

*Review*

# Biosynthesis, regulation and properties of plant monoterpenoids

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Monoterpenes are a big family of natural products which are well-known as the constituents of the essential oils and defensive oleoresins of the aromatic plants. In addition to ecological roles in pollinator attraction, allelopathy and the plant defense, monoterpenes are used extensively in the food, cosmetic and pharmaceutical industries. Monoterpenes are widely spread in plants, from algae and fungi to monocots and dicots. The production of monoterpenes is usually associated with plastids in the plant cells. The biosynthesis pathways of monoterpenes include the synthesis of C<sub>5</sub> precursor isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP). All the levels of monoterpenes are thought to be principally controlled transcriptionally and post transcriptionally. In addition, in response to biotic and abiotic factors, the biosynthesis process of the monoterpenoid in the plants can be spatially and temporally regulated during the development phase. This review summarizes the most recent advancements in biosynthetic pathway, metabolism regulation, medicinal properties and ecological roles of the monoterpenoids.

**Key words:** MEP pathway, ecological roles, medicinal properties, metabolism regulation, monoterpenes.

## INTRODUCTION

Monoterpenes generally have fragrance and other biological activities. Some monoterpenes are used as important flavor agents and are added to foods, drinks, perfumes, cosmetics and tobacco (Aharoni et al., 2005). Therefore, the Monoterpenes are widely used in medicine, industry and agriculture. Moreover, monoterpenes are thought to have essential ecological roles and could be induced by biotic and abiotic stresses, such as pathogen

attack, wounding, ozone and high temperature (Holopainen and Gershenzon, 2010; Loreto and Schnitzler, 2010). Monoterpenes are usually of two types, it means they might be linear (acyclic) or may contain rings (mono and bicyclic). Biochemical modifications such as oxidation or rearrangement produce the related monoterpenoids. They consist of several functions: carbures, alcohols, aldehydes, ketone, esters and ethers (Bakkali

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(Bakkali et al., 2008). Like all terpenoids, monoterpenoids are derived from the isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP) (Burke et al., 1999).

Generally, plants use two separate pathways: plastidial Methyl-erythritol-4-phosphate (MEP) and cytosolic acetate-mevalonate (MVA) pathways to form the IPP (Ganjewala et al., 2008). In plant cells, the MVA pathway synthesizes the cytosolic IPP, which can be transported to mitochondria for the biosynthesis of mitochondrial isoprenoids (Disch et al., 1998). In the plastid, the IPP and DMAPP are synthesized by the MEP pathway. Despite of this compartmentation, there is an ample evidence of a limited exchange of common precursors between the cytosol and plastids (Bouvier et al., 2005; Bick and Lange, 2003). The mechanism of this exchange regulation, however, still remains unknown. The presence of putative transporters of prenyl diphosphates in the plastid envelope has been reported in several plant systems (Bick and Lange, 2003; Flügge and Gao, 2005) but it is likely that, regulating the contribution of the MVA and MEP pathways to the production of particular isoprenoids requires more than a simple shuttle of metabolites between the cytosol and the plastid (Schuhr et al., 2003). The extent of the exchange can be manipulated by supplying intermediates of the MVA or the MEP pathways exogenously and is sensitive to metabolic, environmental and developmental cues (Kasahara et al., 2002; Schuhr et al., 2003). As a consequence of the limited exchange of isoprenoid precursors among cell compartments, sterols, brassinosteroids, sesquiterpenes and polyprenols are mainly (but not exclusively) formed from MVA-derived precursors, whereas the MEP pathway provides most (but not all) precursors for the production of gibberellins, isoprene and to form the side chain of chlorophylls, tocopherols, phyloquinones and plastoquinone (Rodríguez-Concepción, 2010). Several lines of evidence from silencing and over-expression genes, specific inhibitors treatments and Labeling intermediates or precursor feeding, indicate that monoterpenoids are formed predominantly, but not exclusively, from MEP-derived precursors (Besser et al., 2008; Lange et al., 2011; Ramak et al., 2013a).

## BIOSYNTHETIC PATHWAYS

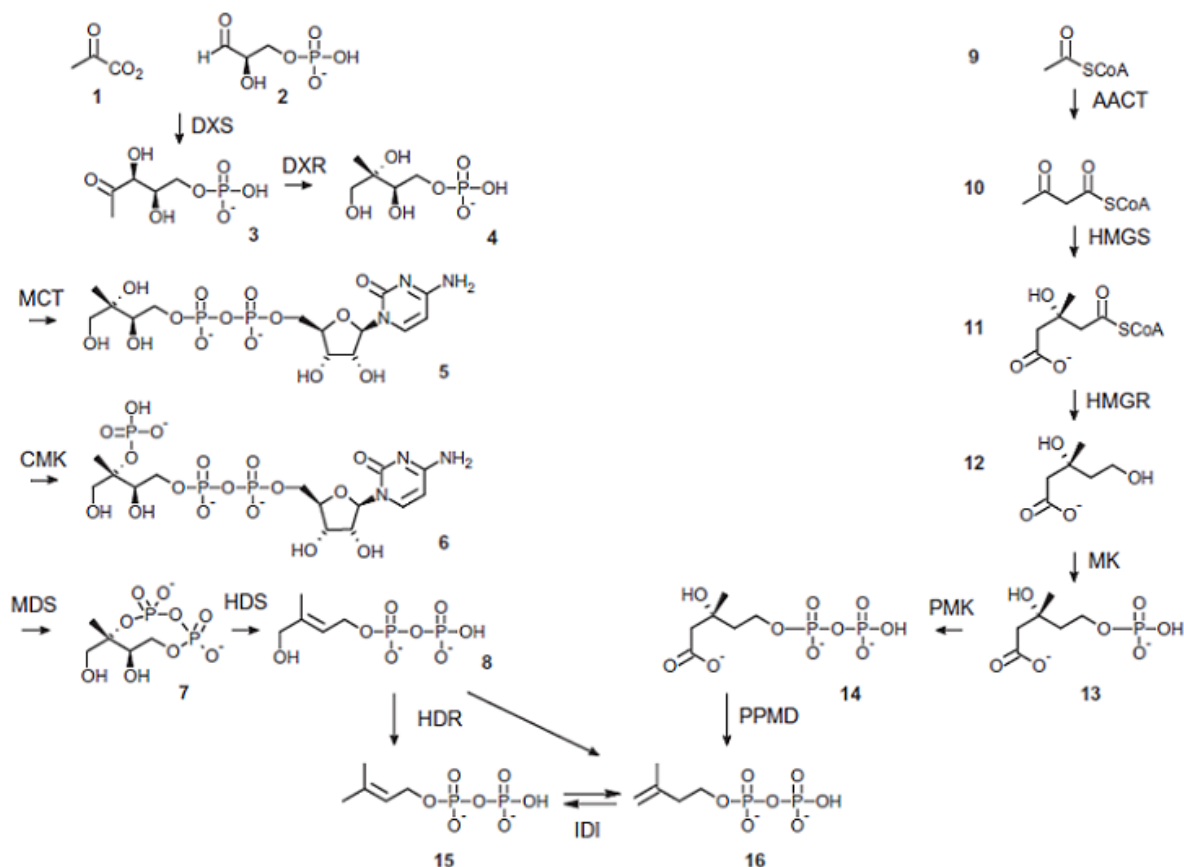
### The MVA pathway

The plant MVA pathway can directly be compared to the one existing in animal and yeast cells. To build up IPP via the MVA pathway in the cytosol, initially two units of Acetyl coenzyme A (Ac-CoA) are condensed into acetoacetyl-CoA through a Claisen-type reaction catalyzed by acetoacetyl (AcAc)-CoA thiolase (AACT) (Figure 1). The following enzyme, 3-hydroxy-3-methylglutaryl coenzyme A

synthase (HMGs), catalyzes the thermodynamically favorable aldol condensation of AcAc-CoA with Ac-CoA to form HMG-CoA. Mevalonate formation is accomplished by HMG-CoA reductase (HMGR). It catalyzes the reversible four-electron, two-step and NADPH dependent reduction of HMG-CoA into MVA (Bochar et al., 1999). The remaining steps towards IPP comprise two phosphorylation reactions to convert MVA to mevalonate 5-diphosphate (MVADP), catalyzed by mevalonate kinase (MK) and phosphomevalonate kinase (PMK), followed by an ATP-dependent decarboxylation of MVADP into IPP, catalyzed by diphospho- mevalonate decarboxylase (PPMD). In archeobacteria, these latter enzymes are missing and it was proposed that these organisms follow a modified MVA route that involves isopentenyl monophosphate phosphorylation into IPP (Grochowski et al., 2006). Finally, in the general scheme, isopentenyl-diphosphate Isomerase (IDI) catalyzes the formation of DMAPP, the chemically active isoprene unit (Hemmerlin et al., 2012).

### The MEP pathway

The MEP pathway consists of seven enzymatic steps involved in the formation of the IPP and DMAPP from pyruvate and D-glyceraldehyde 3-phosphate (Figure 1). The enzymes of the MEP pathway are encoded by nuclear genes and targeted to plastids (Rodríguez-Concepción and Boronat, 2002; Bouvier et al., 2005). The first step in this pathway is the condensation of pyruvate and glyceraldehyde 3-phosphate to form 1-deoxy-D-xylulose-5-phosphate (DXP) by DXP synthase (DXS). DXP is then transformed into MEP by DXP reductoisomerase (DXR), which is also called MEP synthase. MEP is then converted to 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate (HMBPP) by the consecutive enzymatic action of 2C-methyl-D-erythritol 4-phosphate cytidyl transferase (MCT), 4-diphosphocytidyl-2C-methyl-D-erythritol kinase (CMK), 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase (MDS) and 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase (HDS). The last step is the branching of HMBPP to IPP and the DMAPP is then catalyzed by the simultaneous enzymatic action of a single enzyme, 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate reductase (HDR) (Rohdich et al., 2003). Although the HDR in the MEP pathway produces both IPP and DMAPP (Tritsch et al., 2010), the plastid which localized IDI, is also involved in the substrate optimization phase by catalyzing IPP isomerization. IPP and DMAPP can be interconverted by the activity of the enzyme IDI. In plant cells, the IDI is required to produce DMAPP in the cytosol and mitochondria but not in plastids, whereas the MEP pathway produces both IPP and DMAPP (Adam, 2002).

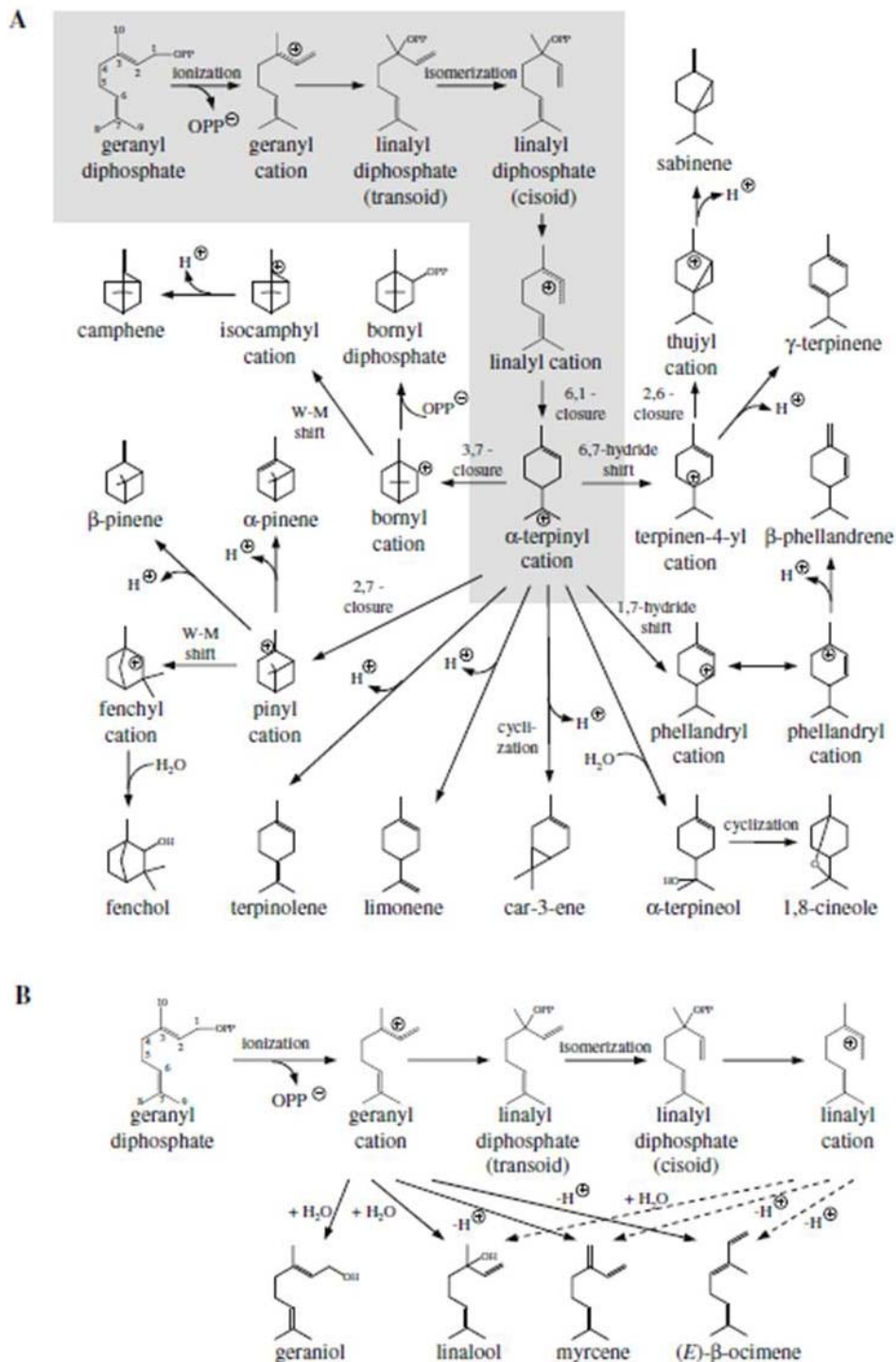


**Figure 1.** Isopentenyl diphosphate synthesis via the MEP or the via the MVA pathway. Intermediates involved in the biosynthesis are as follows: MEP pathway 1, pyruvate; 2, D-glyceraldehyde 3-phosphate; 3, 1-deoxy-D-xylulose 5-phosphate; 4, 2C-methyl-D-erythritol 4-phosphate; 5, 4-diphosphocytidyl-2C-methyl-D-erythritol; 6, 4-diphosphocytidyl-2C-methyl-D-erythritol 2-phosphate; 7, 2C-methyl-D-erythritol 2,4-cyclodiphosphate; 8, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate; MVA pathway 9, Ac-CoA; 10, AcAc-CoA; 11, HMG-CoA; 12, MVA; 13, mevalonate 5-phosphate; 14, mevalonate 5-diphosphate. Enzymes of the MEP pathway are as follows: DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MCT, 2C-methyl-D-erythritol 4-phosphate cytidyl transferase; CMK, 4-diphosphocytidyl-2C-methyl-D-erythritol kinase; MDS, 2C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; HDS, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase; HDR, 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate synthase; IDI, isopentenyl diphosphate isomerase. Enzymes of the MVA pathway are as follows: AACT, AcAc-CoA thiolase; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; MK, mevalonate kinase; PMK, phosphomevalonate kinase; PPMD, diphospho-mevalonate decarboxylase. Both pathways lead to the formation of 15, dimethylallyl diphosphate; 16, isopentenyl diphosphate. The interconversion of IPP into DMAPP is catalyzed by IDI, isopentenyl diphosphate isomerase (Hemerlin et al., 2012).

## Monoterpene synthases

The reaction mechanisms of all monoterpene synthases start with the ionization of the geranyl diphosphate substrate (Figure 2). The resulting carbocation can undergo a range of cyclizations, hydride shifts and rearrangements before reaction is terminated by deprotonation or water absorption. The mechanisms of cyclic (A) and acyclic (B) monoterpene synthases are depicted separately. The formation of cyclic monoterpenes requires the preliminary isomerization of the geranyl cation to a linalyl intermediate, capable of

cyclization. The production of the initial cyclic species, the  $\alpha$ -terpinyl cation, opens the door to secondary cyclizations. The formation of the acyclic monoterpenes linalool, myrcene and (E)- $\beta$ -ocimene might proceed either via the geranyl cation or via the linalyl cation. The numbering of carbon atoms of the intermediates and the products refers to that for geranyl diphosphate (GPP) (Degenhardt et al., 2009). In the last several years, a number of monoterpene synthases has been isolated and characterized from various plant species of both angiosperm and gymnosperm groups (Nagegowda and Dudareva, 2007; Fährnrich et al., 2011). Linalool synthase



**Figure 2.** Conversion of geranyl diphosphate to the monoterpenes: cyclic (A) and acyclic (B) (Degenhardt et al. 2009).

was first isolated and characterized in *Clarkia breweri* flowers (Dudareva et al. 1996). Another two monoterpene synthases' genes, (E)- $\beta$ -ocimene and myrcene synthase, were isolated in snapdragon (Dudareva et al., 2003). Two

flower-specific monoterpene synthases have been isolated from Satsuma mandarins (*Citrus unshiu* Marc), producing an 8-cineole and (E)- $\beta$ -ocimene, respectively (Shimada et al., 2005). A new monoterpene synthase

gene was recently identified from tomato that uses neryl diphosphate as the precursor for monoterpenes synthases (Schillmiller et al., 2009). All monoterpene synthases have similar properties with respect to their native molecular mass and requirement of a divalent metal ion (usually  $Mg^{2+}$  or  $Mn^{2+}$ ) needed for the activity.

Monoterpene biosynthesis occurs in plastids and all known monoterpene synthases are encoded by nuclear genes and possess an N-terminal transit peptide that directs their imports into the plastid (Wise et al., 1998; Turner et al., 1999). The 30 to 80 amino-terminal residues are characterized with a low degree of similarity, typical of targeting sequences, yet those sequences are serine and threonine-rich but with few acidic residues. The DDxxD motif which is found in virtually all deduced sequences for enzymes that utilize prenyl diphosphate substrates (Keegstra et al., 1989; Whittington et al., 2002), is responsible for the coordination of divalent cations and is essential for substrate binding and ionization (Zhou and Peters, 2009). In addition to DDxxD, the monoterpene synthases possess characteristic motifs, including RR(x) 8W and LYEASY, GTLXEL. The RR(x) 8W motif is essential for the enzymatic activity of many monoterpene synthases and LYEASY.GTLXEL motif was also thought to be a part of the active site (Williams et al., 1998; Wise et al., 1998). Most monoterpene synthase genes were characterized in dicotyledon plants and that from monocotyledon plants is especially limited.

Contrary to the general notion that all monoterpenes are localized to the plastids, some recent reports indicate that monoterpene synthases can deviate from this pattern and be localized to the cytosol as was demonstrated in the case of Nerolidol Synthase1 (FaNES1) and pinene synthase (FvPIN) of strawberry (*Fragaria vesca*), or can have a dual plastid and mitochondrial localization, as was reported for Nerolidol Synthase2 (FaNES2) from strawberry and  $\alpha$ -terpineol synthase from Magnolia (Aharoni et al., 2004; Lee and Chappell, 2008). Transgenic Arabidopsis and potato plants expressing the plastid targeted strawberry FaNES1, are capable of forming both linalool and nerolidol from geranyl diphosphate (GPP) and farnesyl diphosphate (FPP), they respectively emitted small amounts of nerolidol in addition to expected high levels of linalool, indicating a small pool of FPP and is presented in plastids (Aharoni et al., 2003). Transgenic tobacco plants over expressing a cytosolic limonene synthase (truncated at the N-terminus) produced low levels of limonene indicating the presence of a small geranyl diphosphate (GPP) pool in the cytosol (Ohara et al., 2003).

## REGULATION OF MONOTERPENE SYNTHASES

Monoterpene production and accumulation in plants are

related to specialize anatomical structures and plants which lack such specialized structures, typically accumulate only trace quantities of monoterpenes. For example, monoterpene biosynthesis in mints originates from the secretory cells of peltate glandular trichomes, which cover the surfaces of the above-ground parts of the plant and the essential oil is stored in the subcuticular storage cavity of these trichomes (Turner et al., 2000). In snapdragon, the production and emission of two main fragrance compounds, the monoterpenes myrcene and (E)- $\beta$ -ocimene, occur in the upper and lower petal lobes (Dudareva et al., 2003).

The rhythmic and developing regulation of volatile terpenoid emission, from different species, has been reported. In *Artemisia annua*, the emission of  $\alpha$ -pinene, fluctuates with the day-night rhythm and is generally higher in the day than in the night (Lu et al., 2002). And so in snapdragon flowers, emissions of monoterpene compounds follow a diurnal rhythm that is controlled by a circadian clock (Kolosova et al., 2001). In *Nicotiana suaveolens*, the nocturnal emission of several monoterpenes from petals and stigmas is a consequence of the transcriptional regulation of the 1,8-cineole synthase by the circadian clock (Roeder et al., 2007). The levels of monoterpenes are thought to be controlled transcriptionally (Mahmoud and Croteau, 2003; Lane et al., 2010; Ramak et al., 2013b). However, not all monoterpenes are controlled at this level (Xie et al., 2008; Schmiderer et al., 2010). In garden sage, seasonal variation was observed in monoterpene formation and in gene expression of the enzymes responsible for the first step of monoterpene formation. The highest correlation between mRNA abundance and the end products was found between (+)-bornyl diphosphate synthase and the borneol, camphor and bornylacetate-branch (Grausgruber et al., 2012).

In addition, environmental conditions such as temperature, day length and light effect quantitative compositions of plant compound mixtures like volatiles and essential oils (Figueiredo et al., 2008). These conditions change during a vegetation period significantly and predictably leading to a pattern of seasonal variation in plant metabolites that is generally repeated every year. Most agricultural crops are harvested only once a year either, at the end-point of development or at the stage of maximum yield. When harvesting herbs like sage, however, the situation is different since, under favorable climatic conditions, several harvests per year are possible. Therefore, basic knowledge about seasonal influences on the composition of plant secondary compounds is essential for determining optimal harvest times for a good quality herb production. The major monoterpenes 1,8-cineole, camphor and the two thujones show pronounced dynamics during a vegetative cycle that has been confirmed under different geographical conditions (Belhattab et al., 2005; Maric et al., 2006). The monoterpene emission

of conifers is generally regarded as light-independent because it mainly originates from compounds stored after syntheses in special organs, such as resin ducts in *Pinus* species (Monson et al., 1995; Lerdau et al., 1997). The effect of elevated temperature on the monoterpene pool size is equally unclear. Elevation of growth temperature has been found to decrease (Snow et al., 2003) or have no effect on the monoterpene concentration in needles of *Pseudotsuga menziesii* Franco (Constable et al., 1999), but increase the concentration in *Pinus sylvestris* L. (Sallas et al., 2003).

## MEDICINAL PROPERTIES OF MONOTERPENES

Many studies have shown that, in the prevention and treatment of cancer, several of the dietary monoterpenes are effective (Elson and Yu, 2004; Crowell, 1999; Kris-Etherton, 2002). Among these, monocyclic monoterpenes D-limonene and perillyl alcohol are known to inhibit the development of mammary, liver, skin, lung, colon, forestomach, prostate, and pancreatic carcinomas (Shi and Gould, 2002). The metabolites of D-limonene such as perillic acid, dihydroperillic acid, limonene-1,2-diol and the oxygenated molecule of D-limonene, carvone, have also been shown to have anti cancer activities (Kris-Etherton, 2002; Carvalho et al., 2006). The mechanism of the monoterpene anti tumor effects is the inhibition of posttranslational isoprenylation of proteins regulating the growth of cells (Crowell et al., 1991). In addition to anti cancer, there is a pool of terpenoids known for their anti inflammatory properties (Look et al., 1986a).

Formation of the free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effects on the organisms (McCord, 2000). Free radicals and other relative species cause the oxidation of biomolecules (for example, protein, amino acids, lipid, and DNA) which leads to cell injury and death (McCord, 2000). In recent years, the antioxidant and free radical scavenging capacity of some monoterpenoids have been reported (Tepe et al., 2004; Tepe et al., 2005). Thymol and terpineol which were present in *Chamaecyparis obtusa*, have been shown to exert potent antioxidant and free radical scavenging capacities in various antioxidant models (Mata et al., 2007). Also the oregano essential oil, rich in thymol and carvacrol, has a considerable neutralizing effect on free oxygen and prevention of lipid peroxidation (Kulisic et al., 2004).

There have been many monoterpenes, such as linalyl acetate, 1,8-cineole, (-)-linalool, and its esters, that possess anti inflammatory activity (Peana et al., 2002; Peana et al., 2003). Particularly, 1,8-cineole was found to be useful in curing chronic ailments such as bronchitis sinusitis and steroid-dependent asthma, or as a preventive agent in returning respiratory infections (Look et al.,

1986b). The monoterpene phenol derivative of cymene, thymol and its structural derivatives also possess an anti leishmanial potential (Robledo et al., 2005). Menthol derivatives have also been described to possess trypanocidal activity (Kiuchi et al., 2002) and Carvacrol inhibits the growth of several bacteria strains; example, *Escherichia coli* (Du et al., 2008) and *Bacillus cereus* (Ultee and Smid, 2001). In addition, in *Pseudomonas aeruginosa* it causes damages to the cell membrane of these bacteria and unlike other terpenes, inhibits the proliferation of this germ (Cox and Markham, 2007). There is also a large group of monoterpenes, which possess anti viral and immunostimulating properties. It was shown that monoterpenes from *Plantago* species enhanced the activity of human lymphocyte proliferation and secretion of Interferon gamma (Chiang et al., 2003). Carvone, limonene and perillic acid were found to increase the total white blood cells count in mice (Raphael and Kuttan, 2003). Moreover, monoterpenes such as linalool, carvone, and thymol were also demonstrated to enhance the permeability of model drugs such as 5-fluorouracil through skin and mucous membranes (Narishetty and Panchagnula, 2004).

## SOME ECOLOGICAL ROLES OF MONOTERPENES

Most Secondary metabolites have significant ecological importance in the interactions between plant and its environment, since they function in protection against herbivores, bacteria and fungi, or as attractants for pollinators, or as allelopathic agents (Croteau et al., 2000). It has been shown that monoterpenes can make the photosynthetic apparatus more resistant to high temperatures, thus protecting it against heat stress (Delfine et al., 2000). Volatilized monoterpenes also have a potential to react with various oxidizing agents (OH, O<sub>3</sub> and NO<sub>3</sub>) existing in the atmosphere (Calvert et al., 2000) and thus could protect plants against internal oxidative damages (Loreto et al., 2004). Herbivory has been found to induce monoterpene emission, which can attract the natural enemies of the herbivores (Kessler and Baldwin, 2001). Furthermore, not only the monoterpenes inside plant organs are detrimental to herbivores (Seybold et al., 2006), but also the volatilized vapor itself, has been found to be toxic (Raffa et al., 1985). The atmospheric lifetimes of individual monoterpenes are variable, mainly depending on the chemical structure of each compound, and also depending strongly on the concentrations of the oxidizing agents in the atmosphere (Atkinson and Arey 2003).

## Conflict of interests

The author(s) have not declared any conflict of interests.

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