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Review Article

A review on biosynthesis, regulation, and applications of terpenes and terpenoids

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ABSTRACT

Essential oils (EOs) are concentrated liquids extracted from various parts of plants and can be classified based on their phytochemical compounds. Terpenes and terpenoids have a wide range of biological activities, including anticancer, anti-inflammatory, antimicrobial, antioxidant, and antiallergic properties. Terpenes are plant-based compounds commonly used in the pharmaceutical, food, biofuel, and chemical industries by humans. In synthetic biology, genomic resources and emerging tools facilitate the production of high-quality terpenoids in plants and microbes. Terpenoids, however, are difficult to produce in large quantities due to their complex chemical structures and the limited amounts found in plants. The regulation of terpenoid biosynthesis has gradually emerged as a research priority. This review presents an overview of the biological activities, synthesis pathways, and key enzymes involved in the biosynthetic pathways and regulation of terpenes or terpenoids. This review will also include references for further research on molecular regulation, biological advancements, and increasing the content of terpenes or terpenoids in plants.

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1. Introduction

Medicinal plants have been used by indigenous populations worldwide to treat human and animal diseases (Dolatkhahi et al., 2014; Mohammadhosseini et al., 2019). Medicinal plants have been the source of many modern drugs, which are derived from their ethnopharmacological uses or applications (A.M. Abdel-Rahman et al., 2022; Ogunlakin and Sonibare, 2022; Thagriki, 2022). Furthermore, nearly 10% of plant species have been explored for their potential use in healthcare, and several important plant species are at risk of extinction, highlighting the urgent need to discover new compounds with therapeutic potential (Lalle and Hanevik, 2018). The therapeutic value of medicinal plants exists in both developing and developed countries, thanks to advancements in pharmacology (Shrestha and Dhillion, 2003; Mohammadhosseini, 2017a). A report by the World Health Organization (WHO) revealed that 80% of people in developed and developing nations rely on plant-based medicine (WHO, 2013). Over the last decade, research has focused on scientifically exploring traditional plant-based drugs for the treatment of various diseases. Medicinal plant parts are also used as preservatives and appetizers (Bonda et al., 2020). Thus, the development of drugs through natural products has proven to be the most successful strategy for discovering new drugs.

Essential oils (EOs) are commonly used natural plant products (Carpena et al., 2021). Essential oils are typically extracted from various parts of plants, such as bark, buds, flowers, fruits, leaves, peels, roots, seeds, and twigs (Stephane et al., 2020). The composition of essential oils is highly complex, as they contain a wide range of highly functionalized chemical substances (Mohammadhosseini et al., 2017; Mohammadhosseini, 2017b). These substances include phenolic acids, alkaloids, isoflavones, flavonoids, monoterpenes,

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aldehydes, terpenoids, and non-terpenoid hydrocarbons (Mohammadhossein et al., 2022). While terpenes and terpenoids are important components of essential oils, in certain species, phenylpropanoids are the primary constituents that contribute to the oils' strong aroma and flavor (Pandey et al., 2016, 2017; Alsherbiny et al., 2018). The common precursors for terpenes and terpenoids are isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The two most common pathways involved in the biosynthesis of isoprenoid units are the plastidal 2-C-methylerythritol-4-phosphate (MEP) pathway and the cytosolic mevalonic acid (MVA) pathway (Bergman et al., 2019). The MVA pathway contributes to the precursor of the terpenoid pathway, IPP/DMAPP, in all eukaryotes and archaea, while the plastidal MEP pathway is active in the plastids of algae and higher plants. The MEP pathway contributes C5 prenyl diphosphates, which play a role in the biosynthesis of monoterpenes, di- and tetraterpenes. On the other hand, the MVA pathway leads to the synthesis of sesquiterpenes, triterpenes, and sterols. The shikimate acid pathway is another biosynthesis pathway of essential oil. The synthesis of phenylpropanoid, a common component of essential oil, occurs via the shikimate acid pathway. The shikimate pathway synthesizes several vital chemicals, such as shikimic acid, quinic acid, chlorogenic acid, gallic acid, pyrogallol, catechol, and other intermediate products of branching pathways. These chemicals have various applications in multiple industries, with a particular emphasis on the pharmaceutical industry (Vogt et al., 2010).

Essential oils are used as antimicrobial, anthelminthic, antiviral (da Silva et al., 2020), antioxidant, and anti-inflammatory (Silva et al., 2015), insecticide (Isman et al., 2006), immunomodulatory (Mediratta et al., 2002), and antinociceptive (Abdollahi et al., 2003) agents. Their potent antimicrobial properties, which are effective against a wide range of microbes, aid in combating drug resistance in a cost-effective manner. The presence of bioactive components in essential oils disrupts the resistance pathway by interacting with cellular enzymes, resulting in an increased mortality rate of microbes. They are also used in medicines, agriculture, hygienic applications, skincare products, perfumes, dental care, farming, and the food sector (de Sousa et al., 2023). In the food industry, essential oils are used as food flavorings and natural preservatives (Bonda et al., 2020). Research has shown that the food and pharmaceutical industries need to incorporate new antimicrobials as natural preservatives (Doost et al., 2020). Essential oils (EOs) are a valuable source for discovering effective and clinically safe bioactive molecules with a wide range of therapeutic and industrial applications.

The current review deals with the biosynthesis, regulation, and applications of terpenes and terpenoids, and provides additional information about their availability in the literature.

2. Structural diversity of essential oil

Essential oils are primarily composed of numerous aromatic compounds extracted from fragrant plants that possess a strong aroma. These aromatic compounds belong to a wide range of structural groups such as terpenoids, monoterpenes, sesquiterpenes, diterpenes, and phenylpropanoids (Aguirre et al., 2013). Examples of major bioactive compounds found in essential oils are listed in Fig. 1.

2.1. Terpenes or terpenoids

The basic unit of terpenes or isoprenoids is 2-methylbuta-1,3-diene (isoprene units), which consists of carbon backbones that can be rearranged to form multiple cyclic configurations (Hyldgaard et al., 2011). The word "terpene" is derived from turpentine, a product of coniferous oleoresins. The structural variation of terpenes depends on the number of isoprene units they contain. For example, hemiterpenes consist of a single isoprene unit, while tetraterpenes are composed of 8 isoprene units. The major component of essential oil is a monoterpene, followed by sesquiterpenes. Apart from diterpenes, triterpenes, and tetraterpenes, their oxygenated derivatives have also been found in an essential oil (Bhavaniramya et al., 2019).

Terpenes are simple compounds with hydrocarbon structures, whereas terpenoids are a modified class of terpenes with distinct functional groups. Terpenoids are oxygenated hydrocarbons that have oxidized methyl groups either added or removed at different positions (Perveen et al., 2018). Terpenes are a class of natural compounds found in plants, animals, fungi, and bacteria. They are composed of five carbon isoprene units joined together with head-to-tail arrangements. The variation among terpenes is contributed by varying degrees of unsaturation, functional groups, and ring closure through oxidation. Terpenoids are commonly found in plants, certain insects, and marine organisms. Terpenoids are volatile compounds that give plants and flowers their aroma (Gozari et al., 2021; Zhou et al., 2020). Terpenes are further classified based on the presence of isoprene units: hemiterpenes hydrocarbons (C_5H_8), monoterpenes hydrocarbons ($C_{10}H_{16}$), sesquiterpenes hydrocarbons ($C_{15}H_{24}$), diterpenes hydrocarbons ($C_{20}H_{32}$), triterpenes hydrocarbons ($C_{30}H_{48}$), tetraterpenes hydrocarbons ($C_{40}H_{64}$), and polyterpenes hydrocarbons $(C_5H_8)_n$.

Terpenoids are a significant group of secondary metabolites specific to plants. Terpenes and terpenoids generally possess antimicrobial properties as they inhibit the growth of bacteria by rupturing cells and inhibiting DNA replication and translation (Alvarez-Martínez et al., 2021). The strong development of research methodologies enhances the analysis of the biological functions, synthetic pathways, and regulatory mechanisms of terpenoids. Several useful terpenoids have been synthesized in recent years through genetic engineering and other methods (Kleine et al., 2014).

Terpenes and terpenoids, which are terpene-like compounds, are classified or grouped based on the number of isoprene units found in the parent nucleus, ranging from one to many.

Monoterpene hydrocarbons ($C_{10}H_{16}$) are ten-carbon atom compounds made up of two isoprene subunits. These compounds, which are generally found in

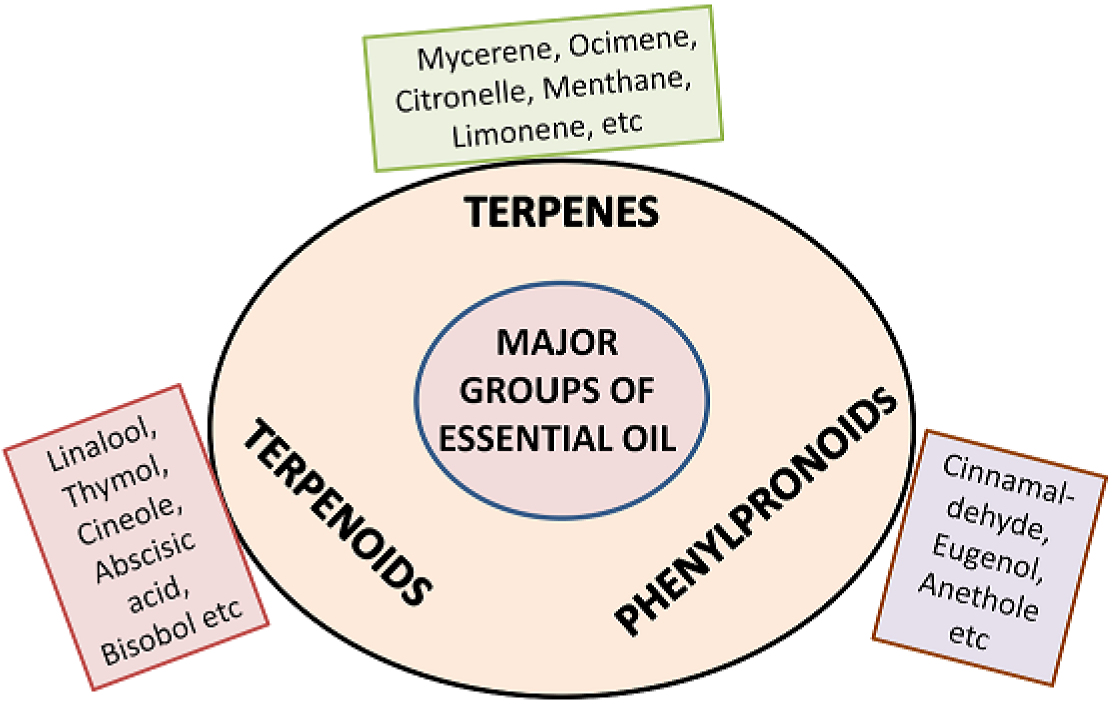

Fig. 1. Major groups of essential oil with examples.

secretory tissues, possess strong aromas and odors. They are used as fragrances in the perfume and cosmetic industry (Marianna et al., 2014). Monoterpenoids exist in approximately thirty carbon skeletons. Out of these thirty, nearly twenty monoterpenoids are further classified into acyclic, monocyclic, and bicyclic types. Common examples of monoterpenes are p-cymene, m-cymene, and pinene. Among monoterpenoids, iridoids are the largest group with a basic skeleton comprising six-membered rings with one oxygen atom fused with a cyclopentane ring. Some common examples of monoterpenoids are linalool, citronellol, thymol, and carvacrol.

Sesquiterpene hydrocarbons ($C_{15}H_{24}$) consist of three isoprene units and exist in linear, cyclic, bicyclic, and tricyclic forms. Sesquiterpenes are also found in the form of lactone rings (Ashour et al., 2010). Sesquiterpenes are the major components of latex and contribute to its antimicrobial and anti-insecticidal properties. Similarly, artemisinin, a sesquiterpene lactone, is an active compound found in the root and shoot tissues of the Artemisia annua plant. The common examples of sesquiterpenoids are blumenol, costunolide, and polygodial. Diterpenoid hydrocarbons are composed of four isoprene units and exhibit structural diversity (Yadava et al., 2014). The different forms of diterpenes include linear, bicyclic, tetracyclic, pentacyclic, and macrocyclic. Diterpenoids generally exist in a polyoxygenated form, containing keto and hydroxyl groups, and are esterified by small-sized aliphatic or aromatic acids (Lanzotti, 2013). The common representatives of these groups are ginkgolides, cephalosporins, drechslerins, and taxol. Carnosic acid, a phenolic diterpenoid found in rosemary leaf extract, possesses antimicrobial, anti-inflammatory, and neuroprotective properties. The oxidation of carnosic acid leads to the formation of carnosol, and together they form the largest class of secondary metabolites (Singh et al., 2023). Diterpenoids exhibit inhibitory action against various pathogenic microbes, pests, and weeds.

Sesterpenes are hydrocarbons ($C_{25}H_{40}$) composed of five isoprene units and exist in monocyclic, bicyclic, tricyclic, tetracyclic, and macrocyclic forms. Some common examples of sesterpenes are sesterstatin, heliocide, and leucosceptrine. Triterpenes, which consist of 30 carbon atoms, form a large class with over 200,000 known members. Two sesquiterpene molecules form triterpenes by joining in a head-to-head fashion (Ludwiczk et al., 2017). Cyclic triterpenes (1-5 rings), such as alcohols, aldehydes, or carboxylic acids (Mander and Liu, 2010). Tetraterpene hydrocarbons ($C_{40}H_{64}$) are composed of eight isoprene units consisting of forty carbons (Takaichi et al., 2013). Triterpenes are more complex because they have several methyl groups that can be further oxidized into alcohols, aldehydes, and carboxylic acids. A few typical examples are ursolic acid, oleanolic acid, α-amyrin, β-amyrin.

Tetraterpene hydrocarbons consist of eight isoprene units, and carotenoids are a commonly found type of tetraterpenoid. They can be either unsaturated hydrocarbon-containing lycopene structures or oxygenated analogues called xanthophylls. Carotenoids are specifically found in the flowers and fruit tissues of higher plants. In higher plants, glycosides are reported very rarely (Maoka, 2020). Some of the most common examples are β-carotene, lutein, crocin, and canthaxanthin. Polyterpenes can also exist with more than eight isoprene subunits. Natural rubber is a polyterpene containing isoprene units in a *cis* configuration. Apart from that, some plants also yield polyisoprene with double bonds in the *trans* form (Grau and Mecking, 2013).

2.2. Phenylpropanoids

The shikimic acid pathway synthesizes phenylpropanoids, which consist of a 6-carbon aromatic phenol group usually linked to a propene tail of cinnamic acid. These compounds are oxygenated at the 3rd, 4th, or 5th position and often contain a carbon-carbon double bond (Stevanovic et al., 2020). Anethole, eugenol, isoeugenol, myristicin, and vanillin are members of the phenylpropanoid group. Contant and group showed in their study that anethole acts as a potent anticancer compound. Previous studies have revealed the role of anethole as an anticancer compound (Contant et al., 2021). A study on safrole revealed the presence of antidiabetic, antibacterial, analgesic, and antifungal activities (Eid et al., 2021).

The objectives of this study are to summarize the recent findings on the biological activities of plant-derived terpenes and other derivatives, the regulatory mechanisms involved in their biosynthesis, the technical advancements involved in enhancing terpene biosynthesis, and the authentication of essential oils using modern techniques.

3. Biosynthesis of essential oils

3.1. Biosynthesis of terpene

The basic steps involved in the biosynthesis of terpenes are: (i) the synthesis of five-carbon isoprenoid units, (ii) the formation of complex compounds from these basic units, and (iii) the synthesis of end products. IPP and DMAPP are components of terpenes and are produced by the isoprenoid unit, converting diphosphate to dimethylallyl diphosphate (Grassmann, 2005; Berthelot et al., 2012). In plants, the most commonly utilized pathways produce isopentyl diphosphate and dimethylallyl diphosphate for the biosynthesis of terpenoids. The MVA and MEP pathways occur in the cytosol and plastids, respectively. The MVA pathway leads to the synthesis of sesquiterpenes, triterpenes, steroids, and terpenes in the cytosol and mitochondria (Henriquez et al., 2016). The dimethylallyl diphosphate pathway leads to the production of hemiterpenes, diterpenes, monoterpenes, carotene, and its derivatives, as well as various plant hormones necessary for growth and proper functioning in the photosynthesis process (Oldfield et al., 2012). A brief introduction to the specific processes and enzymes involved in the two synthesis pathways is provided in Fig. 2.

2.2. Biosynthesis of phenylpropanoid

The shikimic acid pathway is used to synthesize phenylpropanoids. The primary precursors of this pathway are p-hydroxycinnamic acid and cinnamic acid, which are derived from the aromatic amino acids tyrosine and phenylalanine, respectively. Phosphoenolpyruvate and erythrose 4-phosphate enzymes are utilized in the synthesis of shikimic acid. Futher, chorismic acid is produced by removing one ring alcohol in shikimic acid and reacting it with phosphoenol pyruvate. Phosphoenol pyruvate forms the backbone of phenylpropionic acid. Phenylalanine is produced through the amination and reduction of the ketone group, which is the initial step in the phenylpropanoid pathway (Kougan et al., 2013). Phenylalanine is condensed into cinnamate by using the enzyme phenylalanine ammonia lyase (PAL), which initiates the phenylpropanoid pathway. By esterification of the phenylpropanoid pathway, various phenylpropanoids (C6C3 molecules) such as cinnamic acid, caffeic acid, ferulic acid, sinapic acid, and chlorogenic acid esters are synthesized. These compounds have shown various health-beneficial effects and are also used as cosmeceutical ingredients (Said-Al Ahl et al., 2017; Bento-Silva et al., 2020).

2.3. Key enzymes involved in the biosynthesis of terpenoids

The major enzymes in the terpenoid biosynthesis pathways are HMGR, ISPS, DXR, DXS, and TPS. The HMGR enzyme consists of four conserved domains. The gene coding for HMGR was first cloned in Arabidopsis thaliana. Upregulation of that gene leads to an increase in the concentration of isoprene (Shen et al., 2006; Zhang et al., 2020). DXS, found in the thylakoid of plants, is the first rate-limiting enzyme of the MEP pathway. The condensation reaction of pyruvate and glyceraldehyde 3-phosphate, catalyzed by DXS, is the initial step in the MEP pathway. This reaction can lead to the formation of DXP and the subsequent release of CO_2 (Rodriguez-Concepcion et al., 2015; Battistini et al., 2016). In the hairy roots of periwinkle, the upregulation of DXS led to an increase in the accumulation of terpenoids, indoles, and alkaloids, while the amount of isoprene decreased when DXS expression was reduced (Peebles et al., 2011). DXR, a significant enzyme in the MEP pathway, catalyzes the transformation of DXP into intermediate metabolites of IPP. The overexpression of LiDXS and LiDXR genes in tobacco leads to the accumulation of diterpenes (Zhang et al., 2018). IPP and its isomer DMAPP are produced by the enzyme isopentenyl pyrophosphate synthase. GPPS, FPPS, and GGPPS are three distinct synthesized products of this pathway. A single molecule of IPP and DMAPP is catalyzed by GPPS to form GPP (Burke et al., 2002).

The overexpression of the FPS1 gene in Artemisia annae leads to an increase in the isoprene content. The gene coding for GGPPS has been cloned from various plants such as *Salvia miltiorrhiza*, *Solanum lycopersicum*, *Chimonanthus praecox*, and *Catharanthus roseus* (Okada et al., 2000; Ament et al., 2006; Han et al., 2006). The terpene synthases enzyme catalyzes the synthesis of monoterpenes from GPP, sesquiterpenes from FPP, and diterpenes from GGPP. Among the synthesized products, they can be classified as monoterpene synthase, sesquiterpene synthase, and diterpene synthase. The aspartic acid-rich 'DDXXD' conserved

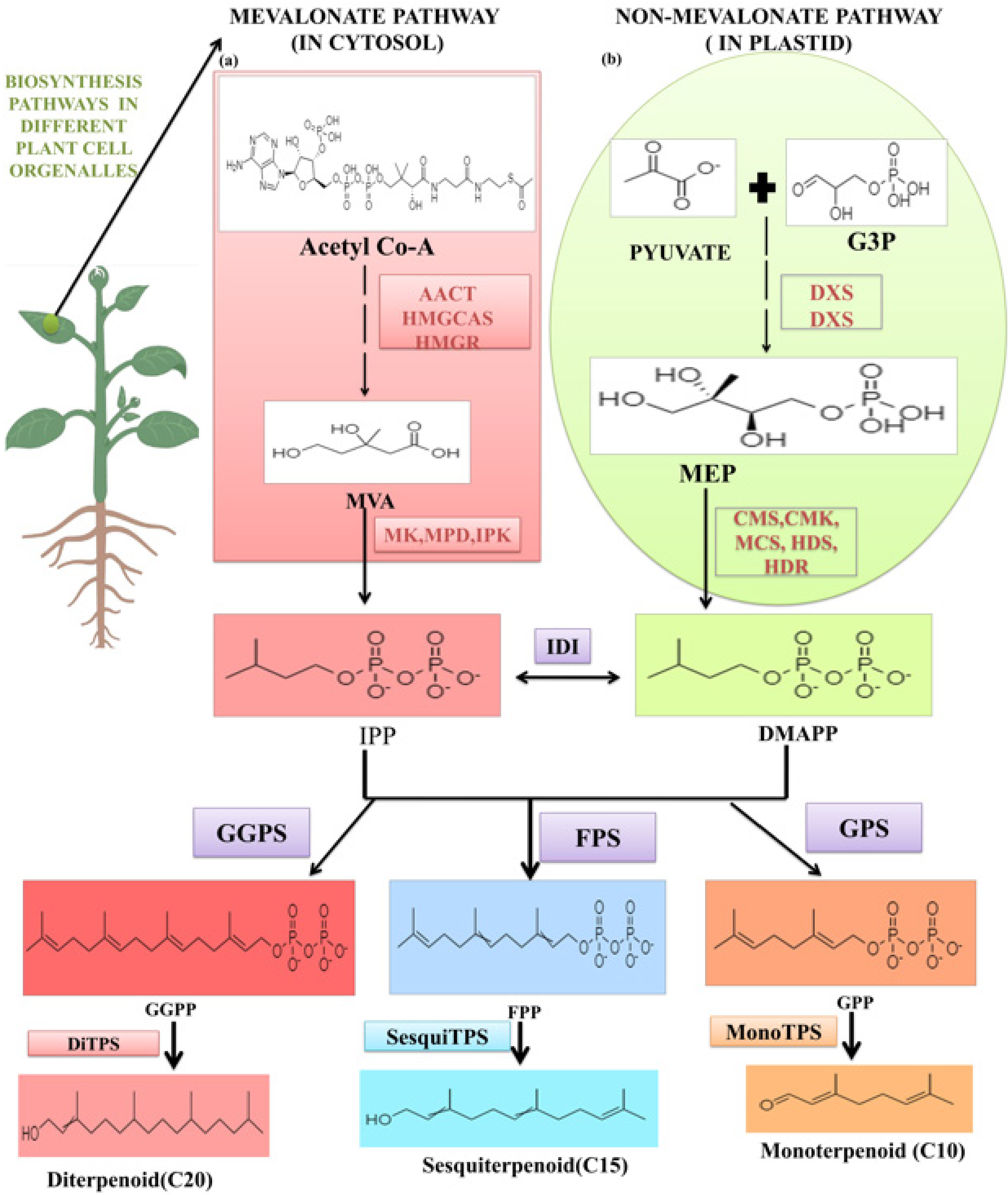

Fig. 2. Terpenoid biosynthetic pathway in plant organelles. (a) Cytosol, (b) Plastid. a) In cytosol acetyl CoA acts as a precursor that leads to the formation of Acetoacetyl CoA then HMG CoA and further mevalonate before entering into the mevalonic acid pathway (MVA). The MVA pathway leads to the synthesis of sesquiterpenes, triterpenes, steroids, and terpenes in the cytosol of mitochondria. b) In a plastid tissue the precursor of methylerythritol phosphate (MEP) pathways are pyruvate and glyceraldehyde 3-phosphate (G3P). In the MEP pathway, an isoprenoid precursor dimethylallyl diphosphate (DMAPP) formation takes place. Further, DMAPP leads to the production of hemiterpenes, diterpenes, monoterpenes, carotene, and its derivatives.

region at the C-terminus, and the arginine-rich'RRx8W' conserved region at the N-terminus, are the two typical conserved domains found in the majority of terpene synthases (Degenhardt et al., 2009) which have been extracted from numerous plants including the carrot (*Daucus carota*), Mexico mint (*Plectranthus amboinicus*), rice (*Oryza sativa*) and celery (*Apium graveliens*), is vital for the interaction between plants and pathogens (Ashaari et al., 2020; Li et al., 2020; Muchlinski et al., 2020). The gene families coding for terpene synthases can synthesize multiple terpene products from a single substrate. This leads to the production of mixtures of structurally diverse molecules, as well as the gene responsible for the diversity in essential oil components (Franz et al., 2009). Further terpene synthase gene family is subdivided into seven subfamilies based on structural characteristics, sequence relatedness, functional evaluation, and gene structures: TPS-a, TPS-b, TPS-c, TPS-d, TPS-e/f, TPS-g, and TPS-h (Chen et al., 2011; Falara et al., 2011). Among these subfamilies, TPS-b and TPS-g contain monoterpene synthases, while the TPS-a gene primarily contains sesquiterpene and diterpene synthases. Identification and characterization of TPS genes have been carried out from plant species, namely Lamiaceae *spp*. and *Lavandula spp*. (Landmann et al., 2007; Lane et al., 2010; Demissie et al., 2011), *Mentha spp*. (Turner et al., 2004), *Ocimum spp*. (Iijima et al., 2004), *Origanum spp*. (Crocoll et al., 2010; Lukas et al., 2010), *Salvia spp*. (Kampranis et al., 2007; Schmiderer et al., 2010) and *Thymus spp*. (Krause et al., 2013; Filipe et al., 2017).

2.4. Regulation of terpenes biosynthesis

The regulation of terpenes or terpenoids biosynthesis is affected by several factors.

2.4.1. Transcription factor

The synthesis of terpenoids is mediated by both structural genes and transcription factors. They regulate the expression level of genes by modifying the transcription rate. The growth, development, and biosynthesis of secondary metabolites in plants are regulated by basic helix-loop-helix (bHLH) transcription factors (TFs) that belong to the MYC family (Huang et al., 2015; Mertens et al., 2016). Members of the MYC family regulate the biosynthesis of terpenoids in plants. A study showed that the transcription factors CpMYC2 and AtMYC2 mediate the synthesis of caryophyllene in Arabidopsis thaliana (Hong et al., 2012; Aslam et al., 2020). AP2/ERF and WRKY are also involved in the biosynthesis of terpenoids. The GA-WRKY1 transcription factor, along with the W-box cis-acting element in the promoter region of the (+)-δ-cadinene synthase gene, activates CAD expression in cotton. In Amomum villosrm, six WRKY transcription factors have been associated with terpene synthases (He et al., 2015).

2.4.2. Plant hormones

Plant hormones can either stimulate or inhibit the biosynthesis of terpenoids. Plant hormones, such as jasmonic acid (JA) and methyl jasmonate (MeJA), activate the accumulation of specific metabolites, including alkaloids, nicotine, and anthocyanins. MeJA also regulates the expression of terpenes and terpenoids when applied externally. In Camellia sinensis, Me induces the expression of CsTPS genes, including CsTPS23, CsTPS25, CsTPS43, CsTPS51, CsTPS52, and CsTPS76. Furthermore, after spraying grapes with MeJA, there is an increase in the concentration of aroma-producing compounds, particularly the number of monoterpenes (D'Onofrio et al., 2018).

2.4.3. Biotic and abiotic factors

Recent research has revealed that a range of volatile terpenoids, such as farnesene, can aid in the interaction between plants and stress-inducing factors. Sesquiterpenoids develop greater resistance in plants against various diseases caused by biotic factors. Similarly, certain volatile monoterpenoids function as insect repellents (Shrivastava et al., 2015; Yoon et al., 2016; Riedlmeier et al., 2017). The infestation of Arabidopsis thaliana by Pieris rapae, a caterpillar, results in the expression of the AtTPS03 and AtTPS10 genes, which produce (*E*)-β-ocimene (Van Poecke et al., 2001). The biosynthesis of terpenoids is also influenced by several abiotic factors, such as light, humidity, temperature, water conditions, nutrient balance, polyethylene glycol, and mechanical damage (Van Poecke, 2001; Hartikainen et al., 2012).

The concentration of terpenoids increases during short-term exposure to high temperatures, whereas long-term exposure to high temperatures decreases their concentration (Duan et al., 2019). Oxidative stress significantly increased the expression of OsTPS20 in rice. In Camellia sinensis, a total of 80 terpene synthase genes have been identified. Among these genes, the expression levels of CsTPS67, 69, and 71 were found to decrease during cold, salt, and polyethylene glycol treatments (Lee et al., 2015). Terpenoids protect plants from photodamage and oxidative stress by supporting photorespiration (Abraham et al., 2021).

2.5. Technical advances in terpene production

Current advancements in genome engineering enable researchers to add, delete, or modify the DNA of a cell or organism, with the aim of improving the production of desired products. Among several techniques, the clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system has been explored for genome regulation through the inactivation of Cas9 (Schultenkämper et al., 2020). The CRISPRi-based system works by binding to genes and repressing the target pathway in order to regulate terpenoid synthesis. In the current scenario, the CRISPRi system is used to decrease the expression level of genes involved in competing pathways for isopentenol (Tian et al., 2019) and valencene (Dietsch et al., 2021).

Synthetic biology is an additive approach to integrating pathway genes from different sources into advanced metabolic pathways (Yadav et al., 2012). The understanding of promoters and ribosome binding sites is crucial for establishing the basis for optimizing both native and modified pathways. Shukal et al. (2019) redesigned ribosome binding sites and increased the yield of viridiflorol synthase, a sesquiterpene, by 50%. A similar approach was used by another group of scientists to enhance the accumulation of amorphadiene (Nowroozi et al., 2014).

Several reports show that the yield of secondary metabolites can be increased through *in vitro* cultures. Taghavi et al. (2020) demonstrated the upregulation of genes involved in the biosynthesis of secondary metabolites. A study on Lithospermum officinale reported an increase in the production of shikonin. Additionally, co-culturing has been used to enhance the yield when a single host cell is unable to support all the enzymes involved in the biosynthesis of secondary metabolites. (Wang et al., 2020). A group of researchers assembled *E. coli*-*E. coli* co-culture system for the synthesis of pinene. In this study, the MEV pathway and a heterologous pathway, which included GPP synthase and pinene synthase, were cloned and expressed in different *E. coli* strains with varying pinene tolerance (Niu et al., 2018).

Hairy root culture supports the biosynthesis of secondary metabolites because it is biochemically and genetically more stable (Chung et al., 2016). Previous reports have revealed that transformation regulated by Agrobacterium rhizogenes leads to the synthesis of more secondary metabolites in transgenic roots (Ismail et al., 2017). Ali et al. (2022) reported that the overexpression of GmFDPS, GmGGPPS, SgGPS, and SgFPPS genes leads to an increased accumulation of terpenes in transgenic soybean roots. Wei et al. (2019) manipulated the MEP pathway to enhance the production and accumulation of tanshinone in transgenic hairy roots of *Salvia miltiorrhiza*.

2.6. Characterization and authenticity of essential oils and their components

The quality of essential oil is evaluated using various biotechnologically advanced techniques, such as gas chromatography-mass spectrometry (GC-MS), electrospray ionization-mass spectrometry (ESI-MS), flow injection analysis (FIA), high-performance liquid chromatography (HPLC), Fourier transform infrared spectroscopy (FT-IR), and quantitative nuclear magnetic resonance (qNMR) spectroscopy (Cebi et al., 2021).

In silico systems such as plantiSMASH, PhytoClust, and METACLUSTER have been developed to detect biosynthetic gene clusters in plants (Huang et al., 2017; Banf et al., 2019). Triterpenoids and diterpenoids exhibit the highest level of gene clustering (Boutanaev et al., 2015). Artemisia annua is another example of successful homologous engineering of specialized terpenoids. The simultaneous expression of HMGR and FPPS genes enhances artemisinin accumulation (Malhotra et al., 2016). The development of golden rice, which involves transferring the genes necessary for tetraterpenoid carotene production, has been one of the earliest and most widely recognized instances of heterologous engineering in plants. Later, to increase the amount of carotene, Golden Rice 2 was produced by inserting more efficient carotene pathway genes (Zhu et al., 2018).

In a study, the extraction of volatile oils from *Cymbopogon schoenanthus* L. Sprengs (CS) leaves was compared using simple grinding (SG) and cryogenic grinding (CG) methods, as well as traditional hydrodistillation. The extraction was carried out using both microwave-assisted hydrodistillation (MAHD) and microwave-assisted steam distillation (MASD). This study revealed that microwave-assisted hydrodistillation is a promising and innovative technique.

Volatile oil extraction and cryogenic grinding can be used to achieve higher extraction yields compared to traditional grinding methods (Bellik et al., 2019). Other extraction techniques, such as hydrodistillation, steam distillation, and Soxhlet extraction, are also used for oil extraction (Gavahian et al., 2015; Pudziuvelyte et al., 2018). However, these procedures take a significant amount of time and require the use of multiple organic solvents and plant samples. To extract essential oil from plants, several effective extraction techniques have been used, including headspace solid-phase microextraction (HS-SPME), gas flow headspace liquid-phase microextraction (GF-HS-LPME), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE). The gas purge microsyringe extraction method was later developed. It is a quick and easy-to-use technique that allows for high-recovery extractions. This innovative equipment integrates the procedures of extraction, cleanup, and concentration. It has been used to test pesticides, environmental hormones, and organic contaminants in various samples (Nan et al., 2015). The appropriate technique has been named gas purge micro solvent extraction (GP-MSE), and this device has undergone technical advancements that now enable more efficient sample extraction and purification.

Essential oils extracted from three different species of *Atractylodes spp*. GP-MSE combined gas chromatography-mass spectrometry was used to compare its capacity to extract volatile fractions from plant tissues. For semi-volatile components, GP-MSE demonstrates enhanced extraction capabilities, resulting in more structured essential oils with high reliability. It also requires smaller sample volumes and reduces extraction times. Therefore, GP-MSE can be regarded as a unique extraction technique due to its simplicity, speed, responsiveness, and eco-friendliness (Zhao et al., 2018).

The commonly used techniques for quantifying the levels of thymol and carvacrol in different matrices are flow injection analysis (FIA) and thin-layer chromatography (TLC). These techniques include two-step gradient elution together with densitometry (Bazylko et al., 2000; Al-Saleh et al., 2006; Mika et al., 2015). Linear sweep voltammetry (LSV) is of significant importance because the analysis of results using this method is simple (Wang et al., 2006). Thus, thymol and carvacol were identified in phytotherapeutic black seed using differential pulse voltammetry on glassy carbon electrodes (Stankovic et al., 2015). Thymol was also identified in the essential oil of Carum copticum using square-wave voltammetry with a boron-doped diamond electrode. Additionally, the total content of biomarkers was detected in Mexican oregano oils using single-walled carbon nanotube screen-printed electrodes (SWCNT-SPE) (Kowalcze et al., 2019). Similarly, gas chromatography coupled with mass spectrometry (GC/MS) was used to characterize 87 compounds in the essential oil of *Setaria parviflo*ra. Among the compounds studied, α-terphenyl acetate (23.6%), β-caryophyllene (16.8%), bicyclogermacrene (9.3%), spathulenol (4.9%), and α-pinene (4.2%) were identified as the major components (Abdullah et al., 2016; Shakeri et al., 2019).

In an experiment, encapsulated peppermint oil was used in conjunction with encapsulated chitosan nanoparticles (CSNPs). The CSNPs were produced through a two-step process that included creating an oil-in-water emulsion and then performing ionic gelation on the emulsion droplets. This process was later confirmed by UV-Vis spectrophotometry and X-ray diffraction (XRD) techniques. The encapsulation efficiency (EE%) and loading capacity (LC%) of CS/PO NPs were found to be approximately 64% and 12.31%, respectively (Rajkumar et al., 2020).

Gas chromatography-mass spectrometry has been extensively used for the analysis of propenyl benzenes. Although this method offers excellent sensitivity and resolution, it is typically time-consuming, taking 25 to 60 minutes for a single run. It reports relative quantification and provides a tentative identification of the constituents based on a library (Raymond et al., 2017; Li et al., 2018). There are few reports available on the analysis of propenyl benzene by liquid chromatography. These reports have revealed that liquid chromatography requires a longer run time compared to other techniques and has low specificity when analyzing mixtures of compounds containing essential oils (Dhalwal et al., 2007; Yun et al., 2010).

Various analytical techniques are used to assess the quality, safety, and authenticity of essential oils. Nuclear magnetic resonance (NMR) spectroscopy is a fast and reliable analytical technique that offers a higher level of confidence in identifying the structural composition of constituents. Additionally, it acts at room temperature, protecting thermolabile analytes from degradation. The quantitative analysis of major chemical markers in essential oils using the 1H qNMR technique is highly valuable for rapidly screening and ensuring quality control in the analysis of essential oils that are abundant in propenyl-benzene compounds.

2.7. Applications of terpenes and terpenoids

Previously published reports have shown the crucial role of terpenes and terpenoids in maintaining human health. They play a significant role in numerous studies, both *in vitro* and *in vivo*, by acting as anticancer agents, antimicrobials, anti-inflammatory agents, neuroprotective agents, anti-aggregators, antioxidants, anticoagulants, sedatives, and analgesics through the activity of terpenes and glycoside compounds. These compounds are widely used in the biofuel industry, as well as in the manufacturing of pharmaceuticals, nutraceuticals, food and beverage-related products, cosmetics, fragrances, synthetic chemicals, and aroma flavor additives (Zhao et al., 2016; Tetali et al., 2019; Yousefi et al., 2020).

2.7.1. Anticancer

Cancer is a disease characterized by excessive and uncontrolled cell proliferation. It either spreads to different body parts or targets them, interfering with the host cells' ability to maintain homeostasis (Sung et al., 2021). Terpenes have a significant impact on reducing and controlling cancer malignancies (Silva et al., 2020). Terpenes and other terpenoids possess a common mode of anticancer activity (Fig. 3). The impact of citral compounds on the growth of human colorectal cancer cell lines, specifically HCT116 and HT29, showed a decrease in the expression level of the anti-apoptotic proteins Bcl-2 and Bcl-xL, as well as an increase in the expression of phosphorylated p53 protein and Bax (Sheikh et al., 2017). A report revealed the potential role of carvacrol as an anticancer agent. Carvacrol possesses genotoxic, ROS-producing, and glutathione-depleting effects that inhibit the proliferation of human gastric adenocarcinoma cells (Gunes-Bayir et al., 2017).

The study conducted by Rodenak-Kladniew et al. (2018) demonstrated that linalool can modify the Ras/MAPK and Akt/mTOR pathways, resulting in anticancer effects against HepG2 cells that are resistant to hepatocellular carcinoma. Similarly, the impact of hinokitiol was examined on the migration of A549 lung cancer cells. It was found that the substance prevented A549 cell migration by inhibiting matrix metalloprotease, promoting antioxidant enzymes such as catalase and superoxide dismutase, and activating caspases 9 (Jayakumar et al., 2018). Pudelek and coworkers reported that thujone shows non-invasive and pro-apoptotic effects on glioblastoma multiforme cells (Pudełek et al., 2019). Thujone not only inhibits the growth and viability of glioblastoma multiforme cells, but it is also linked to malignancy. Another compound, myrtenal, inhibits vacuolar (H^+)-ATPase enzymes. It hinders the transport of H^+ to the extracellular matrix, induces autophagy, and modifies ion gradients. These actions significantly reduce tumor malignancy and metastasis (Martins et al., 2019).

2.7.2. Antimicrobial activity

The antibacterial property of terpene compounds is well-known, and a study by Guimaraes et al. (2019) has reported their ability to combat drug resistance. A comprehensive mechanism of action for the antimicrobial activity of terpenes and terpenoids is illustrated in Fig. 4. Farnesol, a sesquiterpenoid, effectively combats various Streptococcus species by inhibiting biofilm formation (Gomes et al., 2009). Furthermore, limonene and polylysine have been shown to have effective and synergistic effects against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* (Zahi et al., 2017). Terpineol is a natural antibacterial agent used as a food preservative because it can damage bacterial cell walls and membranes (Huang et al., 2021). Terpineol, borneol, and citral also have similar effects on packed food

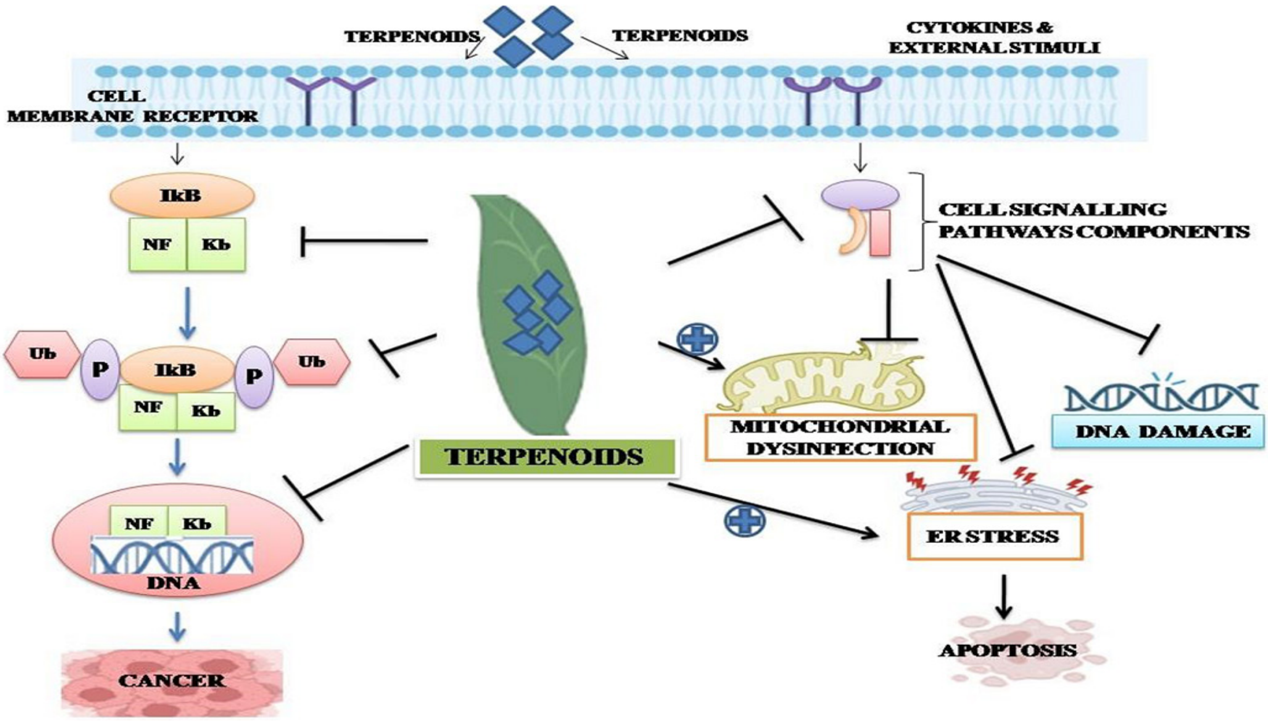

Fig. 3. The schematic representation of the mechanism involved in the anticancer activity of terpenes/terpenoids. Terpenoids inhibit the NF- κB signaling pathway by ubiquitination and dephosphorylation and stop the growth of cancerous cells. Terpenoids after binding to receptors on the membrane of a cancerous cell send signals and lead to disruption of mitochondrial function, DNA damage, and promote cell death by apoptosis. Ub, ubiquitination; P, phosphorylation; NF-κB, Nuclear factor kappa; *IkB, IkappaB.*

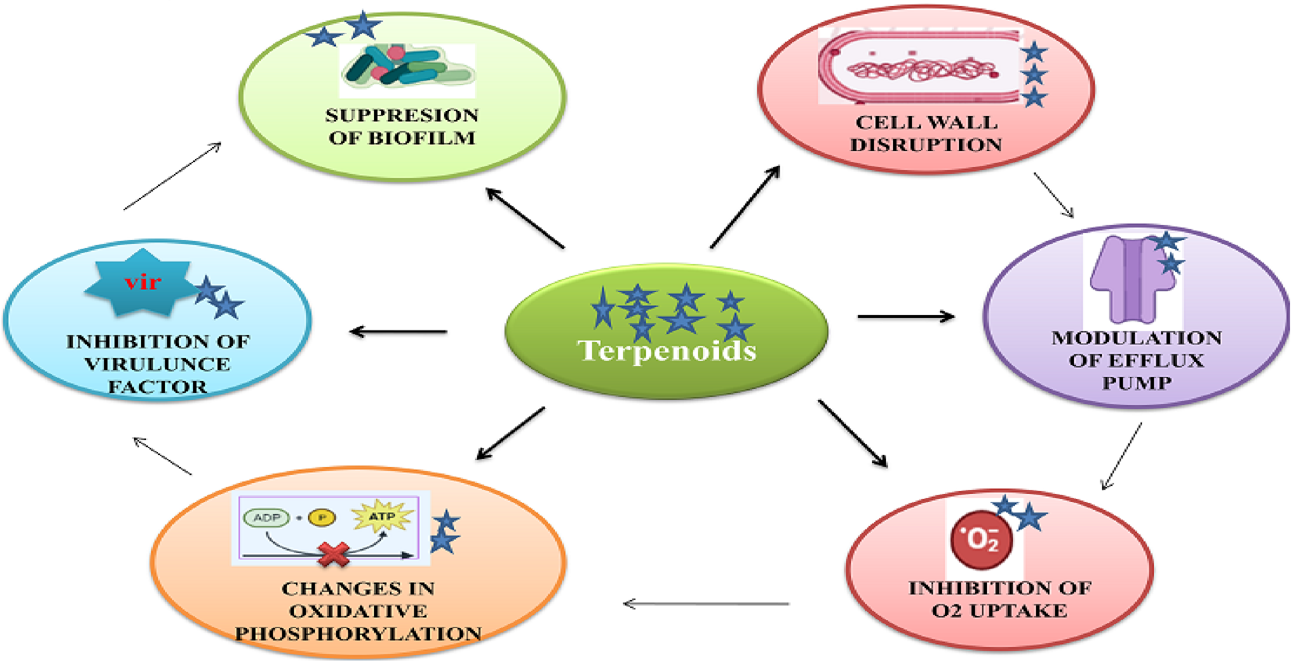

Fig. 4. Antibacterial activity of terpenoids. Terpenoids act on bacterial systems by disrupting cell walls. The damage to the cell wall leads to the modulation of efflux pumps which are required for oxygen uptake and transport of toxic substances out of bacterial cell walls. The damaged efflux pump causes a change in oxidative phosphorylation and inhibits the growth of disease-causing virulence factors including enzymes and toxins. The ability of bacterial cells to form biofilm reduces as it is not able to adhere to host cells.

materials, exhibiting synergistic effects. They also have a positive effect on preventing the formation of biofilms caused by *Listeria monocytogenes* and *Pseudomonas aeruginosa*.

In a study, the potential role of hinokitiol in reducing mitochondrial membrane potential and inhibiting mitochondrial respiratory chain complexes I and II was investigated. This decreases intracellular ATP generation and increases intracellular reproductive stress in *Candida* species. (Jin et al., 2012). Eugenol possesses bactericidal activity against Salmonella enterica. In addition to carveol, citronellol, and geraniol, which have bactericidal effects by impairing the integrity of cell membranes (Devi et al., 2010).

2.7.3. Anti-Inflammatory activity

Inflammation is a protective process against external agents, such as microbial infections or tissue damage. This process can lead to acute or chronic inflammatory responses, depending on the extent of tissue damage Terpenes reduce inflammation (Fig. 5) by influencing pathogenic processes in the inflammatory response (Chen et al., 2020).

One of the most important immune cells, macrophages, is involved in several immune pathological processes that occur during inflammation. A study by Isman et al. (2006) investigated the anti-inflammatory effect of myrcene in rat models during the treatment of renal inflammation. The study found that myrcene upregulated calcium, superoxide dismutase, and glutathione. Another study shows that terpineol and pinene reduce the expression levels of genes associated with inflammation and the release of hexosaminidase in Rat Basophilic Leukemia cells (RBL-2H3) stimulated by lipopolysaccharide. The action of these terpenes against inflammatory reactions can pave the way for the development of new treatments (Yang et al., 2021). While borneol enhances the expression of superoxide dismutase and reduces the expression of cytokines, it has the potential to be used to mitigate the effects of acute pancreatitis (Bansod et al., 2021).

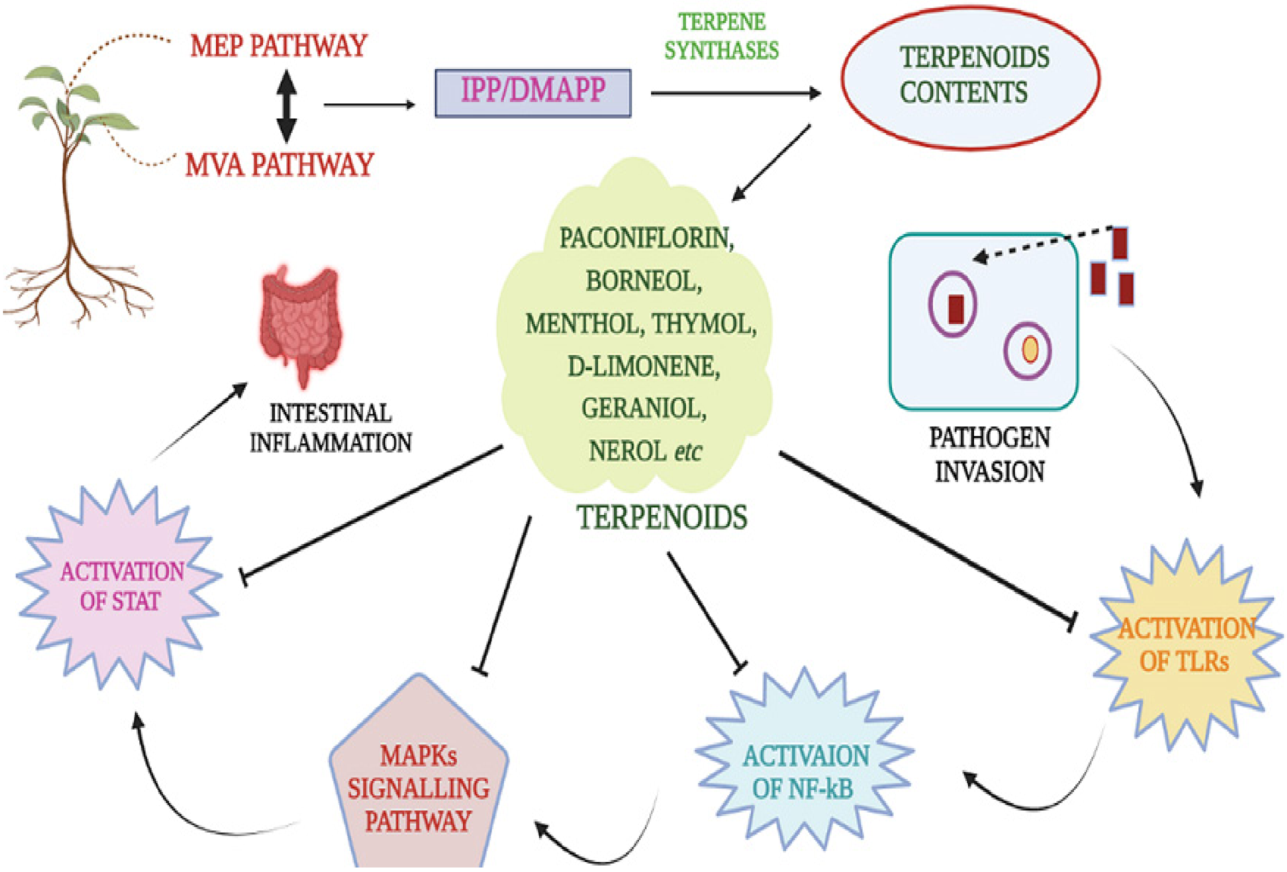

Fig. 5. Various modes of action of terpenoids as an anti-inflammatory agent. When a pathogen invades a cell, terpenoids activate toll-like receptors (TLRs) leading to a cascade of signals and activating factors involved in the inflammatory response of the body like nuclear transcription factor-kappa B (NF-kappa B). Phosphorylation and activation of STAT, a transcription factor leads to the activation of anti-inflammatory compounds which controls inflammation of intestinal cells.

2.7.4. Antidiabetic activity

Diabetes is characterized by high levels of glucose in the blood. The continuous rise of blood glucose levels can be life-threatening, as it can lead to damage to vital organs. A report by Jeppesen et al. (2006) revealed the role of diterpenoid stevioside, an active component extracted from stevia (*Stevia rebaudiana* (Bertoni) Hemsl.). The plant controls the high levels of glucose in rats. The antidiabetic effect of stevioside is related to the expression of genes involved in glycolysis and the addition of a phosphate group to liver mitochondrial ATP, which leads to a hypoglycemic condition.

2.8. Activities of other terpene derivatives

Thymol is a colorless crystalline monoterpenoid phenol with a distinctive odor. Thymol is recognized as a safe (GRAS) component by the US FDA (Boskovic et al., 2019; Chen et al., 2020). Thymol has been reported to possess antimicrobial properties (Xu et al., 2018), antitubercular effects (Dubey et al., 2013), antifungal activity (De Castro et al., 2015), antioxidant properties (Lee et al., 2005), and anticancer properties (Kang et al., 2016). Thymol can induce apoptosis in cancer cells by downregulating Bcl-2 protein expression and upregulating the expression of Bax protein (Blazickova et al., 2022). Thymol possesses antibacterial activity in addition to its bactericidal properties. Thymol breaks down the integrity of the Staphylococcus aureus membrane to enter the interior of the bacterial cell. It then attaches to the minor grooves of DNA, causing a slight disturbance to the secondary structure of DNA (Nafisi et al., 2004). A summary of recent studies on the various biological functions of thymol, published from 2020 to 2022, is provided in Table 1.

Table 1
A summary of a few recent studies on the various biological functions of terpenoids published between (2020-2022).

Sr. No.	Studied Biological Functions	Reference
1	Antineoplastic activity, Antiviral activity, Antifungal activity, Antibacterial activity	Kowalcyzk et al., 2021
2	Anti-inflammatory activity	Wang et al., 2021
3	Antibacterial activity	Chen et al., 2022
4	Antibacterial activity	Li et al., 2020
5	Antibacterial activity	Liu et al., 2021
6	Antibacterial activity	Boye et al., 2020
7	Antioxidant activity	Cheng et al., 2020
8	Antibacterial activity	Chen et al., 2022
10	Antioxidant activity	Havasi et al., 2020
11	Anticancer activity	Hassan et al., 2021
12	Antifungal activity	Kong et al., 2020
13	Antiviral activity	Valliammai et al., 2021
14	Antifungal activity	Miranda-Cadena et al., 2021
15	Antioxidant activity, Antibacterial activity	Liu et al., 2022
16	Anticancer activity	Qoorchi Moheb Seraj et al., 2022
17	Anticancer activity	Blazickova et al., 2022
18	Antifungal activity	Sun et al., 2021
19	Antifungal activity	Liu et al., 2022
20	Antifungal activity	Ranjbar et al., 2022

Menthol, a derivative of monoterpenoid, possesses insecticidal activity against insects that damage crops (Robledo et al., 2005). A study on dihydrocitronellol (3,7-dimethyl-1-octanol) revealed that IC_{50} values of 13-52 μM are highly effective against the development and growth of schistosomes (Mafud et al., 2016). Terpene also acts as a good immunomodulator. Oleanolic acid, ursolic acid, and triterpene acids have shown promising immunomodulatory properties against Gram-positive *Mycobacterium tuberculosis* by activating macrophages

(López-García et al., 2005).
Terpenoids extracted from American ginseng, specifically ginsenoside Re, have been found to possess antioxidant properties that protect cardiomyocytes from oxidative damage (Xie et al., 2006). Ginsenoside Rg1 exhibits anti-aging properties by regulating gene expression at the cellular level. Ginsenoside Rd is also used as a neuroprotective agent. Wang et al. (2017) demonstrated in their study that ginsenoside Rd can alleviate symptoms of cognitive dysfunction. Silibinin has anti-inflammatory and antioxidant characteristics by reducing the production of inflammatory cytokines such as NO, TNF, and IL in various inflammation models (Rigby et al., 2017).

3. Concluding remarks

The essential oils extracted from various medicinal plants have been used as natural products and are popularly known for their physicochemical properties. Their major constituents, namely terpenes and terpenoids, are crucial in both the pharmaceutical and medical industries. Terpenes are simple compounds with hydrocarbon structures, while terpenoids are a modified class of terpenes that contain oxygenated hydrocarbons. Terpenes and terpenoids possess several potential activities, such as anticancer, antimicrobial, anti-inflammatory, and antioxidant properties. It is important to conduct a detailed analysis of the mechanisms underlying the biological properties of terpenoids in order to improve their application in the field of medicine. Terpenoids, along with other secondary metabolites, play an important role in growth, development, and defense mechanisms. However, in plants, the concentration of secondary metabolites is relatively low, which makes their extraction challenging. The extraction of terpenoids through genetic engineering and key enzyme approaches has become an intriguing topic in this research field. CRISPR-Cas9, synthetic biology, *in vitro* techniques, and hairy root culturing have been used in several studies to regulate and increase the production of terpenes. This review focuses on key enzymes involved in the biosynthesis of terpenes. It aims to fill the gap and aid in the identification of alternative pathways for terpene biosynthesis, as well as their regulation under laboratory conditions. The CRISPR-Cas9 technique helps increase the expression of the terpene synthesis gene. Along with this information, this review also discusses the utilization of cost-effective modern analytical techniques for extracting, authenticating, and detecting essential oil compositions.

Conflict of interest

The authors declare that there is that there is no conflicts of interest.

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