1.1 Introduction

Throughout history, mankind has always been interested in naturally occurring compounds from prebiotic, microbial, plants and animals sources. It is estimated that 60% of the world population rely on traditional medicine, mostly plant drugs, for their primary health care needs [Shrestha and Dhillon (2003)]. India has a rich diversity of medicinal plants; more than 8000 species of wild plants are known to be used in India for the treatment of various health problems. The Himalayan region is one of the well defined and better known phytogeographical regions of the Indian subcontinent. It has been a potent source of important medicinal herbs and is extremely rich in plant life and abounds in genetic diversity of medicinal and aromatic plants with large number of peculiar medicinal plants in different habitats. This is largely because of the diverse agro-climatic conditions which exist in the area. Medicinal virtues of the western Himalayan plants are well known from the early times of the great epics of Ramayana and Mahabharata [Singh and Rawat (2011)] and are mentioned in the oldest Hindu scriptures viz. Rigveda, which is said to be source of Ayurvedic Medicine System [Tiwari et al. (2011)]. The high hills are the storehouse of numerous herbs which are exploited not only for the pharmaceutical industries in India but outside as well. In fact, a large percentage of crude drugs in the Indian market come from this Himalayan part. It is believed that out of over 1,600 species of medicinal plants traditionally used in India [Uniyal et al. (2002)], more than 50% species come from the Himalayan region. For thousands of years medicine and natural products (NPs) have been closely linked through the use of traditional medicines and natural poisons. Isolation of active compounds, beginning with the isolation of morphine (1) from opium in the early 19th century and subsequently led to the isolation of early medicines such as quinine (2), aspirin (3) and pilocarpine (4) [Butler (2004)].
The research on microorganism culture led to the isolation of streptomycin (5), chloramphenicol (6) and chlortetracycline (7). Some of the first compounds identified in the early 1970s using mechanism-based screening methods included clavulanic acid (8) and mevastatin (9). A mixture of clavulanic acid (8) and amoxicillin (10) is still being used today as a front line antibiotic, while mevastatin (9) was the lead compound for a series of antilipidemic drugs collectively known as the ‘statins’.

The subject of plant chemistry was revolutionised after the Second World War through the development of a range of sophisticated instrument and techniques for isolation and characterization of plant secondary metabolites. The spectroscopic and analytical methods have resulted into rapid identification and quantification of several kinds of natural products from plants which have led to the discovery of new drug candidates. Isolation and characterization of pharmacologically active compounds from medicinal plants is still the interest of biological chemist. Some possible sources of natural products include plants, marine organisms, microbes and fungi.

Modern approaches to understand the action of plant constituents all the time rely on careful structural characterization that is predominantly done by UV, IR, NMR and mass spectrometry, therefore, without any question natural products chemistry, especially phytochemistry, has proven to be the single most successful approach in finding new medicine. Chemical modification of phytochemicals into biologically active and commercially exploited natural compounds is considered as one of the easier and cheaper alternative to fulfil the world demands and produce more effective
derivatives with less toxicity. The scientific search for pharmacologically unique principles from existing remedies continues and complements the achievements of modern medicine. Many valuable drugs of today came into use through the study of folk medicines. Chemists continue to use plant-derived drugs as prototypes in their attempts to develop more effective and less toxic medicines.

Among these, three important medicinal plant species (*Cedrus deodara*, *Podophyllum hexandrum* and *Albizia chinensis*) were selected from medicinal plants of Western Himalayan region for chemical investigation. The natural products discussed in this thesis are sesquiterpenes, lignans and polyphenols.

### 1.2 Terpenes

Terpenes are among the largest families of natural products comprising of numerous organic compounds with a broad range of physiological properties and immense structural diversity. Over 30,000 terpene hydrocarbons and oxidized or modified derivatives, called terpenoids, have been isolated to date from a wide variety of biological sources. These metabolites serve crucial roles in primary metabolism and ecological interactions of their organisms as well as display medically and economically valued characteristics. For instance, several monoterpenes and sesquiterpenes are important volatile organic compounds that are important in the perfume and flavour industries, whereas some microbial terpenoids are phytotoxic metabolites that have significant impact on agriculture.

#### 1.2.1 Biosynthesis of sesquiterpenes

Sesquiterpenes are the group of terpenoids which are formed by the combination of three isoprene units and are found widely distributed in many essential oil in the high boiling fraction. These represent collection of highly complex and diverse structural systems.

Acetyl-coenzyme A, also known as activated acetic acid, is the biogenetic precursor of terpenes (Figure 1). Similar to the Claisen condensation, two equivalents of acetyl-CoA couple to acetoacetyl-CoA, which represents a biological analogue of acetoacetate. Following the pattern of an aldol reaction, acetoacetyl-CoA reacts with another equivalent of acetyl-CoA as a carbon nucleophile to give \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA, followed by an enzymatic reduction with dihydronicotinamide adenine dinucleotide (NADPH + H\(^+\)) in the presence of water, affording (\( R \))-mevalonic acid. Phosphorylation of mevalonic acid by adenosine triphosphate (ATP) via the monophosphate provides the diphosphate of mevalonic acid which is
decarboxylated and dehydrated to isopentenylpyrophosphate (isopentenylidiphosphosphate, IPP). The latter isomerizes in the presence of an isomerase containing SH groups to \( \gamma,\gamma \)-dimethylallylpyrophosphate. The electrophilic allylic CH\(_2\) group of \( \gamma,\gamma \)-dimethylallylpyrophosphate and the nucleophilic methylene group of isopentenylpyrophosphate connect to geranylpyrophosphate as monoterpene. Subsequent reaction of geranyldiphosphate with one equivalent of isopentenylidiphosphate yields farnesylidiphosphate as a sesquiterpene [Cheng et al. (2007); Dev (1979)].

**Figure 1:** Biosynthesis of sesquiterpenes

The widely studied sesquiterpene constituents of the *C. deodara* essential oil appear to be derived from cis-farnesyl pyrophosphate either by a 1,6-cyclization to give bisabolane derivatives, or a 1,11-cyclization, to give himachalane or longibornane derivatives. The primary product of \( \gamma \)-humulene synthase (Figure 2) results from C\(_{11}\)-C\(_1\) closure of ionized nerolidyl diphosphate (NPP), followed by a 1,3-hydride shift and deprotonation. C\(_6\)-C\(_1\) closure of the humulyl C\(_1\) cation generates the himachalyl cation, with alternate deprotonations affording \( \alpha \)-, \( \beta \)-, and \( \gamma \)-himachalene. Further cyclizations of the himachalyl cation result in tricyclic olefin formation; C\(_3\)-C\(_7\) closure and a Wagner-Meerwein rearrangement lead to longifolene and longicyclene, whereas C\(_2\)-C\(_7\) closure leads to \( \alpha \)- and \( \beta \)-longipinene. The structurally simplest olefin products are generated by alternate deprotonations of the bisabolyl cation (formed by C\(_6\)-C\(_1\)
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closure of NPP) that yield *E*-α-bisabolene, β-bisabolene and γ-bisabolene; direct deprotonation of the nerolidyl cation results in *E*-β-farnesene.

![Diagram of sesquiterpene biosynthesis](image)

**Figure 2:** Biosynthesis of sesquiterpenes

### 1.3 Flavonoids

Flavonoids are a group of common natural occurring polyphenolic compounds that are widely found in the plant kingdom. They occur naturally as plant pigments in a broad range of fruits and vegetables as well as beverages such as tea, red wine, coffee and beer. Many of the known 4000 different flavonoids to date are part of our regular diet. These are polyketide derivatives synthesized exclusively in plants, many of which possess various functions, such as floral pigments, signal molecules, and antimicrobial compounds. In addition, several beneficial effects on human health because of their antioxidant, anti-inflammatory, antitumor and estrogenic activities have been reported. Flavonoids are, therefore, potential targets for nutraceuticals, cosmetics, and pharmaceuticals [Katsuyama *et al.* (2007)].
Flavonoids are C15 compounds that composed of a C6-C3-C6 carbon framework, or more specifically a phenylbenzopyran functionality. They can be subdivided into several classes of flavonoids and flavonoids related compounds such as dihydrochalcone, chalcone, flavone, flavanones, isoflavones and aurones (Figure 3).

1.3.1 Biosynthesis of flavonoids

Plant polyphenols arise from two main pathways: the shikimate pathway which directly provide phenylpropanoids; and the polyketide (acetate) pathway. Most of the phenolics (flavonoids) are derived biosynthetically from the combination of these two pathways which involve the formation of central C-15 chalcone intermediate. The involvement of chalcone on flavonoids biosynthesis has been described in a number of studies.

Flavonoids are synthesized via the phenylpropanoid pathway, beginning with the deamination of phenylalanine by the enzyme L-phenylalanine ammonia-lyase (PAL) to cinnamate. The cinnamate 4-hydroxylase (C4H) catalyzes the synthesis of p-coumarate from cinnamate and 4-coumarate:CoA ligase (4CL) converts p-coumarate to its coenzyme-A ester by thioesterification, activating it for reaction with malonyl CoA. The flavonoid biosynthetic pathway starts with the condensation of one molecule of 4-coumaroyl-CoA and three molecules of malonyl-CoA, yielding naringenin chalcone. This reaction is carried out by the enzyme chalcone synthase (CHS). Naringenin chalcone is converted to (2S)-naringenin, a flavanone, through intramolecular cyclization by chalcone isomerase (CHI), which accomplishes the synthesis of a flavanone scaffold. From these central intermediates, the pathway diverges into several side branches, each resulting in a different class of flavonoids.

Flavanone-3-hydroxylase (F3H) catalyzes the stereospecific 3β-hydroxylation of (2S)-flavanones to dihydroflavonols.
**Figure 4:** Schematic overview of the flavonoids pathway. Enzymes are abbreviated as follows: CHS, chalcone synthase; CHR, chalcone reductase; STS, stilbene synthase; AS, aureusidin synthase; CHI, chalcone isomerase; F3H, flavonone hydroxylase; FNS, flavones synthase; IFS, isoflavone synthase; FLS, flavonol synthase, F3’H, flavonoid-3’-hydroxylase; F3’5'H, flavonoid-3',5'-hydroxylase; DFR, dihydroflavonol-4-reductase; ANS, anthocyanidin synthase; LAR, leucoanthocyanidin reductase; ANR, anthocyanidin reductase; 3GT, flavonoid-3-glycosyltransferase.
By 3β-hydroxylation, flavanone 3β-hydroxylase (F3H) catalyzes the conversion of (2S)-flavanones to (2R, 3R)-dihydroflavonols, which are intermediates in the biosynthesis of flavonols, anthocyanidins, catechins and proanthocyanidins. Flavone synthase I (FNS I) synthesizes apigenin from (2S)-naringenin and flavanone 3-β-hydroxylase (F3H) and flavonol synthase (FLS) sequentially convert (2S)-naringenin into dihydrokaempferol and kaempferol. For the biosynthesis of anthocyanins, dihydroflavonol reductase (DFR) catalyzes the reduction of dihydroflavonols to flavan-3,4-diols (leucoanthocyanins), which are converted to anthocyanidins by anthocyanidin synthase (ANS). The formation of glucosides is catalyzed by UDP glucose-flavonoid 3-O-glucosyl transferase (UFGT), which stabilize the anthocyanidins by 3-O-glucosylation [Schijlen et al. (2004)]. The overview of the flavonoid pathway is presented in Figure 4.

1.4 Lignans

Lignans are phenylpropanoid dimers in which the monomers are linked by the central carbon (C8) atoms and are distributed widely in the plant kingdom. Lignans have gained increasing attention due to their biological effects; antimitotic, antiviral, cathartic, allergenic and antitumour activity. The most important of these is their antitumour activity. The aryltetralin (Podophyllum) group lignans are important compounds showing this activity.

1.1.4 Biosynthesis of lignans

Biosynthetic studies showed that the hydroxycinnamyl alcohols such as 4-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol termed as monolignols, are the basic precursors which form the essential building blocks of natural lignans. The biosynthesis of coniferyl alcohol begins from ferulic acid which is activated with coenzyme A and is accomplished by a 4-hydroxycinnamate:CoA ligase, that requires ATP and Mg²⁺ as cofactors (Figure 5). The reversible reduction of cinnamoyl-CoA esterase to the corresponding coniferyl aldehydes could be achieved by coniferyl-CoA reductase with NADPH as cofactor. Further reduction of coniferyl aldehydes to coniferyl alcohol again requires an NADPH-dependent enzyme, coniferyl alcohol dehydrogenase [Kamil and Dewick (1986)].

Podophyllum lignans contain two series of compounds which are biogenetically distinct. One series is represented by the metabolites containing 3,4,5-trimethoxy substituted pendant aromatic ring such as podophyllotoxin and its derivative podophyllotoxone, while the other series has a 4-hydroxy-3,5-dimethoxy substituted
aromatic pendant ring e.g. 4'-demethyl podophyllotoxin and 4'-demethylpodophyllotoxone). Matairesinol is considered to be the branch point leading to the two series of metabolites as confirmed by the radio labelled studies in *P. hexandrum*. The proposed biosynthetic pathway to *Podophyllum* lignans involve two phenylpropane units with the same 4-hydroxy-3-methoxy substituents derived from the coniferyl alcohol.

![Chemical Pathway Diagram](image)

**Figure 5:** (a) phenylalanine ammonia-lyase (b) cinnamate-4-hydroxylase (c) tyrosine ammonia-lyase (d) 4-coumarate-3-hydroxylase (e) S-adenosylmethionine:caffeate-3-O-methyltransferase (f) ferulate 5-hydroxylase (g) S-adenosylmethionine:caffeate-3-O-methyltransferase (h) 4-coumarate:CoA ligase (i) coniferyl CoA reductase (j) coniferyl alcohol dehydrogenase

The coupling of these free radical mesomers proceeds stereospecifically in the presence of enzymes leading to the formation of a diquinone methide. Reduction of diquinone methide followed by lactone ring formation yields the basic intermediate matairesinol. The modification in the substitution pattern of the aromatic pendant ring in matairesinol produces yatein (Figure 6). In the 1980s, Dewick and co workers conducted a series of feeding experiments and revealed the pathways from yatein to podophyllotoxin via deoxypodophyllotoxin. They also showed that matairesinol was metabolized to podophyllotoxin and proposed yatein as a possible intermediate between matairesinol and podophyllotoxin. The transformation of matairesinol to
yatein involves four steps: 5-hydroxylation, dual methylation at C4-OH and at C5-OH, and methylenedioxy bridge formation between C3’ and C4’. Many possible sequences of these four steps can be envisaged. Based on metabolic profiling and a series of stable isotope tracer experiments using A. sylvestris, a direct pathway and not a metabolic grid was demonstrated from matairesinol to yatein via thujaplicatin [Suzuki and Umezawa (2007); Xia et al. (2000)].

**Figure 6: Biosynthetic pathway of Podophyllum lignans**

The successive steps are presumed to involve the oxidation of dibenzylbutyrolactones and to the quinone methide followed by the cyclisation to the corresponding deoxypodophyllotoxin and 4’-O-demethyldeoxypodophyllotoxin. Finally deoxypodophyllotoxin and 4’-O-demethyldeoxypodophyllotoxin yield podophyllotoxin.
and 4’-demethyl series of aryltetralin lignans, respectively by stereospecific benzylic 4-hydroxylation. The sequence of building up of the substitution pattern in the aforesaid two series of metabolites which was derived by radiolabelled experiments using labelled \([S\text{-methyl}^{-14}\text{C}]-\text{methionine}\). Similarly, the \(\alpha\)-peltatin and \(\beta\)-peltatin were likely to arise by hydroxylation of deoxypodophyllotoxin and 4’-O-demethyldeoxypodophyllotoxin in the aromatic ring as indicated by radiolabelling experiments. In addition, podophyllotoxin and related metabolites can epimerize at C-2 position in the presence of base to give picro derivatives. The two ketones isopicropodophyllone and 4’-O-demethylisopicropodophyllone could be produced by epimerization of C-3 of podophyllotoxone and 4’-O-demethylpodophyllotoxone.

1.5 Abundantly available starting material for synthesis of bioactive molecules

Essential oils are an extremely useful source of starting materials for several industrial processes used for the synthesis of fragrances and pharmaceutical compounds such as verbenone from alpha pinene, rose oxide from citronellol, citral to nerol and geranyl nitrile. A new route to high added value compounds from low cost natural products is therefore a challenge. In fact, many different methods for functionalization of essential oil were developed for preparing new products having biological and perfumery properties.

1.6 Objectives of the present study

In the above context, it is evident that the compounds belonging to above mentioned families are of immense importance in the fields of food, flavors and medicines. Hence, the objective of this research work was to carry out the chemical investigations of Cedrus deodara, Albizia chinensis and Podophyllum hexandrum including isolation, characterization and quantification of bioactive molecules. Further, emphasis will also be given on the development of new analytical methods for the determination of major constituents in these plants. More emphasis will be given on utilization of abundantly available compounds such as himachalenes for the synthesis of novel molecules of biological importance.

**Chapter 1** Isolation, characterization and quantification of bioactive molecules from Cedrus deodara, Albizia chinensis and Podophyllum hexandrum

**Chapter 2** Synthetic modifications of himachalenes isolated from Cedrus deodara for value added products

**Chapter 3** Evaluation of biological activities of natural and synthesized molecules
Each chapter describes introduction and review of literature followed by results and discussion, and experimental section, references are given at the end of the chapter.

1.7 References


