous analysis of their biosynthetic pathways. Various innovative research strategies have emerged to effectively harness Chinese medicine resources. These approaches encompass simulating terpenoid active ingredient biosynthesis pathways, leveraging synthetic biology techniques in host cells (such as *Escherichia coli, yeast,* and *tobacco*), designing and integrating terpenoid active ingredient biosynthesis pathways, constructing and optimizing "cell

factories," and fine-tuning metabolic flux to enable the heterogeneous synthesis of terpenoid active ingredients within host cells[117–119]. A pioneering breakthrough was achieved by the Keasling Research Team at the University of California when they successfully transferred the artemisinic acid synthesis pathway into yeast cells and regulated its metabolism. This yielded yeast strains capable of fermenting and producing remarkable concentrations of artemisinic acid (25 g/L). Subsequently, a sequence of four chemical catalytic steps led to the successful synthesis of artemisinin, marking a significant advancement in artemisinic acid production[120]. This groundbreaking work inspired further efforts to synthesize functional components found in traditional Chinese medicine within microbial systems. These components include cannabinoids, ginsenosides, paclitaxel, tanshinone, scutellarin, glycyrrhetinic acid, and emodin[117–119,121]. For example, Liu et al. heterologously expressed the oxidation squalene cyclase genes *AiOSC1* or *MaOSC1* along with the cytochrome oxidase MaCYP71BQ5 from *A. indica* in high squalene-producing brewing yeast. This pioneering study successfully yielded the corresponding intermediates in the azadirachtin pathway[122]. Similarly, Hou et al. achieved impressive results by over-expressing *AiCYP71CD2* in yeast cells, leading to the synthesis of dihydroniloticin in an impressive yield of 405 mg/L[5].

In addition to synthesizing known compounds, Muranaka et al. made significant strides in 2013 by identifying the pivotal P450 genes, *CYP72A61v2* and *CYP72A68v2*, within *Medicago truncatula*. They subsequently introduced *β-AS*, *CPR*, *CYP72A63*, *CYP93E2*, and *CYP716A12 i*nto yeast cells, effectively enabling the production of P450 genes not naturally found in *M. truncatula* plants[123]. Building on this foundation, Chun et al. accomplished a transformative goal in 2020. Homology modeling and molecular docking techniques were employed to manipulate the *CYP72A63* gene element within the glycyrrhetinic acid synthesis process. This endeavor yielded rare yeast cells capable of producing valuable compounds, such as glycyrrhetinol, glycyrrhetinic aldehyde, and glycyrrhetinic acid[118]. These examples underscore the remarkable success in the realm of synthetic biology, in which the synthesis of structurally analogous compounds with high activity and low content has been achieved. Biosynthesis, in particular, is a viable avenue for the industrial-scale production of azadirachtin intermediates and their derivatives. Moreover, it paves the way for the development of new drugs, including those linked to traditional Chinese medicine and other natural remedies.

Discussion

Many medicinally active ingredients in neem, including azadirachtin, are present in low concentrations. The long growth cycle of neem is constrained by factors such as time, space, and climate, making its cultivation challenging. Another hurdle in extracting target compounds from plants is the presence of numerous structurally similar compounds, which increases preparation costs[62]. Most natural products exhibit intricate structures and numerous chiral centers, rendering the chemical synthesis

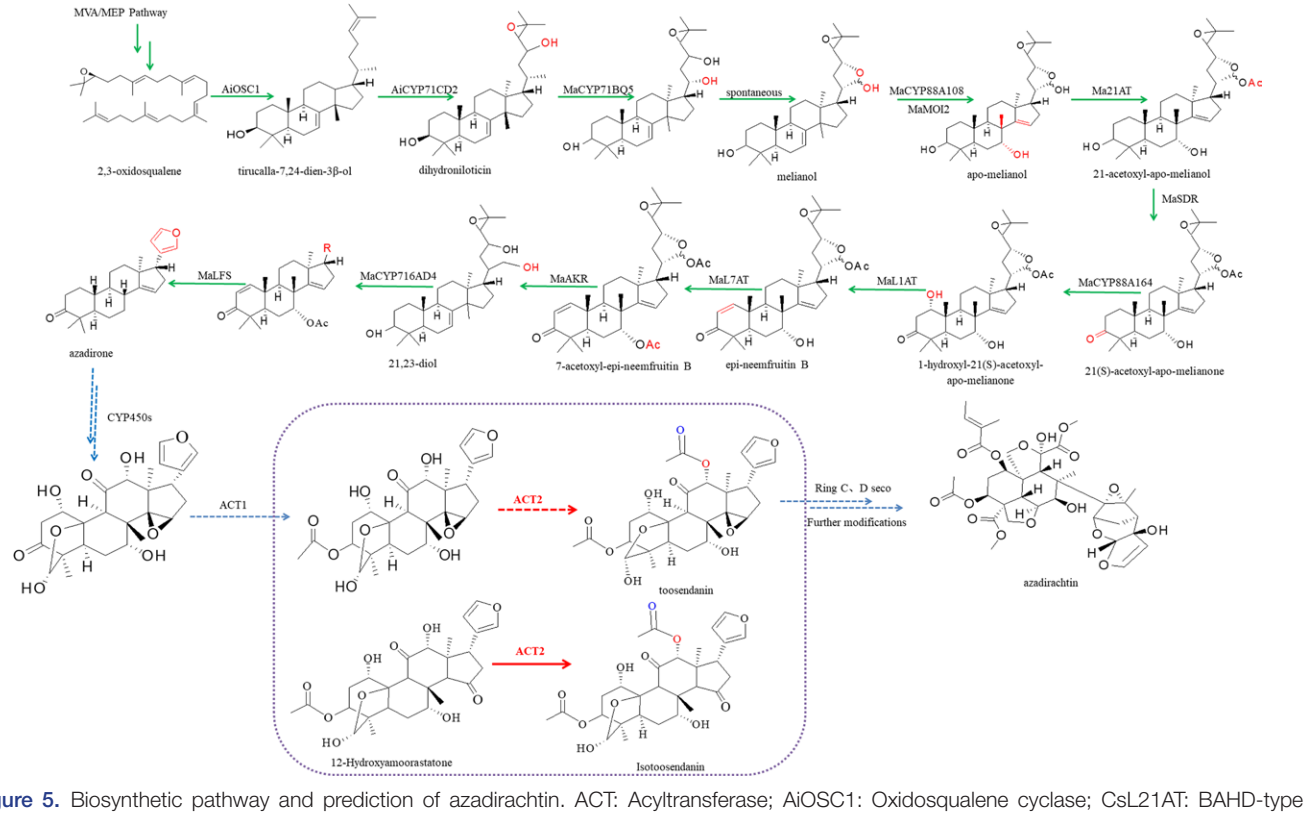

Figure 5. Biosynthetic pathway and prediction of azadirachtin. ACT: Acyltransferase; AiOSC1: Oxidosqualene cyclase; CsL21AT: BAHD-type acetyltransferases; MaAK: Aldo-keto reductases; MaLFS: Limonoid furan synthase; MaMOI2: Melianol oxide isomerases; MaSDR: Short-chain dehydrogenases.

arduous, step-intensive, and inefficient. This complexity leads to the generation of numerous byproducts, high extraction costs, and irreversible environmental damage[19].

Synthetic biology offers a promising avenue for azadirachtin production, with notable recent progress in biosynthesis. However, several challenges remain unaddressed. Complete elucidation of the biosynthetic pathway remains incomplete, requiring further modifications to azadirone, such as C and D ring openings, C12 acylation, and C11 hydroxylation. Additionally, some enzymes struggle to express themselves correctly or fulfill their functions in heterologous hosts, hindering the maintenance of a dynamic balance among multiple components.

To efficiently synthesize target functional ingredients within cell factories, the choice of chassis cells is of paramount importance. Chassis cells possess a clear genetic background, straightforward processing techniques, ease of cultivation, and adaptability to exogenous enzymes and products. Chassis cells frequently studied in the recent literature include *E. coli*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, tobacco, and tomatoes. *S. cerevisiae* stands out for its ability to express enzymes that require a transmembrane protein pathway, such as P450, without the need for extensive transmembrane protein modifications. This yeast species, with its rich membrane structures, such as the endoplasmic reticulum, facilitates the proper localization and folding of P450. Compared with *E. coli*, *S. cerevisiae* has several advantages in azadirachtin biosynthesis. For instance, Liu et al. successfully expressed the oxidation squalene cyclase gene *AiOSC1* or *MaOSC1* and the cytochrome oxidase *MaCYP71BQ5* from *A. indica* in high squalene-producing brewing yeast, synthesizing the corresponding intermediates in the azadirachtin pathway[122]. Hou et al. synthesized dihydroniloticin in yeast cells by over-expressing *AiCYP71CD2*, yielding 405 mg/L[5]. Tobacco is a model plant for foreign gene expression and is widely used in laboratory settings. Plant expression patterns include genes encoding enzymes, organelles with similar structures and functions, coenzymes, coenzyme factors, and precursors that facilitate the expression and post-translational modification of plant proteins. Thus, tobacco is more suitable for the synthesis of functional components from medicinal plants than from bacteria or fungi. For example, Osbourn et al. analyzed the azadirachtin biosynthesis pathway using a tobacco transient expression system, and later validated it in yeast cells and *in vitro*. Future research should explore other transient expression systems, such as *A. indica* hairy roots, to create a more suitable reaction environment for azadirachtin synthesis.

Beyond selecting chassis cells, by transferring the original metabolic pathway or creating a new pathway, biosynthesis in engineered strains is interconnected within a dynamic and balanced metabolic network. When engineering strains, optimizing and regulating this metabolic network is crucial to enhance the synthesis and accumulation of target medicinal components. Biosensors capable of real-time signal outputs enable dynamic monitoring and feedback to balance biosynthetic pathways and improve natural product yields. For example, Yang et al. established a regulatory system using the hexadiene diacid (MA) promoter in *E. coli*. In the absence of MA, two MA-responsive transcription factors (*CatR*) form a tetramer that binds to a 26 bp inhibitory sequence (*rbs*) and a 14 bp activation sequence (*abs*), causing DNA bending and preventing RNA polymerase Rep from activating the catBCA promoter. This effectively halted the transcription of the gene *catBCA*. When MA is present, it triggers a conformational change in *CatR*, reducing DNA bending and enabling the RNA polymerase to activate *catBCA* transcription. Combined with RNAi technology, this dual-function dynamic regulatory metabolic network resulted in an MA yield of 1.8 g/L[124].

After introducing corresponding key enzyme genes into heterologous host cells, challenges such as incorrect folding, post-transcriptional modification, improper positioning, cofactor deficiencies, non-optimal pH, unnatural substrate use, and product feedback inhibition may arise. Enzyme engineering can address some of these problems, such as by modifying the membrane localization sequence of P450 genes to improve organelle/environmental positioning[125] or by expanding the endoplasmic reticulum through engineering. This expansion enhances the synthesis and folding capabilities of related proteins, alleviating metabolic limitations caused by limited enzyme abundance[126]. Additionally, during functional gene screening, computationally biological methods can be employed to mine and study a database of possible functional components within natural product biosynthesis pathways with greater precision. This approach significantly reduces workload and enhances screening efficiency[5].

Conclusions

Azadirachtin is a fascinating compound known for its potent insecticidal effects and a range of pharmacological applications, including tumor growth inhibition, and anti-malarial, anti-bacterial, and anti-inflammatory properties. Analysis of its chemical structure was a painstaking 18-year endeavor, and it took 22 years to achieve chemical synthesis. Our understanding of its genome and transcriptome emerged only in 2011/2012, and its biosynthetic pathway has been clarified, but has not yet been fully analyzed. Despite years of dedicated efforts, our progress in the application, mechanism of action, and acquisition methods of azadirachtin is noteworthy. However, its industrial production and application continue to face limitations imposed by various factors. To unlock its full potential, it is imperative to leverage scientific knowledge for deeper azadirachtin exploration. This will enable broadening and enhancing their acquisition and applications in a more effective and widespread manner.

Using synthetic biology, we can reconstruct the synthetic pathways of specific compounds within chassis cells. This involves designing the genetic elements responsible for the synthesis of high-value natural products. This approach serves multiple purposes: it enables continuous large-scale production of complex plant-derived natural products;

addresses supply shortages; facilitates the synthesis, activity evaluation, and development of rare intermediates; and allows for the creation of structural analogs that are challenging to find in nature. Furthermore, biosynthesis is poised to play a pivotal role in the industrial production of high-value-added natural products.

Conflicts of interest statement

The authors declare no conflict of interest.

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Author contributions

Xinyao Su and Zhipeng Liang are the main drafters of the manuscript. Caixia Wang conceived the manuscript idea, Qiang Xue, Jia Liu and Xuemi Hao revised and provided critical version of the manuscript. All authors contributed to the revision of the manuscript and approved the final manuscript.

Ethical approval of studies and informed consent

Informed consent was obtained from all participants. These authors contributed equally.

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None.

Data availability

All data generated or analyzed during this study are included in this published article.

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