Chapter 1

Introduction, Review of the Literature and Objectives of the Present Investigation

1.1 Introduction

The East Indian Sandalwood, *Santalum album* L., is a tropical, woody and perennial essential oil yielding tree. The fragrant heartwood accumulates the sandalwood oil (SWO), rich in sesquiterpenoids and is reported to have many medicinal properties. Epidemic phytoplasmal 'spike' disease leading to severe destruction, illegal poaching and over exploitation owing to increased global demand are the reasons of it being inducted into IUCN, Red List of Threatened Species (IUCN, 2006) as vulnerable, thus necessitating its *in vitro* micropropagation and biotechnological manipulation. The *Santalum album* wood and oil (rich in sesquiterpenoid santalols) are among the costliest plant products (wood ~ US $ 180 kg^{-1}; oil ~US $ 2600 l^{-1}). The estimated average global annual export is about 2, 000 tons of wood and 100 tons of oil (Gupta, 1998), against a projected demand of 10, 000 tons of wood, involving an international trade of about US $ 360 million; thus implying a huge gap between demand and supply, largely due to dwindling natural population of the plant. In USA alone the total need is 22, 000 kgs yr^{-1} (Burdock and Carabin, 2008). Sandalwood tree is valued for commercial, medicinal, traditional, spiritual, aesthetic and social importance. The traditional curative properties for acne, urino- genital tract infections, bronchitis, congestion etc. are known.

The number of plant species in India is estimated to be over 45, 000 representing about 7 % of the world's flora. Medicinal plants, as a group, comprise approximately 8, 000 species and account for about 50 % of all the higher flowering plant species of India. India is one of the richest countries in the world as regards genetic resource of medicinal and aromatic plants. It constitutes 11 % of the total known world flora having medicinal properties (http:// www. inheritanceindia.co.in). The commercial, traditional and social importances of this medicinal tree are:

1. Cosmetics, perfumes, soaps, musks, face packs for its fragrance, fixative property and its oil base fixes both dry and oily skin.
2. Handicrafts, luxury items and wooden furniture for domestic use due to durability and scent.
3. The unique fragrance of the oil is known as an aid to meditation, spiritual growth, emotionally it relaxes stress, soothes irritation, lifts depression and is aphrodisiac.
4. It has a long history in ethno pharmacology in India and elsewhere, for uses such as a tonic to the immune system, effective against strep throat, gastrointestinal ailments, skin infections, reducing vomiting, fever, thirst and so on, though the perfumery use has outweighed the medicinal uses.

No active principles were implicated in such healthcare purposes. However, in the last decade, the compounds from oil were shown to have antimicrobial, anticancer and antiviral properties. The above facts inspired us to the present study on bioprospecting of the phenylpropanoids and terpenoids. Apart from the healthcare angle, the accumulation of the oil in the heartwood in this plant generates natural curiosity of correlating the profiles of phenylpropanoids and terpenoids.

1.2 Review of literature

1.2.1 Taxonomy

The East Indian Sandalwood, belongs to the angiosperm class of 'Magnoliopsida', specifically to the 'Rosids', in the order 'Santalales', family 'Santalaceae', type genus '*Santalum*' and species '*S. album*'. The common name is East Indian Sandalwood or White Sandalwood as well as Chandan in Hindi.

Tree, semi- parasitic in roots (host plants usually are *Cassia, Dalbergia, Azadirachta, Pongamia, Casuarina, Pterocarpus, Acacia* etc.). Leaves opposite, simple, decussate, shape varies among six morphological types- ovate, lanceolate, elliptic, linear, big and small. Inflorescence axillary or terminal cymose panicles. Flowers: small, purplish brown, unscented. Fruits: drupe, purplish when fully matured, single seeded, shape- round to oblong. Seed: polymorphic, lacking testa, dicotyledonous embryo, endosperm fleshy. Basic chromosome number of the genus is x= 10, few are polyploids. Twenty different species, a few are commercially important; include *S. lanceolatum, S. yaasi, S. spicatum, S. austrocaledonicum.* In India, more than 90 % of sandalwood forest lies in Karnataka and Tamil Nadu covering an area of 8, 300 sq. km. Andhra Pradesh, Kerala, Maharashtra, Madhya Pradesh and Orissa are some other states where sandalwood trees are found (Figure 1.1). A total area of about 9, 600 sq. km. harbors sandalwood plantation (Rao and Bapat, 1992). *Santalum album* L. is distributed throughout Indonesia in the West, Portuguese island of Timor and on Celebes, Ceylon to Juan Fernandez Islands in the East and from Hawaiian Archipelago in the North to New Zealand in the South.

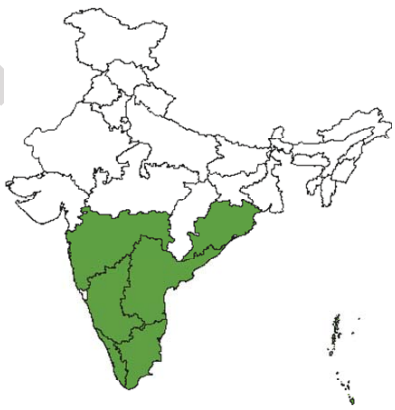

Figure 1.1 Distribution of East Indian sandalwood in Indian subcontinent (modified from Srinivasan et al., 1992)

1.2.2 Diseases and pests

Spike disease, caused by a phytoplasmal organism invading the phloem tissues, is the most destructive among all diseases in sandalwood, resulting into severe destruction of population; as high as 7 million plants in a particular calendar year (Srinivasan et al., 1992). This calls for the necessity of *in vitro* grown disease- free planting stock and the production of SWO or its constituents under *in vitro* conditions.

1.2.3 Sandalwood essential oil

The quality of sandalwood timber, measured by its oil content, is dependent on its species. *Santalum album* has the highest oil content (~6 %, India) followed by *S. yasi* (~5 %, Fiji), *S. austrocaledonicum* (~4 %, New Caledonia, Australia) and *S. spicatum* (~2 %, Australia). Oil content also varies within species, depending upon the genotypes (Vernes, 2001). The essential oil develops in the heartwood of the stem and root. The site factor significantly influence the heartwood formulation. This forest tree yields the much precious SWO that contains over 90 % santalols (α- and β- santalols and their isomers) (Demole et al., 1976). SWO is accumulated in the heartwood only after around 20- 30 years of its growth (Howes et al., 2004) under natural conditions, depending upon the age of tree and the color of heartwood (Srinivasan et al., 1992). The essential oil yield from an old matured tree (> 25 years old) ranges from 2.5- 6 % (the highest among all 20 *Santalum* species) depending upon the individual tree under study, location within the tree and the environment of growth of the tree (Thomson, 2006). There is a compositional difference in oils obtained from young and mature sandal trees and the content and composition of oil varied from heartwood at different levels in the tree (Shankarnarayana and Parthasarathi, 1984).

1.2.4 Santalenes as major constituents

All the C_{15} sesquiterpenoid compounds, including the santalane, bisabolane and campherenane type molecules with the santalane- skeleton, are referred as "santalenes". Santalane type sesquiterpenoids and their biogenetically related bisabolane and campherenane type analogues are characteristic of the family Santalaceae (Valder et al., 2003). The oil components from various species of sandalwood, their uses, and olfactory responses were reviewed (Erligmann, 2001). In particular, plants of the genus *Santalum*, distributed in India, Indonesia, Malaysia, and Australia, are rich in such sesquiterpenoids (Guha and Bhattacharya, 1944; Demole et al., 1976; Buchbauer et al., 1979; Christenson et al., 1981; Ranibai et al., 1986) and phenylpropanoids (Gibbard et al., 1969).

In SWO, α- santalol is twice more abundant than β- santalol (Anonis, 1998). Epi- β- santalol was isolated for the first time from East Indian SWO (Brunke et al., 1980). Trans- β- santalol, epi- cis- β- santalol (Christenson et al., 1981), α- santalol and β- santalol (Brieger, 1963), α- curcumene and β- curcumene (Adams et al., 1975), α- nuciferol (Kim et al., 2005)

were identified in the heartwood. Major SWO constituents are sesquiterpene alcohols like, cis- α- santalol (53 %), and cis- β- santalol (23 %) (Verghese et al., 1990), α- trans-bergamotol, epi- cis- β- santalol whereas minor constituents include trans- β- santalol and cis-lanceol (Howes et al., 2004), hydrocarbons such as, α- santalene, β- santalene, α-bergamotene, epi- β- santalene, α- curcumene, β- curcumene, γ- curcumene, β- bisabolene and α- bisabolol (Adams et al., 1975; Howes et al., 2004; Jones et al., 2006). In East Indian sandalwood tree, 46 compounds including 32 newly identified and 4 novel molecules, inclusive of 17 sesquiterpenoids were detected (Demole et al., 1976). Three new campherenane- type, three new santalane- type sesquiterpenoids, and two aromatic glycosides together with 12 known metabolites including β- santalals, β- santaldiols, α- santalenoic acid were isolated from sandalwood chips (Kim et al., 2006). Other constituents include dihydro-β- agarofuran, santene, teresantol, borneol, teresantalic acid, tricycloekasantalal, santalone, santanol, β- farnesene and dendrolasin (Leung and Foster, 1996). The seed oil contains stearolic acid, santalbic acid and methyl santalbate (Jie et al., 2006). Minor constituents in SWO include phenols, lactones and terpenes. About 2- 4 % of santalol is present as esters (Budavari et al., 1999). This 'santalol content' renders East Indian SWO precious and necessitates further research on its oil biosynthetic pathway.

1.2.5 Chemo- synthesis of santalenes

Growing global demands added to scarcity in natural sources for SWO production, fuelled research related to chemo- synthesis of santalol odor 'homologues' and 'analogues'. First synthesis of α- santalol utilized camphor in a Wittig reaction (Wittig and Geissler, 1953) and required aggressive reagents but the final product obtained was *E*- isomer whereas the natural one is a *Z*- isomer. The natural *Z*- α- santalol is synthesized using, a variant, Wittig-Schlosser reaction (Lewis et al., 1967). Synthesis of β- santalol relies upon, either Claisen rearrangement, asymmetric Diels- Alder reaction starting from norbornanone to yield a final mixture of *Z*- β- santalol and *E*- β- santalol (Krotz and Helmchen, 1990).

1.2.6 Synthetic analogues of santalanes

The SWO substitutes constitute two classes, based on their chemistry of synthesis. First group called terpenophenols are prepared from acid catalysis of camphene (a terpene) and guaicol (a phenol), yielding about 128 isomers with a strong odor of sandalwood. Second class comprised of campholenic aldehydes, prepared by treatment of α- pinene oxide through aldol condensation with another aldehyde or a ketone, yielding unsaturated derivatives, which upon reduction provided sandalwood odor (Sell, 2003). First sandalwood- smelling mixtures of terpenylcyclohexanols were serendipitously found in late 1930's, launched into market in 1960's in the name of Sandela (Givaudan, Switzerland). This was followed by Osyrol (IFF, USA) launched in 1973, a milestone in SWO mimics. Large varieties of synthetic molecules

giving the sandalwood note are extensively reviewed (Bajgrowicz and Gaillard, 2007) and are beyond the scope of this thesis.

1.2.7 Sandalwood odor chemistry

For musky and sandalwood odors, the hydrocarbon group in the molecule must follow a size- constraint to generate odor stimuli. Sandalwood is a typical 'musky'/ 'animalic' fragrance, owing to a size- constrained hydrocarbon group in the molecules, belongs to a typical chemistry with C_{12} to C_{16}/ C_{17}, compact, spherically rigid molecules with free - OH groups located 4 A° of a bulky quaternary carbon, where ramifications from carbon 2 and carbon 6 and the presence of an electron donor either support or generate the sandalwood odor (Chastrette and Zakarya, 1990). Molecular surface and electrostatic properties of β-santalol were rendered critical to aroma, where 3 osmophoric points were held responsible for sandalwood tonalities (Buchbauer et al., 2001) whereas santalophore pattern studies revealed that odor- active compounds possessed only a -OH or t- butyl as a functional group (Chastrette et al., 1990).

1.2.8 Organoleptics and physicochemical properties

Sandalwood oil (EC- 284- 111- 1, CAS- 8006- 97- 9, FEMA- 3005, and FDA- 3301.29.6000) odor type is sweet, woody, medium strength, balsamic and nutty. Appearance is pale yellow to yellow and a clear oily liquid with a specific gravity of 0.97- 0.978 at 25 °C, pounds per gallon weight as 8.071- 8.138 with a refractive index of 1.498 - 1.512 at 20 °C. The optical rotation is - 20 to - 15, boiling point 276 °C at 760 mm Hg, flash point is > 200 °F or 93.3 °C and a shelf life of 24 months. The oral toxicity, LD_{50} values are at 5580 mg/ kg, for rats and the dermal toxicity values are at more than 5 g/ kg levels on the skin of rabbit. Recommendation usage level is 10 % in fragrance. This fragrance cannot be substituted with any synthetic analogues.

1.2.9 Santalol biosynthetic pathway

SWO is a complex mixture of sesquiterpenoids, mostly the abundant 'santalols', followed by santalenes, bisabolenes, bergamotenes, curcumenes and their numerous isomers. The biosynthetic/ biogenetic pathways leading to their accumulation is still far from established) owing to slow onset of oil formation making *in vivo* isotopic feeding difficult and localization of key functional enzymes within so called 'dead' woody tissues impossible (Jones et al., 2006). Farnesyl pyrophosphate (FPP) was identified as a possible precursor for 'santalol' biosynthesis and biosynthetic schemes were proposed (Parker et al., 1967). Enzymes known as 'terpene cyclases' or 'terpene synthases' generate enantiomers from the corresponding diphosphate owing to inherent chirality arising from the folding of an acyclic substrate (Colby et al., 1998). FPP, by change of double bond position forms the *E, Z-*

farnesyl cation, in turn which on several rearrangement intermediates lead to the formation of a nerolidyl cation. On subsequent rearrangement, the later forms the *E*, *Z*- farnesyl cation which forms the bisabolane carbocation. Probably, the initial bisabolane carbocation is being trapped by the double bond in the ring. This leads to the campheranane skeleton. This name draws attention to the fact that the skeleton resembles that of the camphene series of monoterpenoids where an extra isoprene unit is attached to one of the methyl- groups on the one- carbon bridge. A Wagner- Meerwein rearrangement of this carbocation gives the α- santalane skeleton, whilst trans- annular elimination of a proton, leads to the formation of β- santalanes. This β- santalane family of sesquiterpenoids lead to the formation of a plethora of intermediates and final products produced again biogenetically in a very defined manner. The positive charge of the bisabolyl cation can be trapped by the double bond in the six membered rings and this can happen in one of two ways. In one way, it is trapped to leave a tertiary cation by a strained four- membered ring giving carbocation. The carbocation can eliminate a proton to give α- bergamotene. The alternative one is for the cation to add to the other end of the double bond. This gives a less strained ring system but a less stable secondary carbocation. The resultant structure is the intermediate. Transannular elimination of a proton from the intermediate gives α- santalene. A 1, 2- carbon shift in the intermediate gives a new 2, 2, 1- bicycloheptyl carbocation that eliminates a proton to give β- santalene (Sell, 2003). Epi- β- santalene, α- santalene, β- santalene and α- bergamotene could be formed from the cyclization of 3- *S*- nerolidyl diphosphate. Bisabolyl cation intermediate could generate β- curcumene and β- bisabolene, from its R form while the S form could yield α- curcumene and γ- curcumene. Synthesis of β- bisabolene could occur via the initial cyclisation product from farnesyl or nerolidyl diphosphate, followed by loss of a proton from C- 14. Formation of curcumenes, like γ- curcumene and β- curcumene were related to that of β- bisabolene and α- bisabolol whereas biogenesis of β- bergamotene was linked to biosynthesis of santalenes (Kulkarni, 1966).

Quite interestingly, a recent report, claimed that a sesquiterpene synthase from *Santalum album*, upon heterologous expression in *Escherichia coli* yielded, β- elemene and germacrene D- 4- ol as the major products (> 55 % together) and minor constituents such as γ- muurolene, α- selinene, α- bulnesene, helminthogermacrene, δ- cadinene, β- selinene, α- humulene, *E*- nerolidol, patchoulene etc. A sandalwood monoterpene synthase upon heterologous expression produced, α- terpineol and limonene (> 75 % together), *E*- geraniol, linalool, myrcene, sabinene, α- terpinolene (Jones et al., 2008). The activities were localized to heartwood and leaves of matured trees and santalol biosynthesis was addressed as resultants of cytochrome P450 gene family. Cyclization of FPP results into a *E, E*- or *E, Z*- germacradienyl cation, which upon reaction with water yields germacrene D- 4- ol, germacrene A and β- elemene by deprotonation, whereas dehydration of germacrene D- 4- ol

formed δ- cadinene and γ- cadinene. Germacrene A could re- ionize forming α- bulnesene and upon mild acidification yielded α- selinene and β- selinene (de Kraker et al., 1998).

1.2.10 *In vitro* micropropagation and plant development

Somatic embryogenesis is foreseen as a technology for cloning of commercial forest trees while somatic embryos are projected models to study wood formation (Karkonen, 2001), differentiation, embryo development and physiology (Komamine et al., 1992). Secondary metabolism is linked to tissue differentiation (Verpoorte et al., 1999), thus somatic embryos constitute the first stage of differentiation, where secondary products could be screened. From the first report on somatic embryogenesis *in vitro* in 1957 from *Oenanthe aquatic* (Karkonen, 2001), with established zygotic embryogenesis in model plant *Arabidopsis,* somatic embryogenesis in *Daucus carota* and wood formation in model plant, *Populus*, one looks forward to establishment of an *in vitro* model to study wood formation. Among the tropical forest trees concerned, sandalwood could lead as a model tree with well- established somatic embryogenesis stages and events. Somatic embryogenesis offers a potential system for large-scale plant propagation in bioreactors (Paek et al., 2005).

1.2.11 Secondary metabolism in plants

'Secondary metabolites' are compounds produced by plants that are not directly essential for basic photosynthetic or respiratory metabolism, processes which are mainly governed by the 'primary metabolites'. Secondary plant metabolites may not play a fundamental biochemical role in the normal building and maintaining of plant cells (Dixon, 2001), but they too play an ecological role. Number of metabolites present in the plant kingdom is estimated to exceed 3, 00, 000 and are represented in all higher plants in a high structural diversity. Secondary metabolites such as terpenes serve a range of functions i.e., herbivore deterrence, fungal toxicity and pollinator attraction. Developmental, abiotic and biotic signals can directly or indirectly influence changes in biochemical pathways leading to the production of bioactive primary and secondary metabolites (Croteau et al., 2000).

1.2.12 Plant cell cultures as sources of natural products

Plant cell cultures for the production of valuable natural products such as pharmaceuticals, flavors and fragrances, and fine chemicals targets over 30, 000 various chemicals produced by them, with about 2, 000 new plant chemicals added each year (Doran, 1993) that globally includes 121 clinically useful prescription drugs (Payne et al., 1991). According to OECD report, plant derived drugs and intermediates account for approximately US $ 10 billion annually in US (Principe, 1989). The biosynthetic totipotency of plant cells offer attractive alternative source to whole plant for the production of high- value secondary metabolites (Kieran, 2001), with success known for, shikonin, ginseng and berberine

production at commercial scales (Misawa, 1991). Major target products from biotechnological manipulation include, flavonoids, alkaloids, polysaccharides and terpenoids etc. Presently, plants producing immunomodulatory, antiviral, antimicrobial, antiparasite, antitumor, anti-inflammatory, hypoglycemic, tranquilizer and anti- feedant principles are in demand (Yamada, 1991). Callus cultures are easy to establish, manipulate and grow rapidly thus allowing secondary metabolite production *in vitro* in large scale. Somatic embryos consist of well- differentiated cells at organ- level organization with higher growth rates and are amenable to growth in traditional bioreactors. A correlation existed between the expression of secondary metabolism and morpho- / cyto- differentiation, though unclear in relation to development of specific structures and genetic and/ or physiological links. Secondary metabolites are produced by specialized cells and or at distinct developmental stages, and thus pose the biggest challenge. In sandalwood, only a few articles have been published (Pal et al., 2003) indicating major metabolites obtainable in cultured cells.

1.2.13 Approaches to characterize secondary metabolome

Owing to the vastness and types of secondary metabolites synthesized within a plant cell, their over- lapping biosynthetic schemes and turnover rates, its not yet possible to quantify all the metabolites in a cellular system (Fernie et al., 2004). Current approaches in *plant systems biology* are aiming at comprehensively describing the living cell at several organizational levels: genes (The *Arabidopsis* Genome Initiative, 2000), transcripts (Ruuska et al., 2002) and metabolites (Roessner et al., 2001). Metabolite profiling by mass spectrometry (MS) is just a decade old but has a strong footholds within the area of plant biotechnology (Fiehn, 2000; Huhman and Sumner, 2002) and drug discovery (Boros et al., 2002). Box. 1 summarizes various profiling and fingerprinting aspects.

Box 1. Analytical methods for plant metabolite profiling and chromatographic fingerprinting.

Extraction techniques for 'plant metabolites'-

a. Pressurized liquid extraction (Benthin et al., 1999)
b. Supercritical fluid extraction (Blanch et al., 1999)
c. Sonication (Sargenti and Vichnewski, 2000)
d. Subcritical water extraction (Gámiz- Gracia and de Castro, 2000)
e. Microwave techniques (Namiesnik and Gorecki, 2000)
f. Pervapouration (Starmans and Nijhus, 1996).

Vastness of 'plant metabolome' and the 'hyphenated' solutions-

I. Size of plant metabolome- 90, 000- 200, 000 metabolites.
II. ~150 compounds (77 as amino acids, sugars, organic acids) within potato (*Solanum tuberosum*) tuber by GC- MS, first ever metabolite profiling (Roessner et al., 2001).
III. ~326 metabolites (half could be assigned) in two *Arabidopsis* ecotypes (Fiehn, 2002)
IV. Sugars, amino acids and glycosides in phloem exudates of *Cucurbita maxima* by LC- MS (Tolstikov and Fiehn, 2002).
V. ~ 27 saponins in *Medicago truncatula* (Huhman and Sumner, 2002).
VI. ~ 5844 masses (assigned chemical formulae to half) from strawberry (*Fragaria ananassa*) fruits by FT- MS (Aharoni et al. 2002).
VII. ~1000 metabolites (leaf) and 600 metabolites (tuber) of potato (*Solanum tuberosum*) by GC- ToF (Bino et al., 2004).
VIII. ~1400 components from Arabidopsis leaf extracts by LC- ESI- ToF- MS analysis (Roepenack-Lahaye et al. 2004).
IX. ~300- 500 polar/ non- polar extracts and ~400 samples/ week by GC- MS (Lisec et al., 2006).
X. Metabolite profiling have been performed with- tomato, rice, wheat, strawberry, cucumber, lettuce, tobacco, poplar, eucalyptus etc. (Schauer and Fernie, 2006).
XI. Auxins, cytokinins, abscisic acid, gibberellins, jasmonates, salicylates quantification in crude plant extracts, i.e. *Arabidopsis* by HPLC and ESI- MS (Pan et al., 2008).
XII. Profiling of lignin biosynthesis, isoflavone synthesis, isoprenoids, oxylipins, taxane, diterpenoids, alkaloids, flavonoid glycosides and volatiles (Trethewey, 2004).

LC- based metabolite profiling address qualitative and quantitative evaluation for-

i. Saponins (Huhman and Sumner, 2002)
ii. Phenolics (Chen et al., 2003)
iii. Sugar derivatives (Tolstikov and Fiehn, 2002)
iv. Carotenoids (Romer et al., 2000).

Chromatographic fingerprinting methods include-

✓ TLC and HPTLC (Xie and Yan, 1988): fast, easy, cheap but poor precision
✓ GC (Yuan et al., 2003): high precision, limited application.
✓ HPLC (Goppel and Franz, 2004): HPLC- UV- DAD of paramount interest (Engelhardt and Konig, 1989).
✓ CE (Wang et al., 2005): limited to specific groups of metabolites.
✓ ^{1}H- LC- NMR (FAO, 1996): quantity of samples required is a limitation.
✓ HSCCC (Gu et al., 2004): poor resolution and reproduction
✓ UV, IR, NMR (Zhou et al., 2002): inability to resolve complex mixtures.

Hyphenated chromatographic/ mass spectroscopy approaches [GC- MS, LC- MS and CE- MS] offer advantages-

- Correction of retention time shift, selectivity, chromatographic separation abilities
- Measurement precision, elimination of instrumental interference (~ 'dimension advantages') (Gong et al., 2001; Booksh and Kowalski, 1994)

Chromatographic fingerprinting analysis (Ji et al., 2005; Ke and Wang, 2005) **is recommended for botanicals/ plant extracts/ herbal medicines by-**

- WHO, FDA, EMEA,
- German Commission E,
- British Herbal Medicine Association,
- Indian Drug Manufacturers' Association,

(Philipsom, 1996; IDM, 1998; EMEA, 1999; WHO, 2000; FDA, 2000; Calixto, 2000).

1.2.14 Phenylpropanoid biosynthetic pathway

Phenylpropanoids (PP) have important functions in plant defense against pests and predators, as UV protectants and as signal molecules both internally and for communication with other organisms (Lynn and Chang, 1990). They comprise of a group of phenylalanine-derived molecules with general C_6- C_3 skeleton, utilizing common set of chemical transformations, usually through CoA mediators (Noel et al., 2005). Starting from the enzyme mediated deamination reaction of L- phenylalanine to t- cinammic acid, they eventually form a wide variety of natural products, i. e., ~2 000, based on a phenylpropane backbone, from which a wide range of natural products are formed like lignin monomers, coumarins, benzoic acids, flavonoids and simple esters. Figure 1.2 outlines biosynthetic pathway of major groups of PPs leading to lignification, accumulation of secondary depositions and biosynthesis of heartwood.

1.2.14.1 Phenylalanine ammonia lyase (PAL)

Phenylalanine ammonia- lyase (PAL, EC 4.3.1.5) is a key enzyme of PP metabolism catalyzing the first reaction in the biosynthesis by a deamination reaction of L- phenylalanine (Strack, 1997) to give t- cinammic acid. Fluctuations in PAL levels control PP biosynthesis in higher plants. Elevated enzyme levels are characteristic of hormonal changes leading to lignification during xylem differentiation (Haddon and Northcote, 1976). PAL, the bridge between primary metabolism and natural product biosynthesis, is a potential site for pathway regulation both spatially and temporally. A small family of differentially regulated genes encode distinct isoforms of PAL enzyme subunits (Lois et al., 1989).

1.2.14.2 Cinnamyl alcohol dehydrogenase (CAD)

Cinnamyl alcohol dehydrogenase (CAD, EC 1.1.1.195) is an enzyme belonging to zinc- containing, long chain, NADPH- dependent alcohol dehydrogenase family of proteins, existing as a dimer of 40 kDa apiece subunits with broad substrate specificity (Higuchi, 1997). CAD is associated with the formation of lignin, in the last step in biosynthesis of monolignols in a rate- limiting regulatory manner. CAD or, its newly discovered isoform SAD (sinapyl alcohol dehydrogenase), catalyze the reduction of hydroxycinnamyl (or, PP)-aldehydes such as p- coumaraldehye, coniferaldehyde and sinapaldehyde to the corresponding alcohols i.e., p- coumaryl alcohol, cinnamyl alcohol and sinapyl alcohol, respectively, collectively known as "monolignols", the precursors of lignins and lignans (Bomati and Noel, 2005; Tobias and Chow, 2005). CAD activities are assayed by spectrophotometry, radiometry and HPLC (Santos et al., 2006). CAD homologues are multigene families. Structural models revealed zinc- binding motifs and a 'glycine' rich NAD^+- binding motif [Rossmann fold (GXGXXG)] that provides stereo- and aromatic specificity to the functional enzymes (McKie

et al., 1993). CAD enzymes were detected originally in plants and till date, several cDNA's have been studied, which are provided in Appendix A.2.

1.2.14.3 Peroxidase (POX)

Peroxidases (POX, EC 1.11.17) are ubiquitous, heme- containing glycoproteins that catalyze oxidation of diverse organic and inorganic substances at the expense of hydrogen peroxide (H_2O_2). POX isoenzymes of higher plants are classified as anionic, neutral and cationic, based on their isoelectric points (Barz et al., 1990) and are represented by an array of isoenzymes, approx. 10-20 isoforms. POXs have been linked to various physiological processes including lignification, suberization, cross- linking of structural proteins and polysaccharides, auxin metabolism, defense against pathogen attack and plant development (Brownleader et al., 2000). Cell wall associated POXs are involved in cross- linking reactions (Polle et al., 1994). Anionic POXs are specifically involved in the polymerization of the lignin monomers, based on their affinity for coniferyl alcohol, their location in the cell wall and their expression in lignified tissue (Carpin et al., 1999), whereas cationic POXs are associated with somatic embryogenesis (Cordewener et al., 1991). In *Oryza sativa*, 42 expressed sequence tags (ESTs) of POXs and in a commercial preparation of horseradish (*Armoracia rusticana*) POX (HRP) 42 isozymes were anticipated (Hoyle, 1977).

1.2.14.4 Polyphenol oxidase (PPO)

Polyphenol oxidases (PPO) or tyrosinases are enzymes with a dinuclear copper centre, which are able to insert oxygen in a position ortho- to an existing hydroxyl group in an aromatic ring, followed by the oxidation of the diphenol to the corresponding quinone. They show monophenol oxidase (tyrosinase, EC 1.14.18.1) and catechol oxidase or *o*- diphenol: oxygen oxidoreductase (EC 1.10.3.2) activities (Mayer, 2006). Differential, tissue specific, expression of six genes coding for PPO in potatoes and for seven genes in tomatoes (Thipyapong et al., 1997) are known.

1.2.14.5 Phenolics

Plants biosynthesize phenolic substances by the phenylalanine (hydroxycinnamate) pathway (Strack, 1997), yielding a large and heterogeneous group of biologically active non-nutrients which display structures ranging from simple moieties containing a single hydroxylated aromatic ring to highly complex polymers (Harborne, 1994). Biosynthesis and accumulation of phenolics are endogenously controlled processes during developmental differentiation (Macheix et al., 1990).

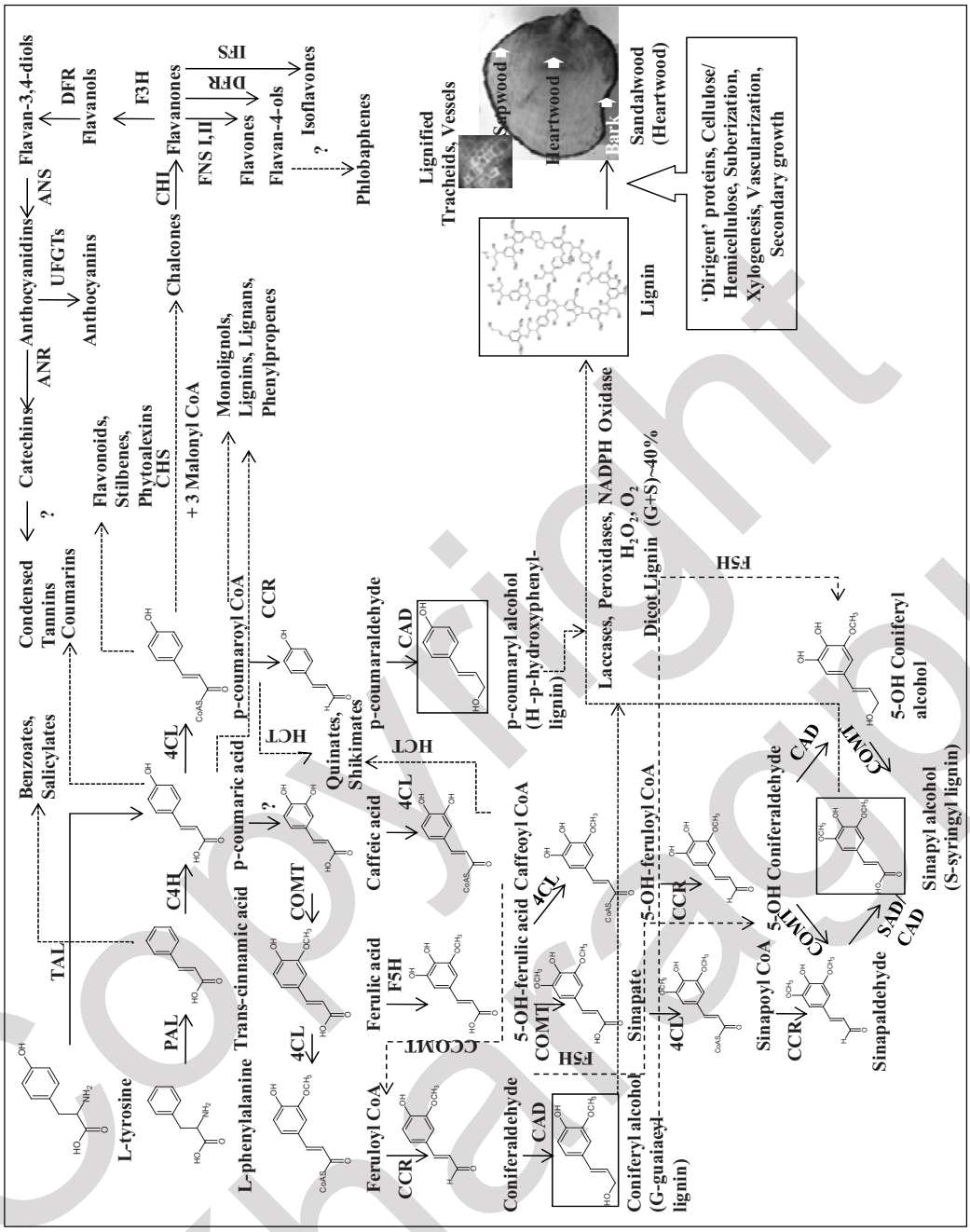

Figure 1.2 General phenylpropanoid biosynthetic pathway leading to a wide range of phenylpropanoids and vascularization (modified from, Ferrer et al., 2008).

The shikimate pathway product, L- phenylalanine, is the common precursor for phenolic compounds in higher plants (Strack, 1997). Hydroxycinnamic acids, their coenzyme A esters, are common structural elements of phenolic compounds, such as cinnamate esters and amides, lignin, flavonoids and condensed tannins (Macheix et al., 1990). Phenolics are cell- wall support materials (Wallace and Fry, 1994) in the form of polymeric materials such as lignins, providing mechanical support and as barrier against microbial invasion.

Embryogenic capacity is well correlated to a balanced concentration of phenolics. High concentrations of hydroxycinnamic acid amides are associated with the non- embryogenic response while hydroxycinnamates are involved in lignin biosynthesis and alterations of the cell wall composition during differentiation and morphogenesis (Kroon and Williamson, 1999). Cinnamic acids upon conjugation with free polyamines regulate somatic embryogenesis (Cvikrova et al., 2003). Phenolic acids in plants are hydroxylated derivatives of benzoic and cinnamic acids. High performance liquid chromatography (HPLC), particularly, reverse phased -HPLC (RP- HPLC) is the method of choice in the chromatographic analysis of phenolic acids. Phenolic compounds absorb in the UV region, thus utilize a variable- wavelength UV or UV- Visible detector allowing analysis of phenolics by HPLC (Robards and Antolowich, 1997). Benzoic acid derivatives (λ_{max}; 246-262 nm), hydroxycinnamic acids [λ_{max}; (i) 225-235 nm, (ii) 290-330 nm], cinnamic acid derivatives (λ_{max}; 320 nm) absorb at variable wavelengths thus rendering a more generalized monitoring at 280 nm (Pussayanawin and Wetzel, 1987). Microscopic inspection under UV light exploiting lignin's inherent autofluorescence in stems or root sections or by histochemical staining with lignin- specific dyes (Wesner, Maüle etc.) represent the simplest way to identify qualitative and/ or quantitative changes in lignification events during development (Boudet, 2000).

1.2.14.6 Lignin biosynthesis and lignification

Lignin is a heteropolymer of hydroxylated and methoxylated phenylpropane units, derived from the oxidative polymerization of different hydroxycinnamyl alcohols (p-coumaryl, coniferyl and sinapyl alcohols, collectively called 'monolignols') connected by labile 'ether' or 'ester' bonds and/ or resistant carbon-carbon linkages (Boerjan et al., 2003). In dicotyledonous angiosperms lignin is built from coniferyl and sinapyl alcohols, incorporated, respectively, as guaiacyl (G) and syringyl (S) units to form heterogeneous G- S polymers. Moreover, lignin, the second most abundant material on earth surface, after cellulose; is the major structural component of secondarily thickened cell walls of water-conducting xylem elements in the vascular system (Lewis and Yamamoto, 1990).

1.2.14.7 Flavonoids and anthocyanins

Flavonoids are PP- derivatives, i.e., diphenylpropanes (Winkel- Shirley, 2002), characterized by a C6- C3- C6 carbon skeletons, i.e., benzo- γ- pyrone derivatives consisting of phenolic and pyrane rings and derive from products of the aromatic amino acid biosynthesis (shikimate or phenylalanine) and the Krebs cycle (acetate- malonate; acetyl CoA) (Gantet and Memelink, 2002). First committed step in the formation of flavonoids is conjugation of malonyl- CoA and coumaroyl- CoA molecules to chalcones, catalyzed by the

enzyme chalcone synthase. Chalcones are converted to flavanones by the action of chalcone isomerase. Flavanones are precursors of all classes of flavonoids (Winkel- Shirley, 2002). Over 4, 000 different flavonoids have been identified within the major flavonoid classes, which include flavonols, flavones, flavanones, catechins, anthocyanidins, isoflavones, dihydroflavonols and chalcones. Flavonoids possess antilipoperoxidant, antitumoral, antiplatelet, anti- ischemic, anti- allergic, and anti- inflammatory activities (Cao et al., 1997).

Anthocyanins are glycosylated derivatives of the 3, 5, 7, 3′- tetrahydroxyflavylium cation and constitute a special class of flavonoids. They are the most important pigments of the vascular plants as they are harmless and easily incorporate into aqueous media, rendering them natural water- soluble colorants (Pazmiño- Durán et al., 2001). They impart orange, pink, red, violet and blue colours in the flowers and fruits of plants and occur in several species of spermatophytes. Anthocyanidins are found in their glycoside forms (bonded to a sugar moiety) as anthocyanins and consist of an aromatic ring bonded to an heterocyclic ring that contains oxygen, which is also bonded by a carbon-carbon bond to a third aromatic ring. In nature, 500 types of anthocyanins, 23 types of anthocyanidins (Andersen and Jordheim, 2006) are found and their biosynthesis is well studied (Martens et al., 2003), with the most common forms being, cyanidin, delphinidin and pelargonidin. Anthocyanins display antioxidant activities, which play vital roles in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes, among others (Konczak and Zhang, 2004). Anthocyanin pigmentation is a cytodifferentiation process and are indicators of stress.

1.2.14.8 Phenylpropanoids in heartwood

Significant progress in the understanding of xylem (sapwood) formation (Lewis, 1999) still lack information on gene expression during heartwood formation, as heartwood does not lend itself to biosynthetic studies or mRNA extraction owing to prevalent dead cells and limited access to transition zones. Ray parenchyma's final function is the synthesis of secondary metabolites, impregnating the wood into ‘heartwood’ (Higuchi, 1997). Similar spectrum of substances are found in heartwood and pathogen, pest- challenged or stressed plants (Harborne, 1994), thereby suggesting common operative pathways for heartwood- and active defense- related secondary metabolism (phytoalexin). PAL is active in sapwood, while chalcone synthase (CHS) is active in the heartwood boundary (Magel et al., 1995). PAL is involved both in the formation of lignin and flavonoid biosynthesis, thus inhibitor feeding of the sapwood prevented the formation of PP metabolites. Cell culture systems allow heartwood- type metabolic differentiation studies in gymnosperms and angiosperm trees (Zhentian et al., 1999).

1.2.15 Terpenoids

1.2.15.1 Importance of terpenoids

Secondary metabolites, also known as 'natural products' from plants comprise of 100, 000 compounds, with about 4, 000 new ones being discovered every year (Verpoorte, 1999). Largest family of secondary metabolites are known as 'terpenoids', comprising more than one third of all known compounds. Over 30, 000- 40, 000 terpenoids are known in plants (Dewick, 2002). Terpenes are hydrocarbons that usually contain one or more C= C bonds and derive from condensation of 'isoprene units' (C_5H_8, 2- methyl- 1, 3- butadiene). Specifically, 'terpenoids' are oxygen containing analogues of terpenes (hydrocarbons). Terpenoids serving as defensive chemicals are secured and accumulated in secretory structures mostly, thus minimizing auto toxicity but play efficient role in defense, i.e., oleoresin in resin ducts/ laticifers in conifers (Gershenzon and Croteau, 1991). Industrially useful polymers (rubber, chicle), pharmaceuticals (taxol, artemisinin, ginsenoside, gingolides, and betulinic acid) and agrochemicals (azadirachtin, pyrethrins) are also terpenoids in origin. Terpenes being volatile are flammable, hence used in industries as solvents for fingerprints and as heavy petroleum greases and oils. Terpenoids usually are very expensive due to their lower level of synthesis in the plants and thus are not obtained in a profitable way through chemical synthesis and hence isolated normally by steam distillation (Lahlou, 2004).

1.2.15.2 Terpenoid biosynthetic pathway

Terpenoids are derived from isopentenyl diphosphate (IPP), an acyclic C_5 precursor, known as 2, 3- methyl butane. Head- to- tail addition of isoprenoid units form the basis of biosynthesis of terpenoids (Ruziicka, 1953). The first biosynthetic pathway identified, is operative in bacteria, fungi, mammals and plants; the 'mevalonic acid pathway'. In plants, this central precursor 'mevalonate' is synthesized in the cytosol via the classical 'acetate'/ 'mevalonic acid'/ 'mevalonate' (MVA) pathway (Newman and Chappell, 1999), by which the sesquiterpenes (C_{15}) and triterpenes (C_{30}) are formed. In plastids and such prokaryotic endosymbiotic ancestors (Cvejic and Rohmer, 2000); via an alternative route, variously called as 'pyruvate'/ 'glyceraldehyde- 3- phosphate' (GAP)/ 'deoxy- xylulose phosphate' (DXP)/ 'methyl- erythritol pyrophsophate' (MEP)/ 'Rohmer'/ 'MVA independent' pathway (Lichtenthaler, 1999) the monoterpenes (C_{10}), diterpenes (C_{20}), and tetraterpenes (C_{40}) are formed. MEP pathway is conserved in both eubacteria and higher plants (McCaskill and Croteau, 1998). Figures 1.3 and 1.4 depict generalized events in terpenoid biosynthesis.

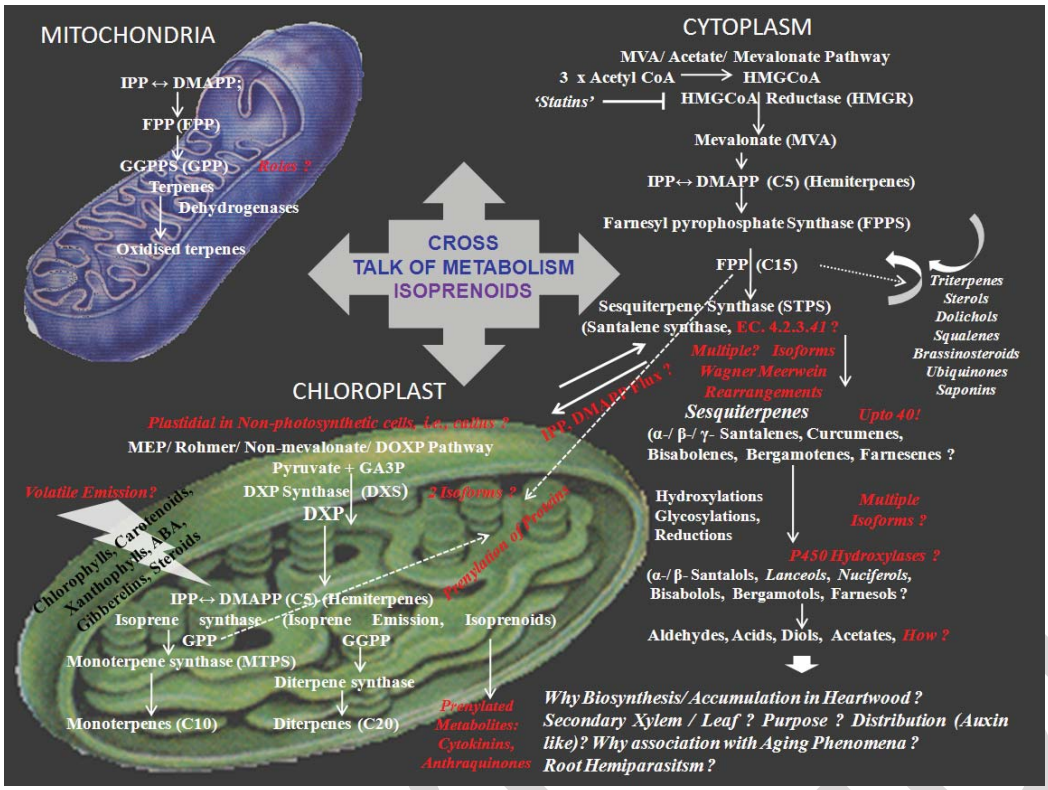

Figure 1.3 General terpenoid biosynthetic pathways operative in plants, with cross talk of intermediates. Sandalwood sesquiterpenoid biosynthetic pathway is highlighted (modified from Laule et al., 2003; Dudareva et al., 2006).

1.2.15.3 Terpenoid production *in vitro*

Terpenoids serve important physiological purpose *in vitro* (Rajaonarivony et al., 1992). Terpene hydrocarbons are emitted into either environment or are metabolized into strong antifungal compounds (Yamaguchi et al., 1999) and thus no volatile monoterpene storage takes place inside cells. *In vitro* volatiles production is rare in cell cultures, due to lower secretion, yields, solubility and different to parent volatile compounds *in vivo*. *In vitro* cultures of sweet orange, myrtle and tomato emit volatile hydrocarbons (Predieri and Rapparini, 2007). Accumulation of monoterpenes resulting from *de novo* synthesis has been demonstrated in tissue cultures of several oil- bearing plants: e.g. in *Mentha*, *Pelargonium*, *Thuja*, *Cupressus lusitanica*, *Tanacetum*, *Perilla*, *Gardenia* species, *Andrographis* and *Ocimum basilicum* and extensively reviewed (Banthrope and Branch, 1985; Matsunaga et al., 2003). Cultured plant cell lines synthesized sesquiterpenoids as in callus cultures of *Pinus radiate* (Banthrope and Njar, 1984). Nevertheless volatiles accumulate in cell suspension cultures of *Achillea millefolium, Artemisia dracunculus, Coleonema album, Cryptomeria japonica, Foeniculum vulgare* and *Pimpinella* anisum were 100 to 1000 times less than that, obtained from matured plants *in vivo* (Figueiredo et al., 2006). PT activity in *Rosa* (Banthrope and Barrow, 1983) and wound inducible monoterpene synthases in *Abies grandis* callus

cultures (Lewinsohn et al., 1994) indicate such operative pathways. Chitosan elicitor and growth regulators (Chang et al., 1998) induced menthol overproduction in suspension cultures of *Mentha piperita*. Individual sesquiterpenoid abundance differed with organs, tissues and developmental stages, i.e., detection of up to ~14 olefins in a pentane extract (Kollner et al., 2004), while qualitative and quantitative changes in sesquiterpenes with development in plants is well known.

1.2.15.4 Chlorophylls and carotenoids

Chlorophylls are molecules directly related to the photosynthetic processes in all photoautotrophs, as the primary photosynthetic pigments, and their structural characteristics [e.g., chlorophylls a and b; chlorophyll a is found in higher amounts than chlorophyll b by a 3 to 1 margin] are taxonomically and evolutionally significant (Delwiche, 1999). Their biosynthesis involves two pathways in photoautotrophs [MVA or MEP pathways for phytol biosynthesis and C_5/ Shemin pathway for chlorophyllide biosynthesis] (Beaumont et al., 2000). Chlorophyll b is a constituent of the light- harvesting system and the reaction centers are rich in chlorophyll a (Thomas, 1997). A close correlation between carotenoids and chlorophyll a+b and between chlorophyll a and chlorophyll b as well as high carotenoid to chlorophyll and chlorophyll b to chlorophyll a ratios is evident in apples (Blanke and Lenz, 1989).

Carotenoids (carotenes and xanthophylls) are colored pigments common to all higher plants that play vital roles in photosynthesis. Carotenoids participate in light harvesting and are recognized as powerful antioxidants in excited states and are singlet oxygen quenchers involved in photoprotection (Edge et al., 1997). They have structural and functional roles in light harvesting in photosynthetic membranes, in protecting their photosynthetic apparatuses from excess light energy, are potent antioxidants and free radical scavengers (Grassman et al., 2002) which can modulate the pathogenesis of cancers and coronary heart diseases (Kritchevsky, 1999). Xanthophylls (oxygenated carotenoids), are accessory pigments in the light- harvesting antennae of the chloroplasts, which are capable of transferring energy to the chlorophylls and are known to provide protection against age- related macular degeneration, mediated by their ability to quench single oxygen and blue light in the retina (Landrum and Bone, 2001).

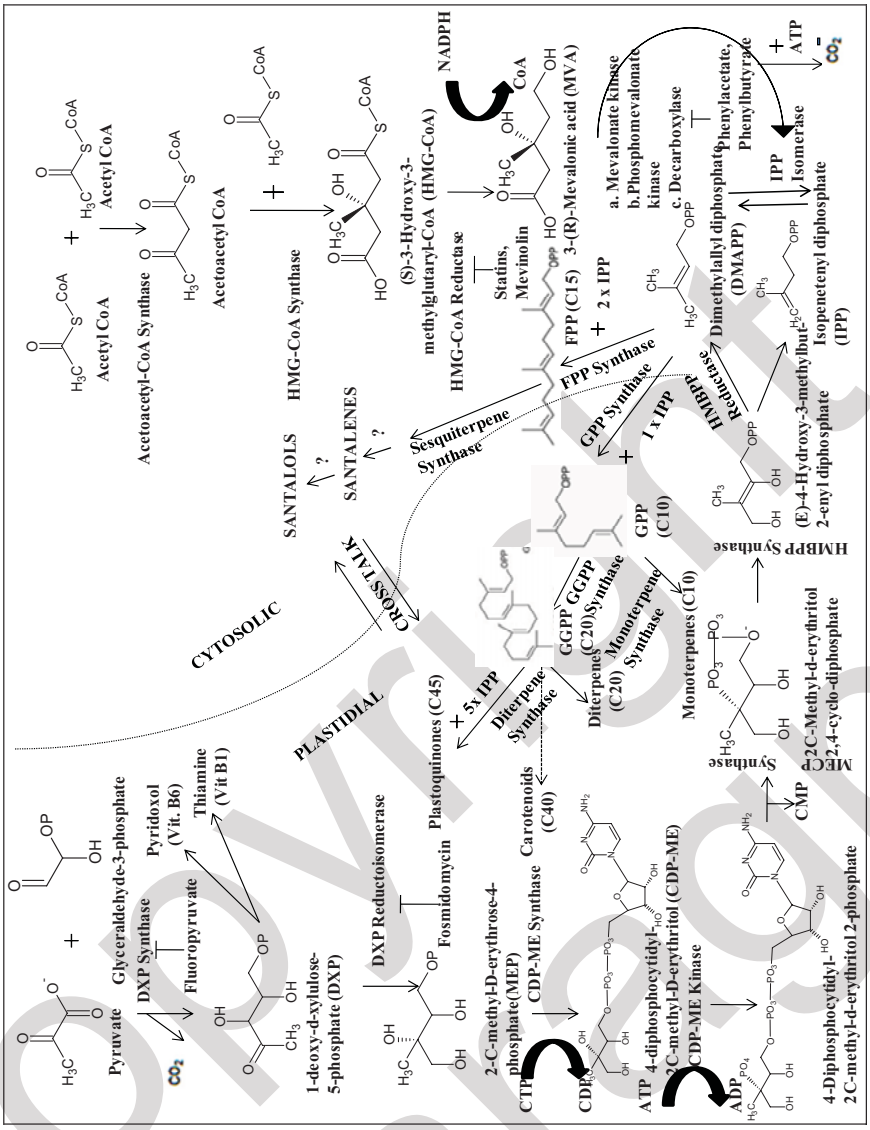

Figure 1.4 Detailed MEP and MVA biosynthetic pathway, showing formation of various terpenoids and several pathway inhibitors (modified from Laule et al., 2003; Dudareva et al., 2006).

1.2.15.5 1- deoxy- D- xylulose phosphate synthase (DXS)

1- deoxy- D- xylulose phosphate synthase (DXS, EC: 4.1.3.37) is an enzyme in the non- mevalonate/ MEP pathway, a thiamine pyrophosphate (TPP, cofactor)- dependent enzyme that carries decarboxylase and transketolase activities. DXS, in presence of TPP and divalent cations such as Mg^{2+} or Mn^{2+}, catalyzes the first step in the biosynthesis of isoprenoids and B vitamins [thiamin (vitamin B1); pyridoxal (vitamin B6) etc.] with the release of CO_2 in most bacteria and plant chloroplasts (Sprenger et al., 1997). Appendix A.3 depicts the DXS characterized till date from various organisms. DXS exhibit two distinct types in higher plants. DXS sequences from *Arabidopsis, Artemisia, Lycopersicon,* and *Capsicum*, fall into the class 1 (DXS 1), while sequences from *Catharanthus, Mentha* and

Tagetes fall into the class 2 (DXS 2) category. In *Zea, Medicago, Lycopersicon, and Nicotiana* the two DXS transcripts correspond to two different genes whilst there is no evidence for multiple *dxs* genes in *Arabidopsis* (Walter et al., 2002).

1.2.15.6 3- Hydroxy- 3- methylglutaryl- coenzyme A reductase (HMGCoAR)

HMGCoA (3- hydroxy- 3- methylglutaryl- coenzyme A) plays key regulatory role in MVA pathway, in cytoplasmic and mitochondrial isoprenoid biosynthesis. HMGCoAR gene families in plants are differentially regulated by various endogenous and exogenous stimuli and physiological conditions. They operate at transcription and translation levels through enzyme degradation and phosphorylation events (Korth et al., 1997). This enzyme is of paramount importance for human cardiovascular diseases as well.

1.2.15.7 Farnesyl diphosphate synthase (FPPS)

Farnesyl diphosphate synthase (*E*- FPP synthase) (EC 2.5.1.10) is a homodimeric protein involved in terpenoid biosynthesis, that catalyzes the stereospecific condensation of IPP with DMAPP via GPP to give (*E, E*)- farnesyl diphosphate (FPP), which eventually lead to formation of isoprenoid lipids such as sterols, ubiquinones, dolichols, heme and isoprenylated proteins. The other form, i.e., (*Z*)- FPPSes catalyze to form (*Z*)- FPP (Schulbach et al., 2001). FPPS at amino acid levels, from plants, yeast and mammals show high similarities that reveal 7 conserved regions (Chen et al., 1994). Regions II and VI are implicated in substrate- binding, with two characteristic aspartate- rich motifs (DDx(xx)xD; x- any amino acid) involved in catalysis. Region II, includes the first aspartate- rich motif (FARM) that determines product chain- length specificity whereas, the second aspartate- rich motif (SARM) in region VI is responsible for IPP binding (Liang et al., 2002). FPPS is localized to cytosol and mitochondria, found as ER- associated and in plastids (Sanmiya et al., 1999) of plants. FPPSes are well conserved among microorganisms and two archeas (Sen et al., 2007). Genes encoding FPP synthase have been cloned from several organism, which are enlisted in Appendix A.4.

1.2.15.8 Terpene synthase (TPS)

Plant 'terpene synthase'/ 'terpene cyclases' (TPS) share a common evolutionary origin based on conserved structural and sequence characteristics, amino acid sequence homology, conserved sequence motifs, intron numbers and exon sizes (Bohlmann et al., 1998; Figure 1.5). Prokaryotic and eukaryotic prenyltransferases (PTs) too share a common ancestral gene (Chen et al., 1994). Phylogenetic analysis of the deduced amino acid sequences of 33 TPSs from angiosperms and gymnosperms allowed recognition of six terpenoid synthase (Tps) gene subfamilies based on clades (Bohlmann et al., 1998). Secondary metabolite producing majority of TPSs are classified into three subfamilies, Tpsa

(sesquiterpene and diterpene synthases from angiosperms), Tpsb (MTPSs from angiosperms of the Lamiaceae), and Tpsd (11 gymnosperm monoterpene, sesquiterpene, and diterpene synthases). The other three subfamilies, Tpsc, Tpse and Tpsf are represented by single angiosperm TPSs, i.e., copalyl diphosphate synthase, kaurene synthase [both involved in primary metabolism, i.e., gibberellin biosynthesis (Macmillan and Beale, 1999)] and linalool synthase, respectively. Bifurcation of terpenoid synthases of primary and secondary metabolism occurred before the separation of angiosperms and gymnosperms (Bohlmann et al., 1998). The functional diversity of chemicals within plants is best demonstrated by terpenoids and the pathway is the most extensively studied metabolic pathway. Prenyl diphosphates of varying lengths are modified through dimerization and cyclization by TPSes to produce a diverse array of terpenoids with an almost equally diverse array of functions. TPSes share two highly conserved regions that contain the Asp- rich consensus sequence DDxxD, the binding site for the divalent metal cation- complexed diphosphate group (Lesburg et al., 1997). Electrophilic reaction mechanisms lead to the generation of a carbocation intermediate, the manner it is quenched, give rise to the great diversity of terpenoids (Bohlmann et al., 1998). Functional diversity i.e., synthesis of a mixture of multiple products or only one major product; can arise with very little change in TPS protein structure and environment. Closely related genes could encode enzymes synthesizing dissimilar monoterpenes and sesquiterpenes. TPSes with > 70- 90 % identity catalyze distinct chemical reactions, whereas TPSes with < 30 % similarity catalyze same reactions (Bohlmann et al., 1998). Levels of TPS activity are correlated with the production of terpenoids, although level of precursors could ultimately be a rate- limiting step.

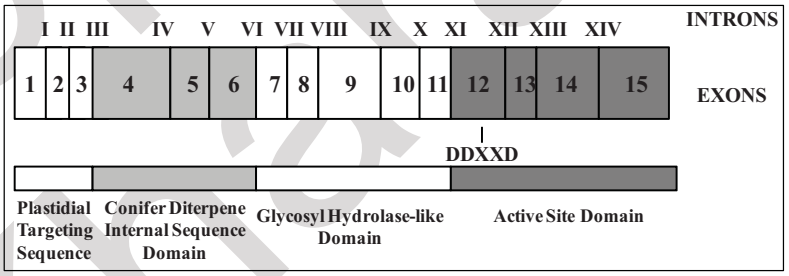

Figure 1.5 Domain architecture of plant terpene synthases (modified from, Trapp and Croteau, 2001).

1.2.15.9 Monoterpene synthase (MTPS)

Monoterpenoids (C_{10}) constitute the simplest class of terpenoids i.e., with 2 joined isoprene units, but constitute the major components of the plant- derived essential oils. Biosynthesized by 'monoterpene synthases/ cyclases', that catalyze the conversion of universal acyclic intermediate GPP ($C_{10}H_{29}N_3O_7P_2$) into cyclic parents of various simple hydrocarbon monoterpene skeletal types, which are then transformed by subsequent

modifications (oxidations) into various derivatives (Gershenzon and Croteau, 1994). MTPSs possess transit peptides in the N- terminus, an aspartate- rich active site DDxxD motif involved in divalent cation binding and the RRx_8W motif involved in isomerization of GPP (Bohlman et al., 1998). Monoterpenes are accumulated in specialized anatomical structures of the Lamiaceae, Rutaceae and Pinaceae members. MTPSs responsible for the synthesis of the menthane, bornane, pinane, camphane, thujane and fenchane families of monoterpenes have been isolated from various members of the Lamiaceae, Asteraceae and Umbelliferae (Wise and Croteau, 1999). Grand fir, *Abies grandis* possess elaborate, monoterpenoid- based chemical defenses owing to richness in MTPSs (Bohlmann et al., 1997) i.e., limonene, myrcene or pinene synthases that are wound- inducible (Bohlmann et al., 1999). In *Arabidopsis*, caterpillars (*Pieris rapae*) induce a myrcene/ β- ocimene synthase, while β- ocimene synthase from lotus (*Lotus japonicas*) is induced by spider mite- feeding (Arimura et al., 2004). In maize, a MTPS is induced in seedling blades in response to herbivory by beet armyworm (Lin et al., 2008). Several such MTPSs characterized till date are provided (Appendix A.5).

1.2.15.10 Sesquiterpene synthases (STPS)

'Sesquiterpene synthases' catalyze the cyclization of farnesyl diphosphate (FPP, $C_{15}H_{28}O_7P_2$) into a myriad of cyclic intermediates, which are the backbone hydrocarbons for biosynthesis of 7, 000 sesquiterpenoids in nature (Bohlmann et al., 1998). Investigated 'sesquiterpene cyclases' (~200 no's), have all proven to be operationally soluble, moderately lipophilic proteins of molecular weight 40- 100 kDa requiring divalent metal cations, usually Mg^{2+} (an occasional Mn^{2+} or K^+) for functionality (Marrero et al., 1992). The first plant STPS was cloned and expressed in *Escherichia coli* and characterized (Back et al., 1994). Subsequent investigations (crystallographic, mutagenesis, domain- swapping approaches) of a wide range of PTs from plants and microbes for achieving transgenic and metabolically engineered plants (Starks et al., 1997) have allowed understanding of structure-function relationship of STPSes. The products of STPSs, i.e. sesquiterpene hydrocarbons and the alcohols etc. are analyzed using various analytical techniques like TLC, GC, GC- MS, FTIR, ^{13}C- NMR, HPLC and ESI- MS (Merfort, 2002). Appendix A.6 provides an exhaustive list of STPSs characterized till date. Few STPSs, interesting in terms of their function, final products and source, are discussed below. Alpha- farnesene, the volatile sesquiterpene on superficial scald (coating on peel) of apple is the product of α- farnesene synthase, was termed 'unusual' owing to presence of both STPS and PT activities in the same protein, requirement of monovalent cations (K^+), production of all 4 isoforms of farnesene, capacity to utilize GDP and phylogenetically more related to isoprene synthases than other STPSs (Yuan et al., 2008). Similarly, α- farnesene synthases from angiosperms, i.e., pear, cucumber (Gapper et al.,

2006), and gymnosperms i.e., loblolly pine, spruce (Martin et al., 2004) while β- farnesene synthases from peppermint (*Mentha piperita*) (Croteau et al., 2000) are known.

1.2.16 Biological activities of natural products

1.2.16.1 Essential oils as bioactives

Essential oils are the volatile fractions of aromatic plants borne in the plant within distinctive oil cells, where the oil is concentrated as a viscous, lipophilic liquid containing a complex mixture of volatile molecules of plant secondary metabolism origin, and the essential oil secreting cell is characteristic of that plant family which taxonomically verify species in a distinctive manner, i. e., " oil of clove" or " oil of sandalwood" (Lawrence, 2000). Essential oil constituents are volatile to semi- volatile, are terpenoids/ PPs in origin, usually of ~400 Da (MW) and are amenable to analysis by ^{13}C- NMR (Demole et al., 1982) and GC- based techniques i.e., GLC, enantioselective/ chiral GC, multidimensional GC, head space GC, GC-olfactory determination, GC- MS, GC- FTIR (Marriot et al., 2001). GC- MS is the benchmark technique for the qualitative analysis of flavor and fragrance volatiles, aided by commercial MS libraries. Antimalarial drug 'artemisinin' and anticancer drug 'taxol' from paclitaxel are hugely successfully with established medical applications.

1.2.16.2 Phytoconstituents as antimicrobials

Multiple drug resistance (MDR) to human pathogenic organisms has renewed the search for new antimicrobial substances from plants (Ahmad and Beg, 2001). Essential oils are well known antimicrobials (Matasyoh et al., 2007). Unlike synthetic drugs, antimicrobials of plant origin display fewer side effects and have an enormous therapeutic potential to treat many infectious diseases. In bacteria, the permeabilization of the membranes is associated with loss of ions and reduction of membrane potential, collapse of the proton pump and depletion of the ATP pool, thereby causing cytotoxicity (Turina et al., 2006).

1.2.16.3 Phytoconstituents as antioxidants

Natural antioxidants of foods derived from fruits, vegetables, spices and cereals (known as *nutraceuticals*) are effective, amenable to defense mechanism of body, safe, nutritional and therapeutic (Ajila et al., 2007). Vitamins C and E, α- tocopherol, β- carotene, glutathione and flavonoids (Nakatsu et al., 2000) prevent the formation of excess free radicals, scavenging them or by repairing damaged molecules (Sanchez- Moreno, 2002). Free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, tissue inflammatory and cardiovascular diseases (Cai et al., 2004). Prevention of chronic diseases and disease progression by either enhancing the body's natural antioxidant defenses or by supplementing with proven dietary antioxidants is possible.

Natural antioxidants from plants include mostly, PPs and terpenoids such as, flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols, ascorbic acid, vitamins, terpenoids, phytoestrogens, carotenoids, tannins, lignans, stilbenes, coumarins, quinones, xanthones, flavones, flavonols, catechins, anthocyanins and proanthocyanins (Calucci et al., 2003) making them increasingly imperative to trap their potential. Polar antioxidants are more active in pure lipids, and non- polar antioxidants most active in a polar substrate ('polar paradox'), thus conferring a wide range of antioxidative activity to medicinal plant constituents that display strong metal ion chelation properties (Rice- Evans et al., 1995). Developing countries like India, where poverty and malnutrition is on rise, plant based antioxidants are cheaper alternative to allopathic medicine as the rich ethnopharmacological history shows use of herbal medicine vigorously. Indian plants showing ethnopharmacological usages, with antioxidant potential are extensively reviewed (Govindarajan et al., 2003).

1.2.16.3.1 Phenylpropanoids as antioxidants

Phenolic compounds and polyphenols are abundant and ubiquitous in plants and hence the upsurge in focus on traditional medicinal plant research since last 30 years (Naczk and Shahidi, 2006). Positive correlations between the high phenolic content and the strong antioxidant activity indicate their roles in, protection from H_2O_2 induced cellular damage, lipid peroxides and absorption and neutralization of free radicals (Cai et al., 2004). Owing to the presence of aromatic rings bearing numerous hydroxyl groups at different locations, they are potentially able to act as reducing agents, hydrogen/ electron donating antioxidants and singlet oxygen quenchers. Flavonoids mostly have antioxidant, capillary protective and tumor inhibitory effects (Czeczot, 2000), i.e., quercetin has potential application as therapeutic drug against free radical reactions (Yao et al., 2007).

Box 2. Assay schemes and methods for antioxidants.

I. ***Chemical methods***

A. ***Hydrogen atom transfer (HAT)- based methods***

- Total Antioxidant Capacity (TAC) by Phosphomolybdenum Method [Mo (IV) reduction to Mo(V); Prieto et al., 1999]
- β- Carotene- Linoleate Emulsion Bleaching System [Linoleic acid and β- carotene; Howard et al., 2000]
- Crocin/ Croton Bleaching System [Crocin; Bors et al., 1984]
- Hydroxyl Radical Scavenging Method [Deoxyribose assay (Sánchez- Moreno, 2002), Scavenging of Hypochlorous Acid (HOCl) (Martinéz- Tomé et al., 2001), Lactate Dehydrogenase (LDH- NADH) Oxidation System (Jun et al., 2001)]
- Lipid Peroxidation Inhibition/ Thiobarbituric Acid Reactive Substance (TBRS) Inhibition [MDA Adducts, Thiobarbituric acid; Lugasi et al., 1998]
- ORAC Assay [β- PhycoErythrin (PE), 2, 2′- Azobis (2- AmidinoPropane) diHydrochloride (AAPH); Glazer's, 1990]
- Total Radical Trapping Parameter (TRAP) Assay [Dichlorofluorescin- diacetate (DCFH- DA); Wayner, et al., 1985]
- Total Oxyradical Scavenging Capacity (TOSC) Assay [α- Keto- γ- MethiolButyric Acid (KMBA) and AAPH; Winston et al., 1998]
- ORACFL Measurement Method [Methylated β- Cyclodextrin; Huang et al., 2002]
- Rancimat/ Conjugated Diene Assay [Sunflower oil; Lee and Shibamato, 2002]
- PhotoChemiLuminescence (PCL) Assay [Luminol; Popov and Lewin, 1994]

B. ***Single Electron Transfer (SET)- based methods***

- Reducing Power Assay [Wong et al., 2006]
- Ferric Reducing Ability Of Plasma (FRAP) Assay [Fe^{3+} reduction to Fe^{2+}; 2, 4, 6- tri- pyridyl- s- triazine (TPTZ), Benzie and Strain, 1999]
- Copper Reduction Assay (CUPRAC) [Apak et al, 2004]
- Metal Chelating Activity [Ferrozine; Decker and Welch, 1990]

C. ***Methods based on both HAT and SET-***

- Trolox Equivalent Antioxidant Capacity (TEAC) Assay [2, 2′- AzinoBis (3- ethylbenzoThiazoline 6- Sulfonate (ABTS) and Persulfate; Miller et al., 1993; Re et al., 1999]
- DPPH Method [1, 1- diphenyl- 2- picrylhydrazyl radical ($DPPH^{\cdot}$); Brand- Williams et al., 1995]
- Nitric Oxide Scavenging Activity [Greiss Reagent; Rao, 1997]

II. ***Physical methods***

- Electron Paramagnetic/ Spin Resonance (EPR/ ESR) Spectroscopy Method [Calucci et al., 2003]
- Cyclic Voltammetry Method [Kohen et al., 1999]

1.2.16.4 Phytoconstituents as tyrosinase inhibitors

Melanins determine mammalian skin color and are biosynthesized in a multi- step biochemical pathway operative in the 'melanosome', known as 'melanogenesis', yielding polymeric structures such as o- dopaquinone catalyzed by tyrosinase (Kim and Uyama, 2005). Melanin in skin provides defense mechanism against the UV- light of the sun, whereas abnormal pigmentation (i.e., melasma, freckles, age- spots and liver spots) and hyperpigmentation pose aesthetic problems (Briganti et al., 2003). Tyrosinase is implicated in enzymatic browning reactions in damaged fruits during post- harvest handling and processing, attributed to oxidation of phenolics in fruits (Gruber et al., 2004) bringing about undesirable changes in color, flavor, and nutritive values. Tyrosinase (monophenol, dihydroxy- L-phenylalanine: oxygen oxidoreductase, EC 1.14.18.1), also known as polyphenol oxidase (PPO) in food industry (Mayer, 1987), is a copper- dependent monooxygenase, multifunctional enzyme that catalyzes two different reactions: the hydroxylation of L-tyrosine to L- DOPA [3- (3, 4- dihydroxyphenyl)- l- alanine, monophenolase activity], which is further oxidized to dopaquinone [diphenolase activity] (Ito, 2003). This highly reactive o-quinone polymerizes spontaneously to form melanin (Seo, 2003). Mushroom tyrosinase is a popular target enzyme for screening and characterizing potential tyrosinase inhibitors (Chen

et al., 2004). Traditional use of plants against skin disease, for cosmeticeutical purposes in domestic medicine of ancient and contemporary cultures might lead to potential anti-pigmentation molecules (Pieroni et al., 2004). Plant based natural tyrosinase inhibitors are now used as cosmetic additives or as medicinal products for hyperpigmentation (An et al., 2005). Owing to their low costs, fewer harmful effects an increased demand in the global market for new plant- based agents as cosmeceuticals, cosmetic vehicles, additives and constituents, and skin- lightening/ whitening principles is anticipated (Aburjai and Natsheh, 2003). They are implicated in treatment of dermatological disorders like, melanin hyperpigmentation, abnormal pigmentation and depigmentation after sunburn (Shiino et al., 2001). Tyrosinase inhibitors also alleviate dopamine neurotoxicity and neurodegeneration associated with Parkinson's disease (Xu et al., 1997). Phenolic compounds sharing structural similarities with L- tyrosine are potential tyrosinase inhibitors and thus act as depigmenting/ skin- lightening/- whitening agents in skin- care products (An et al., 2005). Phenolics owing to their antioxidant nature, prevent or delay pigmentation by scavenging ROS, RNS etc., by reducing o- quinones etc. in melanin biosynthesis leading to delayed oxidative polymerization (Seo et al., 2003). Sulfites (banned by FDA), α- tocopherol, L- ascorbic acid (derivatives), 4-hexylresorcinol, quercetin, morin, kojic acid (octanoates), tropolone, catechins, hydroquinone, arbutin, resveratrols and L- mimosine are established PPO inhibitors (Ohguchi et al., 2003; Xie et al., 2003).

1.2.16.5 Phytoconstituents as antidiabetic principles

1.2.16.5.1 Diabetes mellitus

Diabetes mellitus (DM) is one of the most prevalent chronic, metabolic and hereditary, heterogeneous group of diseases in the world with highest rates of mortality (Barcelo and Rajpathak, 2001). Diabetes is 16th leading cause of global mortality, long- term complications, disability and hospitalization and a risk factor for cardiovascular diseases (Yeh et al., 2003). It is characterized by hyperglycemia (the fasting plasma glucose cut- off level being 7 mM/ l (WHO, 1999) and glycosyuria due to impaired carbohydrate "glucose" resulting from a defective/ deficient insulin secretory response (either absolute or relative lack of it, reduced circulating concentration, poor insulin sensitivity, insulin resistance or poor glucose tolerance resulting in high sugar in plasma), *per se* impairs insulin secretion (Davis and Granner, 2001) and alterations in carbohydrate, fat and protein metabolism associated with complications such as atherosclerosis, neuropathy and cataract formation as well as abnormalities in serum lipids (West, 2000). Hyperglycemia induced by decreased cellular glucose uptake and metabolism, increased utilization and decreased storage of proteins, for energy, instead of glucose, leads to reduction of body weight by depletion of the body proteins (Guyton and Hall, 2000).

1.2.16.5.2 Hypoglycemic potential of plants

Oral hypoglycemic agents and clinical grade insulin are synthetic ones having certain serious adverse side effects as well not suitable for use during pregnancy. Thus promoting alternative therapeutic approaches and to find safer hypoglycemic agents, the ethno pharmacological knowledge of plants which have proved beneficial due to their effectiveness, safety, lower toxicity and fewer side effects compared to synthetic drugs and age old practices as folklore medicine (Pari and Umamaheswari, 2000) have become important. This leads to an ever increasing demand for complementary and alternative medicine with higher achievable antidiabetic/ hypoglycemic activities.

About 200 compounds, in 400- 1200 species, 725 genera and 183 families of plants used ethnopharmacologically or experimentally (Perez et al., 1998), are implicated to have potential blood glucose level lowering activities and in treatment of diabetes mellitus or its symptoms but still remains medically/ scientifically unproven (Grover et al., 2002). Plants possessing hypoglycemic activities, do so by reducing blood sugar, similarly as in sulfonylurea drugs like glibenclamide, effecting hypoglycemia in normal animals by stimulating insulin release from pancreatic β- cells, besides reducing hepatic clearance of insulin hormone and like the biguanides i.e., metformin where they target hyperglycemic but not hypoglycemic condition in normal state and others show insulin sparing action ((Stumvoll et al., 1995; Davis and Granner, 2001). Metformin- like activity of plants augments insulin action by increasing the number of glucose transporters, inhibiting gluconeogenesis, reducing absorption from the intestine but increasing glucose metabolism in liver (Zhang and Tan, 2000). Natural α- amylase inhibitors reduce post- prandial hyperglycemia by slowing down the digestion of carbohydrates and, consequently, the absorption of glucose. However, only a few comprehensive studies of traditional antidiabetic medicinal plants are known (Kobayashi et al., 2000).

1.2.16.6 Cholinesterase inhibitors as neuroprotective agents

Anticholinesterases play a vital role in minimizing the progression of neurodegenerative and chronic diseases like Alzheimer's disease, in the central nervous system of patients through ROS- mediated damage of mitochondrial and nuclear DNA. Pesticides i.e., malathion and parathion, drugs i.e., pyridostigmine, distigmine, neostigmine, physostigmine and ecothiophate used to treat glaucoma etc. are able to inhibit cholinesterases irreversibly. Cholinesterase inhibitors are lipophilic, readily absorbed through the skin, cross the blood barrier causing central cholinergic as well as nicotinic and muscarinic effects (Pappano, 2004). Reports on anticholinesterase activity of essential oils baring a few, i.e., use of *Salvia* essential oils in the treatment of Alzheimer's disease (Savelev et al., 2003) is scanty.

1.2.16.7 Phytoconstituents as cytotoxic agents

Cytotoxicity screens remain the first level of tests to render any extract or constituent safe for further *in vitro* or *in vivo* evaluation. Essential oils and their constituents act non-specifically at various cellular targets (Carson et al., 2002), as their lipophilic nature allows them to pass through the cell wall and cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them causing membrane damage. Essential oils coagulate the cytoplasm, damage lipids and proteins, damage cell wall and membrane leading to the leakage of macromolecules and cellular lysis while permeabilization of outer and inner mitochondrial membranes lead to cell death by apoptosis and necrosis (Armstrong, 2006). Cytotoxic activities are demonstrated in mammalian cells *in vitro* by short- term viability assays using specific cell staining or fluorescent dyes that include; MTT (3- (4, 5- dimethylthiazol- 2- yl)- 2, 5- diphenyl- tetrazolium bromide) test to analyze the apoptotic and necrotic cells (Manosroi et al., 2006).

1.2.16.8 Anticancer drugs from plants

Cancer is caused by uncontrolled growth and spreading of abnormal cells. Cancer remains the leading cause of human mortality. Paclitaxel, doxorubicin, fluorouracil (5- FU), cisplatin, tamoxifen, etc. are some anticancer drugs that are in current practice in chemotherapy of cancer. Cancers in advanced stages are not curable by chemotherapy, thus new drug development continues to play a major role in the fight against cancer, hence a five-decade- old drive for discovery of natural products based or synthetic anticancer drugs. Anticancer drugs may be cell- cycle specific or cell- growth- phase specific while, dose scheduling remains crucial with cell- cycle- specific drugs. For example, paclitaxel blocks cell proliferation in the late G2- mitotic phase of the cell cycle by stabilizing the microtubule cytoskeleton. Chemoprevention of cancers by nutraceuticals and phytochemicals has become a flourishing field of research over the past decade (Wei et al., 2005). In India, M/ s Patanjali Ayurveda Ltd., Haridwar has released several plant- based anti- cancer products (http:// patanjaliayurveda.com/eng/ #).

1.2.16.9 Phytoconstituents as MMP inhibitors

Cancer metastasis consists of a complex cascade of events, which ultimately allow for tumor cell escape and seeding of ectopic environments. For cancer cells to manifest their malignant potential, they must develop the ability to break through and dissolve extracellular matrix (ECM), particularly the delimitating basement membrane (BM). The degradation of the pericellular BM and ECM is catalyzed by the concerted action of several classes of ECM-degrading enzymes. One important class of ECM- degrading enzymes includes the matrix metalloproteinases (MMPs) (Mook et al., 2004), implicated as possible mediators of invasion and metastasis in some cancers. MMPs are produced in latent forms that require catalytic

removal of the pro- peptide domain for function. Expression of MMP- 2 and MMP- 9 is elevated in carcinomas and promote tumor progression in oral carcinoma, lung adenocarcinoma, bladder carcinoma and ovarian carcinoma, implicate their roles in cell adhesion, invasion and migration during the process of tumor cell invasion and metastasis (Schmalfeldt et al., 2001). Synthetic inhibitors of MMPs, such as BB94 (batimastat) and BB2516 (marimastat) are known but carry undesirable side effects, thereby leading to discovery of natural products that are MMP inhibitors (Seo et al., 2005). MMP inhibition implicates antiphlogistic activity and anti- aging effects (Elkington et al., 2005). Flavonoids, such as phloretin, 3- hydroxyphloretin and quercetin showed inhibition of MMP- 1 production in fibroblast cells and catechin, quercetin and phloretin inhibited metastatic activities by reducing MMP production of tumor cells (Leu et al., 2006).

1.2.17 Research and development work in sandalwood

Diseases, poaching and huge global demands necessitated *in vitro* biotechnological means of micropropagation methods. The first *in vitro* work on any woody forest plant was by callus induction from zygotic embryos in sandalwood (Rangaswamy and Rao, 1963). Research on sandalwood mainly focused on *in vitro* and biochemical aspects in the early 70s, as could be inferred from Table 1.1.

Table 1.1 Major achievements in research and development work in sandalwood.

Important Findings	References
In vitro micropropagation	
Callusing, somatic embryogenesis , regeneration from matured explants, embryogenesis in suspension culture	*Rangaswamy and Rao, 1963, 1971; Bapat and Rao, 1988; Lakshmi Sita et al., 1980; Yamashita, 1997*
Somatic embryo maturation and optimization in air lift bioreactors, synthetic seed production, characterization of spent medium	*Bapat et al., 1988, 1990; Das et al., 1998, 1999 ; Pal et al., 2003*
Biochemistry	
Identification of sesquiterpenoids, triterpenoids, phenylpropanoids	*Gibbard and Schoental, 1969; Adams et al., 1975; Demole et al., 1976; Verghese et al., 1990; Howes et al., 2004*
Sandalwood oil quality with tree age, heartwood and oil quality	*Shankarnarayana and Parthasarathi, 1987*
Calcium- dependent protein kinases; calcium signaling/ metabolism during somatic embryogenesis	*Anil and Shankara, 2001*
Polyamine and amino acid metabolism, esp. proline	*Mani and Radhakrishnan, 1976*
Isoenzyme patterns	*Parthasarathi et al., 1985*
Ethnopharmacological evidences	
Stimulant, aphrodisiac, coolant, urinogenital ailments, skin care, detoxification, colitis, ulcerations, enteritis, dysentery, congestion, depression and mental imbalance	*Pande, 1977; Herbal, 2004; Pole, 2006*
Bronchitis, fever, inflammation, antiseptic, antipyretic, expectorant, dysuria, gonorrhea, dermatitis, eczema, acne lesions and pustular acne lesions	*Handa et al., 1951; Dastur, 1962; Jain, 1968; Dikshit and Hussain, 1984; Haque and Haque, 2002*
Hydrolyzed Exhausted Sandalwood Powder (HESP) demonstrated antiremorogenic, anti- inflammatory, antimitotic, antiviral, anticancerous, hypertensive, antipyretic and sedative properties	*Desai et al., 1991*
Elephantiasis and lymphatic filariasis, antidote for poisonous snakebites, schizophrenia, bladder infections, facial paralysis.	*Okugawa et al., 1995; Blumenthal, 1998; Rohadi et al., 2004; Thomson, 2005; Sugiyama, 2007*

This decade, has seen upsurge and rejuvenated interests in exploration of SWO and its molecules for therapeutic applications, hence a host of reports on the screened biological activities, albeit focusing on SWO and heartwood constituents only (Table 1.2).

Table 1.2 Biological activities associated with sandalwood oil and molecules thereof.

Serial No.	Sandalwood Constituents	Biological Activities	Details	References
1	Sandalwood Oil; α- santalol and β- santalol	Antiviral	Human papilloma virus (HPV), DNA pox virus; against HIV and RNA viruses	Haque and Haque, 2002
2	Sandalwood Oil; α- santalol and β- santalol	Skin care	Psoriasis, eczema, warts, dermatitis, allergy, acne, lesions, staphylococcal/ streptococcal acne	
3	α- santalol	Anti- cancer	DMBA/ TPA induced skin papilloma development in CD- 1/ SENCAR mice	Koopman et al., 2003
4	Sesquiterpenoids	Antigastric	Anti- ulceration, against *Helicobacter pylori*	Takaishi et al., 2005
5	Sandalwood Oil	Insecticidal	Acaricide (honeybee), mosquito repellant (*Culex, Anopheles*), against termites	Zhu et al., 2008
6	α- santalol	Cancer chemopreventive	Against UV- B irradiation induced skin tumorigenesis, in SKH- 1 hairless mice,	Dwivedi et al., 2006
7	Sandalwood Oil; α- santalol and β- santalol	Antimicrobial	Antimicrobial activity; axillary bacteria, *Salmonella typhimurium*, *Staphylococcus aureus, Bacillus mycoidess, Candida albicans*	Jirovetz et al., 2006
8	Sesquiterpenoids	Antitumor	Antitumor promoting activity; *in vitro* Epstein- Barr virus early antigen (EBV- EA) activation in Raji cells; suppressed 2- stage carcinogenesis on mice skin	Kim et al., 2006
9	Sandalwood Oil	Antiviral	Herpes simplex viruses (HSV)- 1 and 2, inhibition of replication; in RC- 37 cells	Koch et al., 2008
10	α- santalol	Proapototic	Proapototic and caspase activation, tumor suppression in human epidermal carcinoma A431 cells.	Arasada et al., 2008
11	Sandalwood Oil	Diet	Dietary factors/ supplements, PUFA content increased	Burdock and Carabin, 2008
12	Santalols	Nervous stimulants	Stimulants of CNS; sedative, aphrodisiac, insomnia, alertness, olfactory stimulation through calcium refluxes	Bieri et al., 2004; Ohmori et al., 2007
13	Sandalwood Oil	Physiology and metabolism	Alertness, attentiveness, calmness, mood, relaxation, vigor, alertness, sound- hearing, sedation,	Kovatcheva et al., 2003; Heuberger et al., 2006

1.3 Objectives of the investigation

In spite of numerous traditional and modern uses of oil and wood constituents of sandalwood, specific compounds have not been identified with these medicinal properties. The present investigation has been undertaken with the prime interest of profiling of major wood and oil-related metabolites and exploring biological activities of major phenylpropanoids and terpenoids. Guided by the literature presented herein, the following objectives of the present investigation have been set.

1. Profiling of phenylpropanoids.
2. Profiling of sesquiterpenoids.
3. Screening of biological activities of extracts rich in phenylpropanoids and terpenoids.
4. PCR- based cloning and evaluation of phenylpropanoid and terpenoid biosynthetic pathway genes.