2.1 Pharmacotherapy for asthma

The pharmacotherapy for asthma majorly involve the use of bronchodilators and anti-inflammatory drugs in the form of controllers and relievers (Figure 2.1). Controllers comprise of anti-inflammatory drugs that are required to be administered on regular basis, mostly daily, for long term in order to control asthma. These include inhaled and systemic glucocorticosteroids, leukotriene modifiers, long acting inhaled β_2 agonists combined with inhaled glucocorticosteroids, sustained release theophylline, cromones and anti-IgE agents. Among them inhaled glucocorticosteroids remain most effective controllers so far. Relievers are used on as needed basis; these are short acting by reversing bronchoconstriction and thereby relieving symptoms. They include rapid acting inhaled β_2 agonists, inhaled anticholinergics, short-acting theophylline and short-acting oral β_2 agonists (GINA Guide, 2006 & 2011). Global Strategy for Asthma Management and Prevention update (2011) has given the outline about the drugs used in asthma which are classified as follows

A. Bronchodilators:

1. β_2 agonists: Metaproterenol, Salbutamol, Formoterol and Salmeterol
2. Anticholinergics: Ipratropium and Tiotropium
3. Methylxanthines: Theophylline, Aminophylline and Acepiphylline

B. Anti-inflammatory agents:

1. Corticosteroids: Prednisolone, Dexamethasone, Budesonide and Fluticasone
2. Antileukotrienes: Montelukast, Zafirlukast, Probilukast and Iralucast
3. Mast cell stabilizers: Cromolyn sodium and Nedocromil sodium

2.1.1 Side effects associated with treatments:

Although having such large number of modern medications, the healthcare system has expanded and changed remarkably in recent years because of limited curative potential as well as adverse effects associated with current asthma therapy. Inhaled corticosteroids (ICS) used for long-term treatment with high doses produces adverse effects like hypothalamic-adrenal axis suppression & adrenal insufficiency, decreased bone mineral density, easy bruising, cataracts, growth retardation, dysphonia etc. (Kelly and Nelson, 2003). β2 adrenergic agonists has the most common adverse effects tachycardia, skeletal

muscle tremor, hypokalemia, prolongation of the QTc interval in overdose (Guhan et al., 2000). Excessive long term use of β2 agonists associated with worsening of asthma control and death due to asthma (Sears, 2002). Sustained release theophylline preparations have a narrow therapeutic index and its pharmacokinetics is largely depends on factors that influence its hepatic metabolism. Severe, life-threatening toxicity of theophylline includes seizures and arrhythmias are also reported (Hendeles et al., 1977).

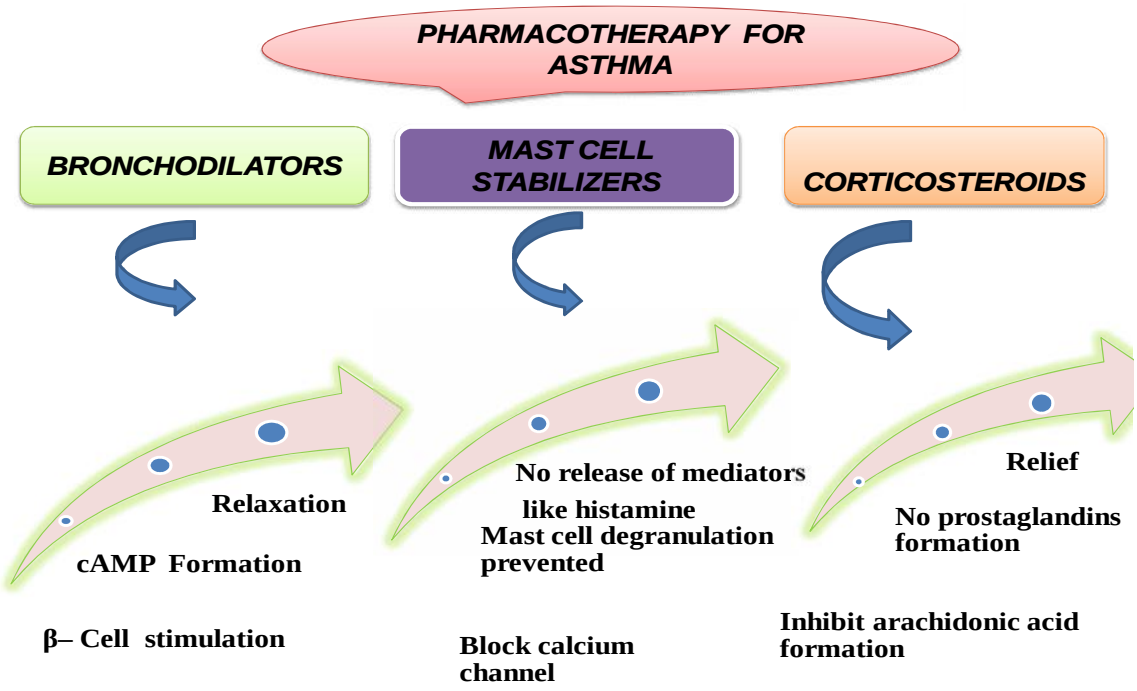


Figure 2.1: Pharmacotherapy for asthma

Mast cell stabilizers like cromolyn sodium and nedocromil recommended as second-line therapy only in mild persistent asthma (NAEPP, 2007). The anti-cholinergics (Ipratropium bromide and oxitropium bromide) have been used only for relief of acute bronchospasm and remain ineffective against allergen and exercise-induced bronchospasm (Rodrigo and Rodrigo, 2002; Larsson, 1982). Leukotrienes (LTs) modifiers (zileuton, zafirlukast and montelukast) are used for the treatment of mild persistent asthma (Zeiger et al., 2005). Drug interactions occur with the LTs modifiers zileuton and zafirlukast but have not been found with montelukast (Bisgaard, 2001; McGill and Busse, 1996). The incidences of Churg-Strauss syndrome and liver dysfunction have also been occurring in patients treated with

leukotrienes modifiers (Wechsler et al., 2000). The features of chronic adverse events or reactions due to the use of currently available anti-asthmatic medication are summarized in Table 2.1

Table 2.1: Side effects associated with asthma pharmacotherapy

S.No.	Pharmacotherapy	Side effects (GINA Guide, 2011)
Controllers		
1.	**Glucocorticosteroids Inhaled(ICS):** Beclomethasone, Budesonide, Ciclesonide Flunisolide Fluticasone, Mometasone and Triamcinolone **Tablets or syrups:** Hydrocortisone, Methylprednisolone, Prednisolone and Prednisone	**Inhaled:** High daily doses may be associated with skin thinning and bruises, and rarely adrenal suppression. Local side effects are hoarseness and oropharyngeal candidiasis. Low to medium doses had produced minor growth delay or suppression (av. 1cm) in children. Attainment of predicted adult height does not appear to be affected. **Tablets or syrups:** Used long term, may lead to osteoporosis, hypertension, diabetes, cataracts, adrenal suppression, growth suppression, obesity, skin thinning or muscle weakness. Consider coexisting conditions that could be worsened by oral glucocorticosteroids, e.g. Herpes virus infections, Varicella, tuberculosis, hypertension, diabetes and osteoporosis
2.	**Mast cell stabilizers:** Sodium cromolyn and Nedocromil cromones	Cough may occur upon inhalation.
3.	**Long-acting β- 2 agonists beta-adrenergic sympathomimetics LABAs** **Inhaled:** Formoterol (F) Salmeterol (Sm) **Sustained-release Tablets:** Salbutamol (S) Terbutaline (T)	**Inhaled:** fewer and less significant, side effects than tablets. Have been associated with an increased risk of severe exacerbations and asthma deaths when added to usual therapy. **Tablets:** may cause tachycardia, anxiety, skeletal muscle tremor, headache, hypokalemia.
4.	**Methylxanthine xanthines sustained-release:** Theophylline Aminophylline	Nausea and vomiting are most common. Serious effects occur- ring at higher serum concentrations include seizures, tachycardia, and arrhythmias. Theophylline level monitoring is often required.

5.	**Antileukotrienes (Leukotriene modifiers):** Montelukast (M) Pranlukast (P) Zafirlukast (Z) Zileuton (Zi)	Elevation of liver enzymes with Zafirlukast and Zileuton and limited case reports of reversible hepatitis and hyperbilirubinemia with Zileuton and hepatic failure with Zafirlukast
6.	**Immunomodulators:** Omalizumab Anti-IgE	Pain and bruising at injection site (5-20%) and very rarely anaphylaxis (0.1%).
Relievers		
7.	**Short-acting β –2 agonists (Sympathomimetics):** Albuterol/salbutamol Fenoterol Levalbuterol Metaproterenol Pirbuterol Terbutaline	Inhaled: tachycardia, skeletal muscle tremor, headache, and irritability. At very high dose hyperglycemia, hypokalemia. Systemic administration as tablets or syrup increases the risk of these side effects.
8.	**Anticholinergics:** Ipratropium, Tiotropium Oxitropium	Minimal mouth dryness or bad taste in the mouth.
9.	**Short-acting** Theophylline Aminophylline	Nausea, vomiting, headache. At higher serum concentrations: seizures, tachycardia, and arrhythmias.
10.	**Epinephrine/ adrenaline injection**	Similar, but more significant effects than selective β –2 agonist. In addition: hypertension, fever, vomiting in children and hallucinations.

There is an urgent need for redefining research in the field of pharmacotherapy for asthma as it involves multicomponent pathophysiology. We should urge to counter the maximum components in the complex pathophysiological cascade of asthma with low toxicity and minimum side effects in the prevention and management of asthma in particularly for children. In low and middle income countries, asthma still being underdiagnosed, undertreated and underestimated and urgently requires quality-assured and affordable essential asthma medicines. Current therapeutic regimens used in pharmacotherapy of asthma are unable to cure all stages and progression of asthma (GINA Guide, 2011).

2.2 Concept of herbalism

Herbalism is the use of medicinal herbs for prevention and treatment of diseases and ailments or for promotion of health and healing. Since time immemorial mankind has made use of herbal drugs to treat various disorders which offer an alternative to the synthetic compounds along with advantages like lesser cost, strength, effectiveness, better tolerance, safety, ready availability and being ecofriendly (Atmakuri, 2010). Indian medicinal plants are widely used directly as folk medicine in different indigenous systems of medicine like Siddha, Ayurveda and Unani and indirectly in the pharmaceutical preparations like extracts or crude powders (Vaidya, 2007). World Health Organization estimates that presently about 80% of the world populations use herbal based medicine for primary health care. According to the WHO "a medicinal plant" is any plant, which in one or more of its organ contains substances that can be used for the therapeutic purposes or which, are precursors for the synthesis of useful drugs. The herbal medicines have been defined as the quality ensured finished and labeled medicinal products containing active ingredients or standardized extracts including aerial or underground parts of plant or other plant material or combinations. WHO encourages, recommends and promotes traditional/herbal remedies in natural health care programs and also has emphasized in a number of resolutions the need to ensure quality of herbal products by using latest techniques and applying suitable standards to assess their safety, efficacy and quality. For this purpose WHO has set specific guidelines for quality control of medicinal plants (WHO technical report series, 1996). Active principles from medicinal plants, i.e. secondary metabolites are likely candidates for drug development or other technological development, or for lead molecules. Naturally derived or originated compounds play a major role as drugs or as leads for the development of a new semi-synthetic molecules (Kunle et al., 2012). About 50% of drugs introduced to the market during the last two decades were derived directly or indirectly from natural molecules. Leads from natural products will continue playing a major role in the discovery and validation of new drug targets. Using multidisciplinary approach to drug discovery involving novel phyto-molecules along with total and combinatorial synthetic methods provides the better chance to improve the drug discovery and development process. For competing with the fast growing pharmaceutical market, there is an urgent need to scientifically validate more medicinally useful herbal products (Atmakuri, 2010; Mazid et.

al, 2012). So rational screening, including extraction, isolation and separation, phytochemical investigation and screening for biological activities are the need of the hour. These investigations will be adding new medicinal plants to the world's pharmacopoeia as important drug before they get lost forever (Pal and Shukla, 2003).

Standardization can be considered as code of conduct for ensuring the consistent efficacy, such that manufacturers can ensure the batch-to-batch consistency of herbal products. Standardization and evaluation of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility (Kunle et al., 2012). Standardization is used to describe all measures, which are taken during the manufacturing process and quality control. Maintenance of the quality and efficacy ensured herbal drug by adjusting defined the content of constituents with known therapeutic activity after adding excipient in the herbal drug preparations is required (Mukherjee, 2002). Evaluation of a drug means confirmation of identity, determination of quality and detection of its nature of adulteration (Panchawat et al., 2009). WHO prescribes that important aspect of plant drug standardization is using physicochemical parameters followed by use of other highly sophisticated analytical methods as described in Figure 2.2. Good Manufacturing Practice (GMP) should be carried out for herbal medicine to ascertain the safety, identity, strength, purity and quality (Mukherjee, 2009). The quality of herbal medicines can be ensured by the standardizing of crude plant material, plant preparations and finished products (WHO 1992; 1996). Different pharmacopoeias like Pharmacopoeia Committee, Chinese Herbal Pharmacopoeia, British Herbal Pharmacopoeia, British Herbal Compendium, Japanese Standards and Ayurvedic Pharmacopoeia of India for Herbal Medicine are available internationally which lay down monograph for individual herbs and herbal products for maintaining their quality in their native nations. The safety of herbs is based on their toxicological studies and efficacy being based on the pharmacological and clinical performance of active ingredients (Sane, 2002; Raina, 2003; Chauhan, 2006; Rawat et al., 2007). Therefore the herbal principles which prove to provide symptomatic relief and assist in inhibition of disease progression with better efficacy, safety and acceptability being supported by controlled clinical trials should

be preferred. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims (Malviya et al., 2011).

"Regained interest in Herbalism, the need for standardization and proper scientific evaluations are the criterion for acceptance of herbal health claims from Indian system of medicine"

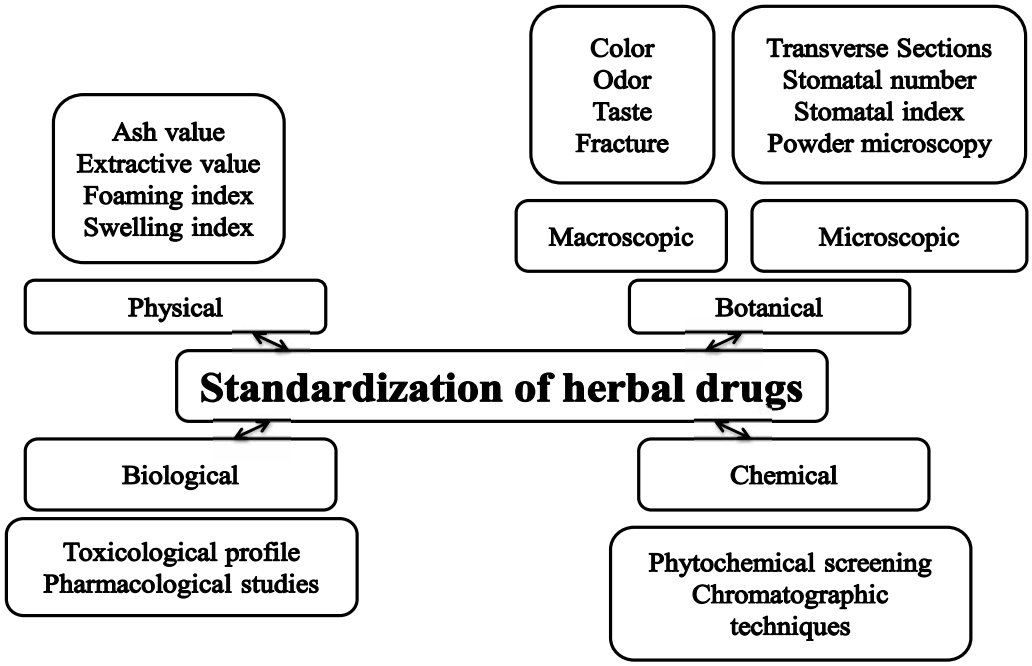


Figure 2.2: Herbal drug standardization

2.2.1 Bio-diversity of Indian Himalayan region

A Himalayan region of India covers an area of about 250,000 km^2 with its unique topography, exhibit diverse habitat within a altitudinal range of approximately 200 - 8,000 m (Samant et al., 2007). There are about 18,440 plant species growing in different habitats (Singh and Hajra, 1997), 1,748 medicinally active plants (Samant et al., 1998), 675 wild edible plant species (Samant and Dhar, 1997), 118 aromatic plants (Samant and Palni, 2000), 155 sacred plants (Samant and Pant, 2006), and 279 fodder plants (Samant, 1998).

This data justifies the uniqueness in biodiversity of this region. The diversity of medicinal plants is evident due to the presence of 31% native, 15.5% endemic and 14% threatened plants species from plants mentioned in Red Data Book for the Indian Himalaya region (Dhar et al., 2000). The richness of this plant wealth explored by the natives and tribes incorporating them various forms including food and medicine. Important contributions made during the past several years, on ethnobotanical knowledge and medicinal plant identification of Central Himalaya, which has opened a new window to natural products (Samant et al., 1998). Himachal Pradesh a hilly state area of North West India, where nature comprises of an appreciable heritage of ethno botanical flora with natural wealth supporting the large biodiversity of plant species which belong to European, Tibetan, Chinese and Indian traditional medicine growing in the different subtropical, temperate, alpine and cold desert regions. Plant species in Himachal Pradesh having Anti-asthmatic potential due to their chief chemical component include *Glycyrrhiza glabra* (Glycyrrhizin), *Adhaotda vasica Nees* (Vasicine), *Albizzia lebbeck* (Saponins), *Ephedra sinica* (Pseudoephedrine), *Solanum xanthocarpum* (Solasodine), *Curcuma longa* (Curcumin) and *Tylophora asthmatica* (Tylophorine) representing a huge biodiversity, bioactivity and phytochemicals (Chauhan, 2006).

2.2.2 Ayurveda and asthma

The word Ayurveda is derived from Ayu (means life) and Veda (means knowledge) and extensively uses the plant-derived compound formulations for treatment of various ailments after a careful study into the type of the disease. The traditional Indian system of medicine, Ayurveda is based on the principle of balance and counterbalance. Ayurveda, accepted to be the oldest treatise on medical systems, came into existence in about 900 B.C. According to Indian Hindu mythology, there are four Vedas written by the Aryans– Rig Veda, Shama Veda, Yajur Veda and Atharva Veda. The Ayurveda is said to be an Upaveda (part) of Atharva Veda, whereas the Charaka Samhita (1900 B.C.) is the first recorded treatise fully devoted to the concept and practice of Ayurveda. The Indian government and private sectors are trying their best to explore all the possibilities for the evaluation of these systems to bring out the therapeutic approaches available in the original

system of medicine as well as to help in generating data to put these products on the national health care program (Mukherjee, 2003).

Ayurveda defines asthma as Svasa (increased or difficult breathing, dyspnea) refers to disorders of the respiratory system and Tamaka svasa refers to asthma. General etiology of svasa roga is that all things, materials, and conditions that could help to increase vata dosa and kapha dosa which are causally responsible for tamaka svasa. This develops from an increase in cough (kasa), undigested materials (ama), diarrhea, vomiting (vamathu), poison (visa), anemia (pandu), and fever (jvara); coming into contact with air containing dust, irritant gases, pollens, or smoke; injuring vital spots; using very cold water; and residing in cold and damp places. Ayurveda suggest the pathology of respiratory allergies as dysfunction immune system, due to the formation of (undigested intermediate product) Ama, and Kapha dosha. Ayurveda has got potential with herbal drugs possessing immunomodulatory, anti-allergic, anti-inflammatory and mucolytic effects which can be employed for breaking the complex pathology of respiratory allergy at various levels thus giving prompt symptomatic relief to the patient. The approach of Ayurveda for management of respiratory allergies is to potentiate immune system of a person in order to decrease the susceptibility of the individual towards the specific allergens and simultaneously providing symptomatic relief to the patient. The pathogenesis of asthma, according to Ayurvedic texts, appears to arise from an abnormal interaction between vata and kapha. The initial step is the increase in vata followed by vitiated condition of vata and kapha. Because of the obstruction to normal movements of vata by kapha, vata begins to move in all directions. This disturbs the channels of respiration (prana), food (anna), and water (udaka) located in the chest, and produces dyspnea (svasa) originating from the stomach (amasaya). This suggests that the root cause of asthma is related to the digestive tract. As the pathogenesis of this disorder involves an imbalance between the vata and kapha, the therapy is directed to correct this imbalance. In addition, there are a few therapies for controlling the acute symptoms of asthma (Sengupta, 1984; Sharma, 1994; Goyal, 1997).

2.2.3 Plants reported for asthma

Asthma is a syndrome that involves a complex pathophysiological cascade including potentially permanent airway obstruction, airway hyperresponsiveness, and multicellular inflammation. Inflammatory reactions of asthma involve multiple cells and cellular mediators which play a major role, making airway epithelium to become fragile and denuded, epithelial subbasement membranes thicken with increased mucus production, consistency and endothelial leakage leading to mucosal edema. The progression of asthmatic disorders involves oxidation, inflammatory and immunogenic events majorly facilitated by activation of mast cells, T lymphocytes, eosinophils, basophils, macrophages, neutrophils, and epithelial cells, production of excessive oxidative stress, release of pro-inflammatory mediators like histamine, bradykinin, cytokines, chemokine, leukotriene and interleukins etc. To counter various targets of this multicomponent syndrome, several herbal product/constituents has been suggested, but their pharmacologic/therapeutic basis has not yet been documented. Moreover, the mechanisms of action are also partially explored or seem to be unexplored. The reported anti-asthmatics plants are classified on the basis of the probable mechanism of action as bronchodilators, mast cell stabilizers, anti-allergics, anti-inflammatory, antispasmodic and immunomodulators summarized in Table number 2.2 to 2.7 (Sharma et. al., 2014; Kale et. al., 2010; Jalwa, 2010; Savithramma, 2007).

Table 2.2: Plants used as herbal bronchodilators

Herbal Bronchodilators			
S.No.	**Plant**	**Part used/ extract/ fraction**	**Major chemical constituent (s)**
1.	*Artemisia caerulescens*	Aerial parts/Butanol	Quercitin, isorhamnetin
2.	*Alstonia scholaris*	Leaves/Ethanol	Ditamine, Echitamine and Echitenines
3.	*Benincasa hispida*	Fruits/Methanol	Triterpenes, Glycosides, Sterols
4.	*Belamcanda chinensis*	Leaves/Ethanol	Tectorigenin
5.	*Coleus forskohli*	Roots	Forskolin
6.	*Cissampelos sympodialis*	Leaves and root bark/Aqueous	Warifteine, α- bisbenzylisoquinoline
7.	*Clerodendron serratum*	Stem Bark/Aqueous	Phenolic glycosides

8.	*Elaeocarpus sphaericus*	Fruits/Aqueous, Pet ether, Benzene, Acetone and ethanol	Alkaloid, Flavanoids
9.	*Galphimia glauca*	Aerial/Alcohol extract/Ethyl-acetate	Tetragalloylquinic acid, Quercitin
10.	*Ginkgo biloba*	Leaves	Ginkgolides

Table 2.3: Plants used as herbal mast cell stabilizers

Herbal Mast Cell Stabilizers			
S.No	**Plant**	**Part used/ extract/ fraction**	**Major chemical constituent (s)**
1.	*Albizzia lebbeck*	Stem bark/Aqueous	Saponins
2.	*Aquillaria agallocha*	Stem/Aqueous extract	Triterpenoids
3.	*Azadirachta indica*	Leaves/Juice	Nimbin, Nimbinine, Nimbandiol, Quercitin
4.	*Bacopa monniera*	Leaves/Ethanol	Bacosides, Alkaloids, Glycosides
5.	*Cassia alata*	Leaves/Ethanol	Anthraquinones, Flavanoids Gentiobiosides
6.	*Cassia torosa*	Seeds	Anthraquinones,
7.	*Cassia obtusifolia*	Seeds/Glycosidal fraction	Betulinic acid Flavanoids
8.	*Citrus unshiu*	Peels	Himacholol
9.	*Cedrus deodara*	Wood oil	α- amyrin, β- amyrin
10.	*Calotropis procera*	Latex	Calotropin
11.	*Curcuma longa*	Rhizome	Tumerones, Curcuminoids
12.	*Gleditsia sinensis*	Fruits/Ethanol	Saponins
13.	*Inula racemose*	Roots/Alcohol	Inulolide
14.	*Mentha piperita*	Leaves	Flavanoidal glycosides
15.	*Magnolia officinalis*	Bark/Aqueous	Honokiol, Magnolol
16.	*Ocimum sanctum*	Leaves	Myrcenol, Nerol, Eugenol
17.	*Vitex negundo*	Leaves/Ethanol	Casticin, isoorientin, Chrysophenol D, Luteolin

Table 2.4: Plants used as herbal anti-allergics

Herbal Anti-Allergics			
S.No	**Plant**	**Part used/ extract/ fraction**	**Major chemical constituent (s)**
1.	*Asiasarum sieboldi*	Roots/Methanol	Methyleugenol, gamma-asarone, Elemicin, Asarinin
2.	*Albizzia lebbeck*	Stem bark/Aqueous	Saponins
3.	*Alisma orientale*	Rhizomes/Aqueous, Methanol	Alisol β- monoacetate, Alismaketones- β-23-acetate and C 23- acetate
4.	*Camellia sinensis*	Leaves	Flavanoids
5.	*Centipeda minima*	Aerial parts	Flavanoids, Pseuodoguainolide, sesquiterpene lactones
6.	*Cnidium monnieri*	Fruits/Ethanol Aqueous	Osthol
7.	*Desmodium adscendins*	Aerial/Alcohol extract/ Ethyl- acetate	Triterpenoid saponin
8.	*Hydrangea macrophylla*	Leaves	Glycosides
9.	*Solanum xanthocarpum*	Roots/Alkaloidal fraction	Solasodine
10.	*Terminalia chebula*	Fruits/Aqueous	Ellagic acid, Tannins, Chebulagic acid

Table 2.5: Plants used as herbal anti-inflammatory

Herbal Anti-Inflammatory			
S.No	**Plant**	**Part used/ extract/fraction**	**Major chemical constituent(s)**
1.	*Aloe vera Tourn.ex Linn*	Leaves/Aqueous, Chloroform and ethanol	Anthraquinones, Sterols, Saponins and Carbohydrate
2.	*Asystasia gangetica*	Leaves/Methanol, Ethyl acetate	Isoflavone glycoside, Dalhorinin

3.	*Bryonia laciniosa*	Leaves/Chloroform extract	Flavanoids
4.	*Cinnamonun zeylanicum*	Oil	Eugenol, Cinnamic aldehyde and α-terpeniol
5.	*Curcuma longa*	Rhizomes	Tumerones, Curcuminoids
6.	*Dalbergia odorifera*	Heartwood	Flavanoids, Tannins
7.	*Elaeocarpus spharicus*	Fruits/Aqueous, Pet ether, Benzene, Acetone and ethanol	Glycoside, Steroids, Alkaloid, Flavanoids
8.	*Nelsonia canescens*	Leaves/ethanol extract	Flavanoids
9.	*Ocimum sanctum*	Leaves/Aqueous	Myrcenol, Nerol, Eugenol
10.	*Ophiopogon japonicas*	Root/Aqueous extract	Ruscogenin and Ophiopogonin
11.	*Pavetta crassipes*	Leaves/Aqueous	Flavanoids, Tannins, Anthraquinones
12.	*Tylophora asthmatica*	Leaves/Alkaloidal	Tylophorine

Table 2.6: Plants used as herbal anti-spasmodic

Herbal Antispasmodic			
S.No.	**Plant**	**Part used/extract/fraction**	**Major chemical constituent(s)**
1.	*Asiasarum sieboldi*	Roots/Methanol	Methyleugenol, gamma- asarone, Elemicin, Asarinin
2.	*Aegle marmelos*	Leaves/Ethanol	Aegelin, Aegelemine, Aegeline
3.	*Asystasia gangetica*	Leaves/Methanol/Ethyl acetate	Isoflavone glycoside, dalhorinin
4.	*Belamcanda chinensis*	Leaves/Ethanol	Tectorigenin
5.	*Cissampelos glaberrina*	Leaves, Root bark/Aqueous	Warifteine,α-bisbenzylisoquinoline alkaloid
6.	*Cnidium monnieri*	Fruits/Ethanol	Osthol

7.	*Drymis winteri*	Bark	Terpene
8.	*Ferula ovina*	Aerial parts/Ethanol	Carvacrol, Alpha-pinene, Geranyl isovalerate and Geranyl propionate
9.	*Ferula sinica*	Roots/Ethanol	Resins
10.	*Saussurea leppa*	Alkaloidal fraction	Sesquiterpene lactone, Terpenoids
11.	*Thymus vulgaris*	Ethanol	Flavanones

Table 2.7: Plant used as herbal immunomodulators

Herbal Immunomodulators			
S.No	**Plant**	**Part used/extract/ fraction**	**Major chemical constituent(s)**
1.	*Picrorhiza scrophulariiflora*	Rhizomes/Pet. Ether, Diethyl ether and methanol	Apocynin, androsin and picroside II
2.	*Trichilia glabra*	Leaf/Aqueous	Polysaccharides
3.	*Cedrela tubiflora*	Leaf/Aqueous	Gallic acid, polysaccharides
4.	*Ipomoea carnea*	Leaf /Aqueous	Nortropane alkaloids, calystegines β_2
5.	*Withania somnifera*	Coded extracts	Withanolides
6.	*Clausena excauata*	Wood/Aqueous	Phenolic compounds, Furanocoumarins, Flavanoids and
7.	*Magnifera indica*	Bark/Alcohol, ether	Magniferin
8.	*Cleome viscosa*	Aerial parts/Aqueous, ethanolic	Alkaloids Saponins
9.	*Typhae angustifolia*	Pollen/Ethanol	Phenolic compounds Flavones
10.	*Angelica sinensis*	Roots/Aqueous and ethanolic	Polysaccharides
11.	*Boerhaavia diffusa*	Roots/Ethanol	Alkaloids

2.3 Literature for plants

The selected plants *Hedychium spicatum var. acuminatum* and *Pistacia integerrima* J. L. Stewart ex Brandis are native to Himachal Pradesh. These contain flavonoids, alkaloids, steroids, tannins, essential oils and terpenoids. These plants have been suggested for the treatment of asthma and constituted into various formulations (Chauhan, 2006).

2.3.1 *Hedychium spicatum var. acuminatum*

It belongs to family Zingiberaceae and comprises of about 52 genera and approximately 1500 species, distributed throughout tropical region of Asia (Sirirugsa and Larsen, 1995). The family Zingiberaceae is a vital group of rhizomatous medicinal and aromatic plant species which can be characterized by the presence of volatile oils and oleoresins. Some are used for nutrition as food due to starch in major quantity while others yield juice containing astringent and diaphoretic principles (Joy et al., 1998). *Hedychium* genus is consisting of about 50 species is important being one of the most popular genera of family Zingiberaceae due to its attractive foliage, diverse and showy flowers, and sweet aromatic fragrance (Hamidou et al., 2008). Kirtikar and Basu reported eight species in the Western Himalaya which includes *H. ellipticum*, *H. thyrsifoxine*, *H. elatum*, *H. coccineum*, *H. autantiacum*, *H. cotonarium* and *H. spicatum var. acuminatum.*

Plant profile: Detailed description of vernacular names, synonyms, and scientific classification of *H. spicatum* is given in table 2.8.

Table 2.8: Vernacular names, synonyms, and scientific classification of *H. spicatum*

H. spicatum **(Royal Botanic Gardens, Kew)**		
Vernacular Names (API, 1999)	**Synonyms**	**Scientific Classification**
English - Spiked ginger lily.	**Homotypic Synonyms:** *Gandasulium spicatum* (Sm.)	Kingdom: Plantae.
Hindi - Kapurkachari.	**Heterotypic Synonyms:** *Hedychium spicatum var. acuminatum* Roscoe, *Hedychium flavescens* Lodd.	Division: Magnoliphyta.
Sanskrit- Gandhamulika, Gandharika, Gandhavadhu, Prithu palashika,		Class: Liliopsida.

Gandhapalashi.	*Hedychium album* Buch.-Ham. *Hedychium spicatum var. trilobum* (Wall. ex Roscoe) *Hedychium tavoyanum* Horan. *Gandasulium sieboldii* (Wall.) *Hedychium spicatum var. khasianum* *Hedychium sieboldii* Wall.	
Bengali - Shati, Kachri, Kapurakachari.		Order: Zingiberales.
Gujarati - Kapurkachari, Kapurkachali, Kapur		Family: Zingiberaceae.
Kannada - Goul kachora, Seena Kachora, Gandhashati.		Genus: *Hedychium*.
Malayalam - Katcholam, Katchooram.		
Marathi - Kapurakachari, Gablakachari.		Species: *spicatum.*
Punjabi - Kachur, kachoor, Bankela, Kachur, Banhaldi, Shalwi, Sheduri.		
Tamil - Poolankizangu, Kichili Kizongu.		
Telugu - Gandhakachuralu.		
Oriya - Gandhasunthi.		

Botanical description

The species *H. spicatum* is perennial, rhizomatic, robust herb up to 1 m tall and up to 7.5 cm in diameter, with elongated straight stem. Stem bears leaves which are glabrous beneath and the white ascending flowers are borne in dense terminal spikes (ginger lily). Leaves are 30 cm or more in length and 2.5-3.8 cm broad, oblong, lanceolate and variable breadth. Terminal spikes are 30 cm in length, densely flowered with bracts large, oblong, obtuse and green. Flowers are white with an orange red base in a dense terminal spike and ascending. Fruits are capsule form being glabrous and globose on ripening the three valves are reflexed to expose number of small black seeds embedded in a red aril. Flowering season is in the months of July- August and seed formation occur in September–October. Rhizomes are 15-20 cm long; 2.0-2.5 cm in diameter, knotty, taste bitter and camphor-like strong aromatic odor. Found spreading horizontally under the soil surface, externally yellowish-brown, but change to dark brown on storage, and have long, thick fibrous roots. The drug is marketed as pieces of 2.5 cm diameter. Each piece has one edge covered by a rough, reddish brown layer and marked with numerous scars, circular rings and rudiments of rootlets (Naithani, 1984; Thakur et al., 1989; Chauhan, 2006, Nadkar, 1976; Kirtika and Basu 1999; Handa and Kaul, 1997). Photograph of *Hedychium spicatum* flowering, seed

formation, freshly dug rhizomes and cleaned rhizomes shown in Figure 2.3.

Figure 2.3: Photographs of *H. spicatum.* (1) Flowering (2) Seed formation (3) Freshly dug rhizomes (4) Cleaned rhizomes.

Distribution

It is found growing in parts of Western and Central Himalayas with wide distribution in Nepal, India and Pakistan. In India it is found in the central Himalaya region at an altitude of 1500–2000 m (API, 1999). In Himachal Pradesh, it is found growing in wild in isolated patches and frequent groups in the forest areas at 1600-2800 m elevations (Chauhan, 2006).

Cultivation

The plant grows well in a rich, moist soil accompanied by sunny weather. It can be grown in a sunny border as a sub-tropical bedding plant, it withstands low temperatures even

tolerating temperatures down to -2^{o}C. The rhizomes spread horizontally under the soil surface, so the tubers should only be just covered by the soil (Handa and Kaul, 1997).

Chemical constituents

α-pinene, β-pinene, limonene, 1,8-cineole, 2-alkanones, linalool, camphor, linalyl acetate, β-terpineol, borneol, β-caryophyllene, γ-cadinene, humulene, terpinolene, p-cymene, benzyl cinnamate, benzyl acetate, lindyl acteate, γ-terpinene, β–phellandrene, methyl paracumarin acetate, cinnamic ethyl acetate, ethyl-p-methoxy cinnamate, ethyl cinnamate, d-sabinene and sesquiterpene like cadinene, sesquiterpene alcohols and sesquiterpene hydrocarbons.

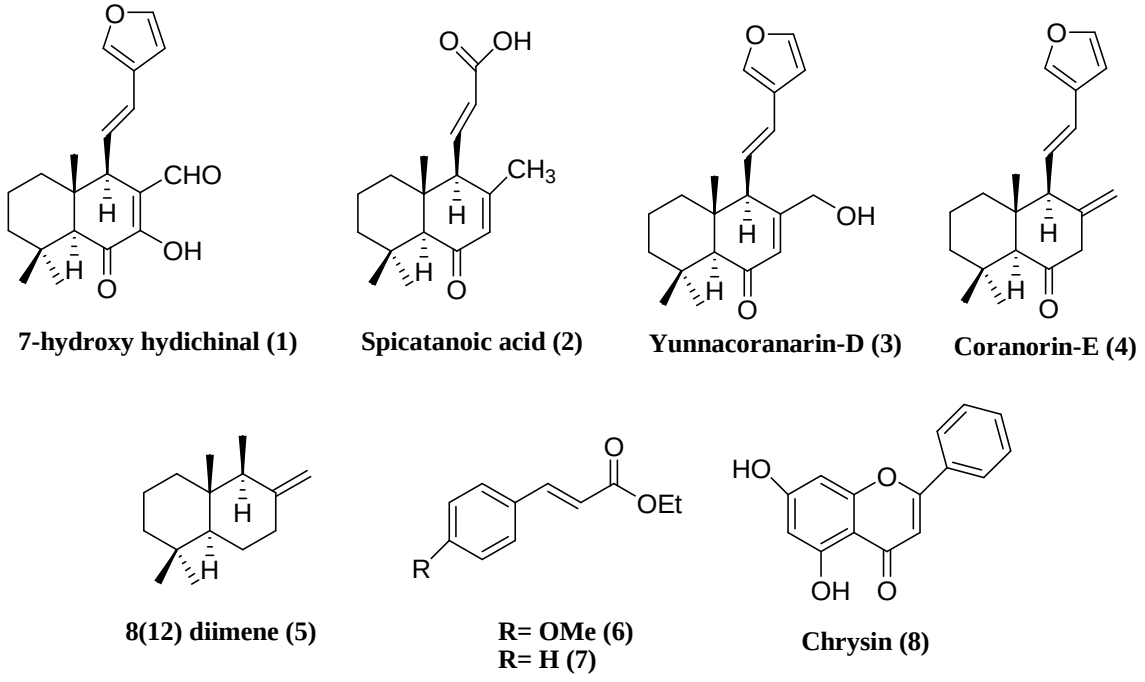


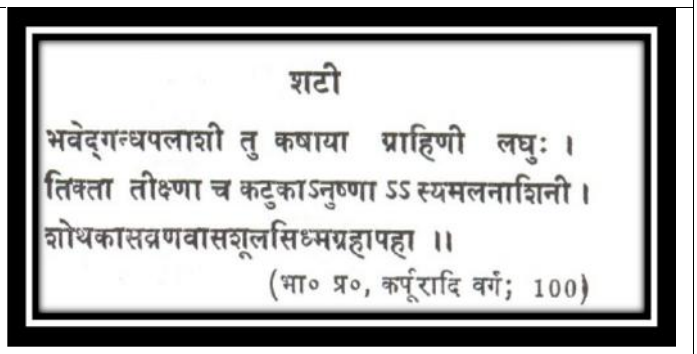
Figure 2.4: Structures of compounds (1) 7-hydroxy hedichinal, (2) spicatanoic acid, (3) yunnacoranarin D, (4) coronarin-E, (5) 8(12) drimene, (6) 4-methoxy ethyl cinnamate, (7) ethyl cinnamate and (8) chrysin.

Drimane and labdane derivatives, drim-8(12)-en-11-al, 11-nordermi-8-en-12-al, trans-5,5,8-alpha-trimethyldecal-2-one and γ-bicyclohomofarnesal, drim-8(12)-ene, 15,16-bisnorlabda-8(17)-dien-14-al (Balas,1967; Dixit et al., 1977; Garg et al., 1977; Nigam et al.,1979; Rastogi and Mehrotra, 1979; Rastogi and Mehrotra, 1984) diterpene 6-oxo-labda,

7,11,13-trien-16–oic acid lactone, hedychenone (Sharma et al.,1975), 7–hydroxyhedychenone (Sharma et al., 2008); 6-oxo-labda-7, 11,13-trien-16-oic acid lactone (Sharma and Tandon,1983) spicatanol and spicatanol methyl ether (Reddy et al., 2009a). 7-hydroxy hedichinal (1), spicatanoic acid (2), yunnacoranarin D (3), coronarin-E, 8(12) drimene (5), 4-methoxy ethyl cinnamate (6), ethyl cinnamate (7) and chrysin (8) (Reddy *et al*., 2009b) chemical structures are represented in Figure 2.4.

Ayurvedic properties: Details of Ayurvedic properties of *H. spicatum* are summarized in Table 2.9.

Table 2.9: Ayurvedic properties of *H. spicatum*

Ayurvedic Properties (Anonymous, 1987; API, 1999)	**Ayurvedic Text**
Rasa - Katu, Tikta, Kashaya.	
Guna - Laghu, Teekshna.	
Veerya - Ushna.	
Vipaka - Katu.	
Doshagnata-Kaphavatashamaka.	
Rogaghnata-Sandhishotha, Shoola, Dantashoola, Mukhadurgandha, Vrana, Apatantraka, Amavata, Aruchi, Agnimandhya, Adhamana, Udarashoola, Atisara, Arsha, Hriddaurbalya, Raktarikara, Pratishyaya, Kasa, Shwasa, Hikka and Twagdosha.	
Karma-Shothakara, Vedansthapana, Durgandhanashana, Mukhashodhana, Keshya, Rochana, Deepana, Shoolaprashamana, Grahi, Uttejaka, Rakthashodhaka, Jwaraghna, Shwasahara and Hikkanigrahana.	

Traditional and ayurvedic uses

The rhizome is used as an insect repellent (for preserving clothes), dyeing (to impart a pleasant smell to fabrics) and for perfuming tobacco. It is also used as stomachic, carminative, stimulant, tonic, aromatic, anti-arthritic, appetizer, stimulant, carminative, deodorant, hair tonic, diarrhea, dysentery, dropsy, headache, ulcers, liver complaints (Tushar, 2010), rheumatoid arthritis, rheumatism skin diseases, as blood purifier, in bronchitis, indigestion, treatment of eye disease, inflammations, and vomiting (Chopra et al., 1956), dyspepsia (powder or decoction), and preparation of cosmetic powders (promoting hair growth). It is useful in colic, cough and asthma (Savithramma, 2007). The rhizomes are reported to be boiled and eaten with salt. The roasted powder is used in asthma, and a decoction of the rhizome with sawdust is used in tuberculosis (Badoni et al., 2010). The essential oil of the rhizome used for seed-borne diseases of crops and have mild tranquilizing activity. It is used in conditions like poor circulation due to thickening of blood vessels. It forms one of the ingredients of the herbal vanishing cream (Anonymous, 1994 & 1987).

Pharmacological activities reported

Pharmacological activities of *H. spicatum* are as explained as under:

Tranquilizer: *H. spicatum* rhizome (essential oil) was reported to possess mild tranquilizing action of short duration depressing conditioned avoidance response, rota rod performance and potentiated the phenobarbitone hypnosis and morphine analgesia in rats (Chopra, 1979).

Analgesic and anti-Inflammatory activities: *H. spicatum* rhizome (benzene extract) possessed significant analgesic activity in acetic acid induced writhing in mice whereas *H. spicatum* rhizome (50 % ethanol and hexane extracts) was found to possess significant anti-inflammatory activity in carrageenan induced hind paw edema in mice (Tandon et al., 1997).

Pediculicidal activity: *H. spicatum* rhizome (essential oil) evaluated for *in-vitro* pediculicidal activity showed that, the essential oil showed more significant activity at 5 %, 2 %, 1 % concentration than 1 % permethrin based product (Jadhav et al., 2007).

Antimicrobial activity: *H. spicatum* rhizomes (essential oil) reported for antimicrobial activity. *H. spicatum* rhizomes (pet ether and chloroform extracts) showed inhibition of growth against gram (+), gram (-) bacterial cultures, including a strain of methicillin and vancomycin resistant *Staphylococcus aureus* and fungal cultures (Bishit and Awasthi, 2006).

H. spicatum rhizomes (terpenoids) also showed significant antimicrobial activity against *Staphylococcus aureus, Shigella flexneri, Pasteurella multocida* and *Escherichia coli* (Joshi et al., 2008).

H. spicatum fruits (ethanol extract) reported to possess antibacterial and antifungal properties against *Salmonella* species, *Escherichia coli* and filamentous fungi (Ray and Majumdar, 1976).

Antioxidant activity: *H. spicatum* rhizomes (terpenoid) possesses antioxidant activity. *H. spicatum* rhizomes (essential oil) collected from three different regions exhibited different relative content of essential oils, which studied for their antioxidant activity by DPPH radical scavenging method, and reducing power assay. It was also tested for effect on the chelating properties of Fe2+ which showed moderate to good Fe2+ chelating activity (Joshi et al., 2008).

Antimalarial activity: *H. spicatum* rhizomes (50% extract) reported for antimalarial activity against *Plasmodium berghei* strain (NK 65) (Misra, 1991).

Cytotoxic Activity: Two new labdane type diterpenes from $CHCl_3$ extract of *H. spicatum* rhizomes reported for cytotoxic activity on cell lines against Colo-205 (Colo-cancer), A-431 (Skin- cancer), MCF-7 (Breast- cancer), A-549 (Lung -cancer), and Chinese hamstar ovary cells (Reddy et al., 2009).

Anthelmintic activity: *H. spicatum* rhizome (methanolic extract) reported against, *Pheretima posthuma* called as adult Indian earthworms. The time taken for each worm for

paralysis and death compared with piperazine citrate. *H. spicatum* showed better anthelmintic effect than standard drug in a dose dependent manner (Sravani and Padmaa, 2011).

Pharmacological activities for bronchial asthma: *H. spicatum* rhizome powder, 10 g in divided doses for 4 weeks in 25 patients with recurrent paroxysmal attacks of dysopnea (bronchial asthma), completely relieved dysopnea, cough and restlessness in all the patients. The ronchi completely disappeared in 36 % of the patients. The mean respiration rate was reduced by 25 % and the vital capacity was increased by 20 %. The mean absolute eosinophil count also declined by 55.6 % (Chaturvedi and Sharma 1975).

H. spicatum rhizome (aqueous and ethanolic extracts) exhibited protection for anti-histaminic and ulcer-protective potential in guinea pig (GP), anti-inflammatory and analgesic activities in rat and acute toxicity in mouse (Ghildiyal, 2012).

Pulmonary eosinophilia: *H. spicatum* rhizome (powder 6 g twice a day for 4 weeks) in 15 patients of tropical pulmonary eosinophilia reported eosinophil count reduced by 60.54 % (Sahu, 1979).

H. spicatum rhizome (powder 70 mg/kg body weight) in children suffering from tropical pulmonary eosinophilia reported with relief (signs and symptoms) and reduces (blood eosinophil level). Most of the symptoms were relieved within one to three week period; radiological findings and lymphadenopathy were normalized on prolonged administration (Shaw, 1980).

Formulations and preparations: The rhizome of *H. spicatum* has been admixed in several herbal formulations like Chyawanprash (anti-asthmatic), Chandraprabhavati (anti-asthmatic), Bharangyadi (anti-asthmatic), Satyadi- churna (anti-asthmatic), Hinguvacadi churna (anti-inflammatory), Katphaladi churna (anti-asthmatic), Eranda paka (anti-inflammatory), Agastya Haritaki Rasayana (anti-asthmatic), Pippalyadi taila (anti-asthmatic), Ushirasava, (anti-asthmatic) Sarivadyasava (anti-inflammatory), Amritaprasha ghrita (anti-asthmatic) and Pradarantaka lauha (anti-asthmatic) (Anonymous, 1987).

Substituents and adulterants

Rhizomes of *Kaempferia galanga* Linn are also used under the same name Shati (Sharma 2002).

2.3.2 *Pistacia integerrima* J. L. Stewart ex Brandis

Zohary (1952) reported 11 species of genus *Pistacia* L. belonging to the family Anacardiaceae. *P. integerrima* was proposed as a recently diverged subspecies of *P. chinensis* on the basis of results from plastid restriction site analyses and its flowering phenology (Zohary, 1952).

Figure 2.5: Photographs of *P. integerrima* (1) Adult tree (2) Leaf gall formation (3) Leaf gall development (4) Dried leaf gall.

Pistacia genus thus comprises 11 species distributed in the northern hemisphere, with seven species (*P. atlantica, P. integerrima, P. khinjuk, P. lentiscus, P. palaestina, P. terebinthus, and P. vera*) distributed from the Mediterranean basin to central Asia, two

species (*P. chinensis and P. weinmannifolia*) in eastern Asia, and two species (*P. mexicana and P. texana*) distributed from the southwestern United States to Central America (Parfitt and Badenes , 1998).

Plant profile: Detailed description of vernacular names, synonyms, and scientific classification of *P. integerrima* are given in Table 2.10.

Table 2.10: Vernacular names, synonyms, and scientific classification of *P. integerrima*

<table>
<tr><th colspan="3">Pistacia integerrima J. L. Stewart ex Brandis
(Missouri Botanical Garden, Tropicos)</th></tr>
<tr><th>Vernacular Names (API, 1999)</th><th>Synonyms</th><th>Scientific Classification</th></tr>
<tr><td>English Name: Crab's claw</td><td rowspan="10">Pistacia formosana Matsum.,
Pistacia philippinensis Merr. &Rolfe,
Rhus argyi H. Lév.,
Rhus gummifera H. Lév.
Pistacia integerrima J. L. Stewart ex Brandis</td><td>Kingdom: Plantae</td></tr>
<tr><td>Hindi: Kakra, Kakra-singi, Kareran, Kakare, Kakkar</td><td>Division: Magnoliophyta</td></tr>
<tr><td>Marathi: Kakra Kaakada, Kaakad shingee</td><td>Class: Equisetopsida C. Agardh</td></tr>
<tr><td>Tamil: Kakkata-shinigi</td><td>Subclass: Magnoliidae Novák ex Takht.</td></tr>
<tr><td>Telugu: Kakarashingi, Kaakara Shingi</td><td>Super order: Rosanae Takht</td></tr>
<tr><td>Kannada: Chakrangi, Kaakada shringi, Karkaataka shringi</td><td>Order: Sapindales Juss. ex Bercht. & J. Presl</td></tr>
<tr><td>Urdu: Kakra, Mastagi desi</td><td>Family: Anacardiaceae R. Br.</td></tr>
<tr><td rowspan="2">Sanskrit: Chakrangi, Chandraspada, Ghosha, Karkata.</td><td>Genus: Pistacia L.</td></tr>
<tr><td>Species: Pistacia chinensis Bunge</td></tr>
</table>

Botanical description

P. integerrima (deciduous tree) adult tree is 10 to 25m in height. Adolescence is achieved after a juvenile period of about 12 years before flowering. The tree may grow for more than 300 years. During the dry season leaves undergo shedding. Flowering (wind pollinated) occurs from March to May and fruits from June to October (Padulosi et al., 1996). Leaves are papery; base is oblique, margin entire, apex acuminate or long acuminate. Both sides of the leaf are little pubescent with midrib and lateral veins prominent. Petiole is minutely pubescent with flattened above; leaf blade: is imparipinnately compound, 1-14 opposite leaflets. Flowers are in form of panicles (small) with reddish color. Buds are red with special odor. Fruits are drupes, irregularly glabrous, wrinkled and 6 mm in diameter, which appear pink (unripe) initially and change to gray (ripe) (Qin et. al., 2007; Liu et al, 1999). Gall: Hollow hard horn shaped thorn like growth formed by an insect of Pemphigus species, rugose, excrescences are on the leaves and petioles (Qin et. al., 2007; Chauhan, 2006; Liu et al, 1999). *P. integerrima* gall (Kakrashinghi) hollow, hard horn shaped, thorn like out growth formed by reformation of leaves and petioles by insect of Pemphigus species is rugose excrescences (Chauhan, 2006). Crushed galls have a very sharp, bitter in taste and terebinthine odor. On cutting leaves, flowers, panicles and stems exude viscous and aromatic fluid containing volatile oil (Chopra, 1982; Chauhan, 2006). Photographs of *P. integerrima* adult tree, leaf gall formation, leaf gall development and dried leaf gall shown in the Figure 2.5.

Distribution and habitat

P. integerrima (Anacardiaceae) grows in many parts of Asia at an altitude of 350-2400m in China, India, Nepal and Pakistan (Duthie, 1903-29). It is native to the ranges of hills and mountain forests on rocky soils in the warm valleys in the Himalayan region from Indus to Kumaon (Wealth of India, 1998). In Himachal Pradesh, it is found in the districts of Chamba, Kangra, Mandi, Kinnaur, Shimla, Sirmour and Solan (Chauhan, 2006).

Cultivation

P. integerrima grows well on dry slopes with shallow soils. Trees are wind firm, termite resistant, frost tolerant (to about -25°C), hardy and moderately drought resistant. *P.*

integerrima grows in sunny area in warm climates, being intolerant of shade. Management is done by pruning, lopping and pollarding which improves the growth of *P. integerrima* tree. Seedlings are needs strongly alkaline soils with nursery time of 12-18 months under controlled conditions, the addition of pentachloronitrobenzene in planting soil medium under greenhouse. Best seedlings can be raised when sown to 2 cm depth and treatment of seeds with concentrated sulfuric acid for 20 min. The germination rate is 30% pretreatment (water soaking for 24 hours) improves germination. *P. integerrima* rootstock seedlings is susceptible to *Verticillium wilt* and *Rhizoctonia solani* AG-4. Some leaf diseases effecting tree are leaf spot caused by *Cercospora megaspermae* and *Septoria pistaciae*, yellow leaf rusting by *Uraecium* species and brown leaf rusting by *Pileolaria pistaciae* (Chopra, 2006).

Chemical constituents

Phytochemical investigation of the galls of *P. integerrima*showed the presence of alkaloids, tannins and flavonoids. The essential oil is obtained by steam distillation of the galls of the plant 'kakrashinghi'. The indigenous *P. integerrima* contains α-pinene (25%), camphene (27%), dilimonene (4-5%), 1:8 cineol (10%), α-terpineol (20%), aromadendrene (4-5%) and small percentage of a lactonic stearoptene (15%). Ghosh (1945) extracted the galls with benzene and obtained two acids 'A' and 'B'. Acid 'A' is a needle shaped compound and 'B' was rhombic crystals. It contains ethyl gallate, 14'-phenoxyteteradecany 3,5-dihydroxy benzoate (pistiphloro-glucinyl ester), 4'-phenoxy-n-butyl-1'-(3-oxy-5-hydroxy) benzoic acid (pistaciaphenyl ether), 3'-(1,3-dihydroxy-5-phenoxy-1'5'-dimethoxbenzene (pisticiphloro-glucinyl ether). Structure for (1) Ethyl 3, 4, 5-trihydroxybenzoate, (2) Hydroxydecanyl arachidate, (3) N-Octadecan-9, 11-diol-7-one, and (4) 17-(5-Ethyl-6-methylheptan-2-yl)-10,13- dimethyl-2,3,4,7,8,9,11, 12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (β-sitosterol) are represented in Figure 2.6 (Ahmad et al., 2010, Ansari, 1993, Monaco et al., 1982; Ansari et al., 1994 a,b).

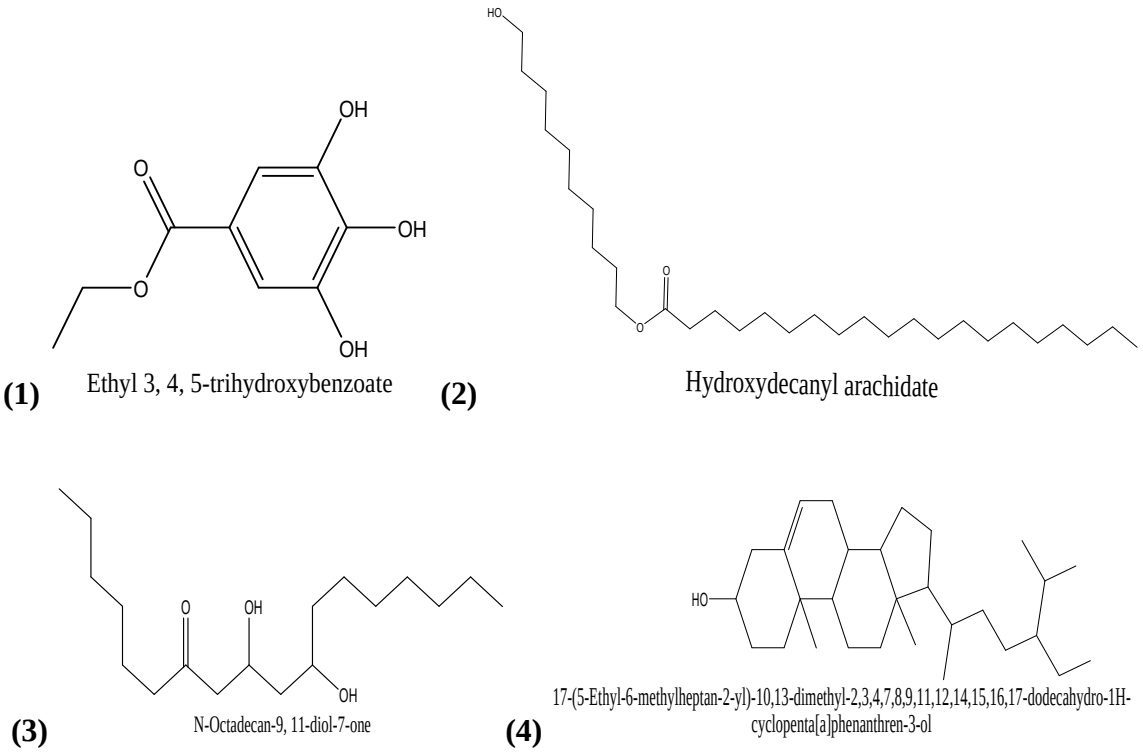
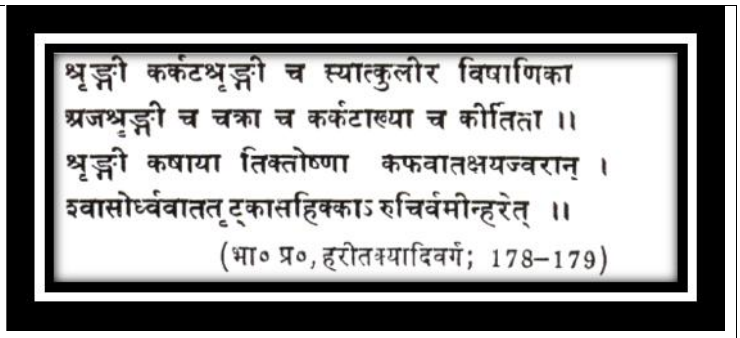


Figure 2.6: Structure of compounds (1) Ethyl 3, 4, 5-trihydroxybenzoate, (2) Hydroxydecanyl arachidate, (3) N-Octadecan-9, 11-diol-7-one, and (4) 17-(5-Ethyl-6-methylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol (β-sitosterol).

Ayurvedic properties: Details of ayurvedic properties of *P. integerrima* are summarized in Table 2.11.

Table 2.11: Ayurvedic properties of *Pistacia integerrima* J. L. Stewart ex Brandis

Ayurvedic Properties (API, 1999)	**Ayurvedic Text**
Rasa (Taste) – Kashaya (Astringent) and Tikta (Bitter) Guna (Characteristics) – Laghu (Light) and Ruksha (Rough) Veerya (Potency) – Ushna (Hot)	कर्कटशृङ्गी .....शृङ्गी कासहराणि भवन्ति । (च० सू०; 4/36) कुलीरशृङ्ग्य इति दशेमानि हिक्कानिग्रहणानि भवन्ति ।। (च० सू०; 4/30)

Vipaka (Post digestion effect) – Katu (Pungent)	

Traditional and ayurvedic uses

P. integerrima pacifies Kapha and Vata. So it can be used effectively in the management of diseases which are of Kapha or Vata origin or of both. In Ayurvedic medicines *P. integerrima* is used from ancient time. Vangasens Samhita suggest that karkatshringi churna and radish powder should be administered with honey and ghee to manage different respiratory distress of infants. The galls are aromatic, astringent, expectorant, asthma, phthisis and used in other disorders for the respiratory tract, chronic bronchitis, hiccough, diarrhea, dysentery, vomiting, appetite loss, nose bleeding, snakebite, scorpion stings, skin diseases, psoriasis, and fever. For pulmonary effect essential oil is responsible; while the large amount of tannins in the drug provides its strong astringent action. *P. integerrima* (Anacardiacea) leaf galls has a great reputation both in veds (Hindu Ayurveda practitioners) and hakims (Mohmmedan unani practitioners) as medicine for tonic, expectorant and other conditions of the respiratory tract (Chopra, 1965; Ahmad et al., 2010; Pant, 2010; Uddin et al., 2011).

Pharmacological activities reported

Pharmacological activities of *P. integerrima* are as follows

Hepatoprotective activity: Hepatoprotective effects of *Berberis lycium, Galium aparine* and *Pistacia integerrima* in Carbon tetrachloride (CCl_4)-treated rats (Muhammad et al., 2008).

Antioxidant activity: The compounds from the ethyl acetate fraction of methanolic extract of *P. integerrima* were reported for antioxidant activity in DPPH free radical method, reducing power assay, scavenging of hydroxyl radical method (Joshi, 2009).

Anti-nociceptive and analgesic activities: *P. integerrima* leaves and gall (hydroalcoholic and aqueous extract) showed antinociceptive and analgesic effects and no apparent acute toxicity on oral administration in mice (Ahmad et al., 2010).

Cytotoxic activity: The extracts/fractions of different parts of *P. integerrima* were found to have profound cytotoxic effect against *Artemia salina* (Leach) shrimp larvae. Among the various parts, the galls reported most cytotoxic agents and among the tested extracts/fractions hexane was found to be least cytotoxic (Uddin, 2013).

Antiasthmatic activity: The aqueous extract of *P. integerrima* gall in dose 1 mg, 23.25 mg and 46.50 mg/kg b. w. *p.o.* reported for mast cell stabilization in *ex vivo* challenge of antigen in sensitized albino rats, and reported to have antihistaminic and spasmolytic activities. The results showed significant protection against bronchospasm induced by histamine aerosol in experimental guinea pigs and also showed the spasmolytic activity in isolated guinea pig tracheal chain preparation against contractions induced by histamine. Antiasthmatic activity of the can be correlated to the membrane stabilizing potential, suppression of antibody synthesis and inhibition of antigen induced by histamine release (Surendra et al., 2013).

The essential oil from *P. integerrima* (5-30 µg/ml) was reported for antioxidant activity, mast cell degranulation, angiogenesis and soyabean lipoxidase enzyme activity and inhibiting 5-lipoxidase enzyme activity with IC_{50} of 19.71 µg/ml. It also inhibited inflammation of bronchioles in rats and airway hyperresponsiveness in sensitized guinea pigs. Essential oil inhibited angiogenesis and at 10, 30 and 100 µg/ml showed anti-allergic activity by inhibiting mast cell degranulation to an extent 19.08 ± 0.47%. The essential oil (7.5, 15 and 30mg/kg i.p.) inhibited neutrophil count, nitrate-nitrite in bronchoalveolar lavage fluid and myeloperoxidase concentration levels in lung homogenates (Shirole, 2014).

Antibacterial activity: The ethanol and aqueous fractions of *P. integerrima* galls was reported for antibacterial activity in concentration dependent 25, 50, 100, 250, 500µg /100µl against Gram positive bacteria and moderate activity on Gram negative bacteria. The ethanol extract had better activity than aqueous extract and as compared with standard antibiotic Ciprofloxacin (Ramachandra, 2010). Crude extracts and aqueous fractions of *P. integerrima* showed varying levels of bactericidal activity with maximum activity (16 mm

zone of inhibition) against *B. subtilis* (Bibi et al., 2011).

Antifungal activity: The ethanol and aqueous fractions of different parts of *P. integerrima* (gall, bark and leaves) were reported as antifungal against *Microsporum canis, Aspergillus flavus* and *Fusarium solani* (Uddin et al., 2013).

Formulations and preparations: The Galls of *P. integerrima* has been constituted in various herbal formulations like Chyawanprash (anti-asthmatic)**,** Katphaladi churna (anti-asthmatic), Kumari asava, Kumari kalp Shringyadi-churna, Karkatadi-Churna (anti-asthmatic), Balchaturbhadra-churna, Ashtaangaavaleha (anti-asthmatic), and Dashmool arishta (anti-asthmatic) etc.

2.4 Bio active leads for asthma

2.4.1 Coumarin and its derivatives

Coumarins are phenylpropanoids which may be naturally occurring or synthetic oxygen-containing heterocyclic compounds with benzopyrone framework (1-benzopyran-2-one). Benzopyrone structure (Figure 2.7) in the derivative molecules helps for interaction with a diverse enzymes and receptors of biological system through weak bonds thus making them potential medicinal drugs. Coumarin based NSAIDs is potentially active against inflammation. Heterocycle containing coumarin derivatives have been found to exhibit potential antiinflammatory and analgesic abilities by acting on COX-2 which is involved in inflammation condition as it catalyze the bioconversion of arachidonic acid to prostaglandins. Thereby is considered as an important target of antiinflammatory agents. Some naturally found coumarin derivatives are umbelliferone (7-hydroxycoumarin), aesculetin (6, 7-dihydroxycoumarin), herniarin (7-methoxycoumarin), psoralen and imperatorin (Bairagi et al., 2012, Gomez et al., 2012).

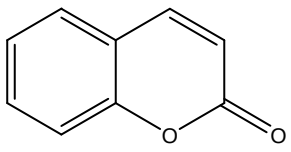


Figure 2.7: Structure of 1-benzopyran-2-one

Coumarins as anti-asthmatics: The root of *Peucedanum praeruptorum* Dunn used in Chinese medicine for the treatment of asthma having major constituent's coumarins. Coumarins of *Peucedanum praeruptorum* Dunn (CPPD) had anti-asthmatic potential which was investigated in female mice challenged with ovalbumin (OVA) to induce allergic airway inflammation. Airway hyperreactivity to the intravenous administration of acetylcholine chloride after 48 hours of final OVA inhalation was evaluated by leukocyte counts in bronchoalveolar lavage fluid and histopathological studies for lung lesions. Amount of interleukin (IL)-4, IL-5, IL-10, IL-13 and IFN-γ in bronchoalveolar lavage fluid, OVA-specific immunoglobulin (Ig) E in serum, and activity of eosinophil peroxidase (EPO) in lungs were also determined. Flow cytometry was used to analyze the percentage of CD4+CD25+Foxp3+ regulatory T cells among CD4+ T cells in the spleen. Coumarins reduced significantly the airway hyperreactivity, airway eosinophilic inflammation, improved pathologic lesion of the lungs, reduced levels of IL-4, IL-5, IL-13 in BALF and OVA specific IgE in serum, inhibited the activities of EPO in the lung, and up-regulated levels of IL-10 and IFN-γ in BALF as well as the percentage of CD4+CD25+Foxp3+ regulatory T cells in the spleen cells. This showed that coumarins from this drug can significantly suppress OVA-induced airway inflammation, airway hyperreactivity and Th2 predominant response in experimental animals, showing its great therapeutic potential for the treatment of allergic asthma (Xiong et al., 2012).

Coumarin derivative IMMLG5521 have been evaluated for sephadex induced lung inflammation in rats where pretreatment with IMMLG5521 (6.25 and 12.5 mg/kg) showed inhibition of massive granulomas and infiltration of neutrophils and eosinophils, with decreased TNF-α level in BALF and lung tissue, and also down-regulated VCAM-1 and ICAM-1 expressions in the lung tissue. Thus coumarin derivative IMMLG5521 showed suppression of the lung injury induced by Sephadex, due to the prevention of the up-regulation of VCAM-1 and ICAM-1 expressions and decreasing levels of TNF-α (Li et al., 2012).

Plant derived coumarin esculetin (6, 7-dihydroxy-2H-1-benzopyran-2-one) was found to have potent bronchodilator in carbachol induced bronchoconstriction by restoring mitochondrial dysfunction and structural changes, also showed good anti-asthmatic potential in an experimental mouse model by reducing airway hyperresponsiveness, Th2

response, lung eotaxin, bronchoalveolar lavage fluid eosinophilia, airway inflammation, and OVA-specific IgE. Esculetin reduced the expression and metabolites of 15-lipoxygenase and lipid peroxidation essential for mitochondrial dysfunction with additional reduction of subepithelial fibrosis and TGF-1 levels in the lung. These results suggest that esculetin both restores mitochondrial dysfunction and its structural changes and alleviates asthma (Mabalirajan et al., 2009).

The antiallergic properties of a new coumarin compound (BM 15.100) were tested in two separate trials, using bronchial provocation tests in asymptomatic volunteers with extrinsic allergic bronchial asthma. Study I was a blind study of 10 subjects with placebo controls and three additional non-medicated controls. Study II was an open study with nonmedicated controls. Body plethysmography (study I) and spirometry (study II) were used for the assessment of bronchial obstruction. A significant protective effect of a single oral 20 mg dose of BM 15.100 administered 60 min prior to allergen inhalation was found in both trials. The effect of 10 mg BM 15.000 was not significant. Skin test, pulse rate, and diastolic blood pressure were not influenced. The results indicate a possible therapeutic value of BM 15.100 (Gonsior et al., 1979)

The SAR studies of a series of 18 coumarins derivatives and ex vivo relaxing evaluation was carried out. The results showed that the ether derivatives 1–3, 7–9 and 13–15 was active (Emax = 100%), where compound 2 (42 μM) was the most potent, being 4-times more active than theophylline (positive control). The SAR analysis showed that it is necessary the presence of two small ether groups and the methyl group at position 4 increase biological activity through soft hydrophobic changes in the molecule, without drastic effect on the cLogP (Recillasa et al., 2014).

2.4.2 Terpenoids (Phytoncides)

Volatile oils or essential oils or ethereal oils or odorous principles of plants which contain numerous volatile terpenoids, which can be extracted both by hydro distillation and extraction with non-polar solvents like hexane and pet ether. Phytoncides are natural volatile compounds biosynthesized by trees and plants either as a protective mechanism against the harmful insects, animals and microorganism or to attract insects, animals and microorganism for pollination. Plants being capable to biosynthesize many different types

of secondary metabolites, which have been exploited for their beneficial role through various applications by humans (Balandrin et al., 1985). Terpenes are most numerous and diverse and also a major group among other plant secondary metabolites (terpenes 55%, phenolic 18% and alkaloids 27%) there by used for a number of medicinal, pharmaceutical and food products being exploited for their potentials as medicines and flavor enhancers (Croteau et al. 2000). An isoprene (C_5H_8) unit bonded with a second isoprene unit (C_5H_8) head to tail defining characteristic of terpene (monoterpene 2 isoprene C_{10}, sesquiterpenes 3 isoprene units C_{15}, two terpene units diterpenes C_{20} and 3 terpene unit triterpenes C_{30}). Chemically terpenes are hydrocarbon and/or their oxygenated derivatives. The major ingredients of phytoncides are highly volatile terpenoids such as alpha-pinene, carene and myrcene (Hisama, 2008). Chemical and pharmacological evaulations prooved that some species produce active phytomolecules that exert carminative, anti-inflammatory, anti-oxidant and radical scavenging activity (Nam et. al., 2006; Oikawa, 2005; Nose 2000). Phytoncide have been reported to improve the activity of human NK cells (Li et al., 2006). Aroma and fragrance from plants have a regulatory effect on immune function in humans and a restorative effect on the stress-induced immune suppression in mice (Komori et al., 1995; Shibata, 1991).

Terpenoids probable role on components of asthma: Though Terpenoids been neglected class as antiasthmatic evidences its remarkable role in countering various pathological pathways, sign and symptoms and severity involved with asthmatic syndrome. Various plants containing terpenoids in their volatile oil through aromatherapy and systemic administration are reported for their actions in various respiratory system disorders like *Osimum basilicum* (Expectorant), *Melaleuca leucadendron* (Expectorant, chronic laryngitis and bronchitis), *Eucalyptus globulus* (Anti-inflammatory, expectorant, bronchitis and asthma), *Lavendula officinalis* (Acute allergic reactions), *Mentha piperita* (Cough, expectorant and anti-inflammatory), *Ravensara aromatic* (Rhinopharyngitis, sinusitis and bronchitis) *Rosa damascene* (Laryngitis, bronchitis and asthma) *Rosemarinus officinalis* (Bronchitis and asthma), *Origanum majorana* (Allergic rhinitis), *Salvia sclarea* (Asthma) and *Cupressus sempervirens* (Asthma, bronchitis and cough).

Terpenoids as NOS inhibitors: Inflammatory processes are associated with the uncontrolled overproduction of nitric oxide (NO), which can easily diffuse into cells without a membrane transporter. Plant derived natural product affect NOS activity and NOS gene expression, terpenoids being one of the richest sources for mammalian NOS inhibition. Sesquiterpenes inhibit the NO production by inactivating the nitric oxide synthase isoforms (iNOS, eNOS and nNOS) and/or by decreasing the iNOS protein and mRNA level. Alpha eudesmol, Beta eudesmol and Gamma eudesmol IC_{50} for inhibition of NOS were found to be 37.0, 44.0, 53.0 and alpha eudesmol being most potent isomer. The diterpene isolated from the rhizome of *Hedychium coronarium* was found to decrease vascular permeability induced by acetic acid in mice and inhibit the nitric oxide production in lipopolysaccharide activated mouse peritoneal macrophages (Matsuda, 1999; Matsuda, 2000; Matsuda 2002).

Terpenoids as anti-Inflammatory: Inflammation resulted as a protective measure for eliminating the injurious stimuli; anti-inflammatory substances should be used for therapeutic treatment of the diseases. Herbal drugs and their isolated compounds are of use in folklore medicine to treat different lung inflammations. Terpenoids are bioactive natural products used against inflammation, volatile oils being referred as a rich source for the anti-inflammatory monoterpenes. Essential oil of *Helichrysum odoratissimum, Heteropyxis natalensis* and *Lippia javanica* showed 5-lipoxygenase inhibitory activity which converts essential fatty acids into leukotrienes, proinflammatory mediators from myeloid cells. Major components of these oils 1,8-cineole and limonene, contribute to the anti-inflammatory activity where1,8-cineole caused partial potentiation of the anti-inflammatory action of limonene (Frum and Viljoen 2006). Immunostimulating activity of different essential oils of lavender, lemon, eucalyptus, juniper, thyme, mentha, rosemary, geranium, pine, and salvia along with monoterpenes 1,8-cineole, menthol, citral, limonene, linalool, thymol,α-pinene, camphor, and borneol showed that the pine and lemon volatile oils were strongest immunostimulator, while α-pinene displayed the strongest action, followed by borneol and 1,8-cineole among the monoterpenes (Kedzia, et al,. 1998).

Terpenoids as immunomodulator.: *In vitro* evaluation for immunomodulation of terpenoids from volatile oils from *Boswellia carteri, Pelargonium graveolens, Matricaria recutita, Lavandula angustifolia, Citrus limon, Santalum spicatum, Melaleuca alternifolia,*

Melaleuca viridiflora, Cedrus atlantica and *Thymus vulgaris* along with monoterpenes α-pinene, (S)-(−)-limonene, linalool, geraniol, thymol, 1,8-cineole, linalyl acetate, and (+)-terpinen-4-ol for potential immunomodulatory effects on activity of the natural killer cell and lymphocyte activation through CD69 expression. Linalyl acetate was the only monoterpene that exhibited a dose-dependent stimulation of natural killer cells. (Standen, et al., 2006) Immunosensitizing potential evaluation of monoterpenes performed in the rat popliteal lymph node assay (PLNA), for screening molecules with potential of inducing allergic and auto-immune-like reactions in humans was found positive for few of the monoterpenes citral, α-terpinene, β-myrcene and (−)-α-pinene, and negative for (−)-menthol, 1,8-cineole, (±)-citronellal, (+)-limonene, (±)-camphor and terpineol. Secondary PLNA, a T-cell priming test, with the four substances tested positive in the primary assay showed that citral, α-terpinene, β-myrcene and (−)-α-pinene were negative in the secondary assay, so these monoterpenes induced an immunostimulatory effect due to their irritant properties, but none them seemed to be a sensitizing agent in the PLNA (Friedrich, et al., 2007). Leukotriene (inflammation mediator) production inhibition evaluation of (−)-menthol, (+)-limonene, α-terpinene, γ-terpinene, terpineol, β-myrcene, (±)-linalool, geraniol, citral, α-cyclocitral, (+)-α-pinene, (−)-α-pinene, and (+)-cis-verbenol revealed them to be effective as an inhibitor of leukotriene production thus should be used for variety of inflammatory diseases, including asthma, chronic bronchitis, and allergic rhinitis (Ahn et al., 2001).

Terpenoids as anti-asthmatic: Anti-asthmatic potential of the essential oil of *Artemisia argyi* (leaves) and its monoterpene constituents trans-carveol, α-terpineol/l-α-terpineol, α-phellandrene, camphene, borneol acetate, isoborneol, and carvone besides the sesquiterpenes elemol and α-cedrene evaluated in guinea pigs indicated that α-terpineol/l-α-terpineol and trans-carveol were antiasthmatic and that α-terpineol was more effective than the essential oil (Sun, 1981; Anonymous, 1982). Antiasthmatic potential of the essential oil of *Ocimum basilicum* Benth and its monoterpene constituents oil such as estragole, 1,8-cineole, ocimene, linalool acetate, 1-epibicyclo sesquiphellandrene, menthol, menthone, cyclohexanol, cyclohexanone, myrcenol and nerol were also tested and myrcenol and nerol exhibited antiasthmatic activity (Huang et al., 1981).

In addition, there are several isolated bioactive terpenoidal chemical compounds which are reported to act in various stages of respiratory and asthmatic pathological conditions and also able to prevent chronic progression of this disease. Some of the compounds with potent biological effect are described below

1, 8-Cineol:

1, 8-cineole (eucalyptol) 200 and 400 mg/kg rectally, investigated by using the trinitrobenzenesulfonic acid induced colitis model (Male Wistar rats) for human anti-inflammatory bowel disease (Chen et al., 1999). Trinitrobenzenesulfonic acid is inducer of an extensive inflammation and ulceration in the colon which is associated with increased myeloperoxidase activity an indicator of neutrophilic infiltration (Kettle et al., 1997). Only pre-treated and not the post-treated animals with 1, 8-cineole (Figure 2.8) showed a reduction in the gross damage scores and wet weights of the inflamed colonic tissue, which are the parameters taken as reliable and sensitive indicator of the severity and extent of the inflammation (Rachmilewitz, et al., 1989).

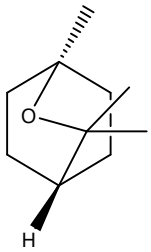


Figure 2.8: Structure of 1, 8-cineole

Additionally, 1, 8-cineole also significantly reduced myeloperoxidase activity, thus indicating anti-inflammatory action against gastrointestinal inflammation and ulceration (Santos, et al., 2004). The anti-inflammatory potential of 1,8-cineole (100, 200 and 400 mg/kg, p.o.) using carrageenan-induced paw edema in rat models showed significant reduction in paw edema 46% . In cotton pellet-induced granuloma rat model 1,8-cineole (100, 200 and 400 mg/kg, p.o. for 7 days) reported maximum anti-inflammatory action exhibiting reduction in both the wet and dry weights of granulation by 37% and 40% (Santos and Rao, 2000). In bronchial asthma patients (double-blind placebo-controlled trial) anti-inflammatory action of 1,8-cineole evidenced by a mucolytic and steroid-saving

effect of 1,8-cineole was reported (Juergens, et al., 2003). *In vitro* Inhibitory effect of 1,8-cineole on lipopolysaccharide (LPS)-and interleukin (IL)-1β (IL-1β)-stimulated mediator production by human monocytes, showed a decreased production of tumor necrosis factor α (TNF-α), IL-1β, leukotriene B4 (LTB4) and tromboxane B2 indicating use of 1,8-cineole for long term treatment of airway inflammation in bronchial asthma (Juergens, et al., 1998). The evaluation of 1,8-cineole effect on metabolism of arachidonic acid in blood monocytes of asthmatic patients showed that the production of LTB4 and prostaglandin E2 (PGE2) was significantly inhibited in patients (−40. 3% and −31. 3%, respectively) and health subjects (−57. 9% and −41. 7%, respectively). The *in vitro* effect of 1,8-cineole (1.5 µg/mL) on monocyte mediator production compared to the effects of inhaled budesonide (108 M), showed both have inhibitory effects similar as evidenced by the reduced production *in vitro* of LTB4 (−27.9%, −23%), PGE2 (−75.5%, −44%), and IL-1β (−84.2%, −52%) (Juergens, et al., 1998). The anti-inflammatory potential of 1,8-cineole in inhibition of polyclonal stimulated cytokine production by human unselected lymphocytes and LPS stimulated monocytes. 1,8-cineole at concentration of 1.5 µg/ml caused significant inhibition in cytokine production in lymphocytes of TNF-α (92%), IL-1β (84%), IL-4 (70%) and IL-5 (65%), and monocytes of TNF-α (99%), IL-1β (84%), IL-6 (76%) and IL-8 (65%), and at 0.15 µg/ml, it significantly suppressed the production of TNF-α and IL-1β by monocytes (77% and 61%, respectively) and of IL-1β and TNF-α by lymphocytes (36% and 16%, respectively). The results indicated 1,8-cineole acts as a strong inhibitor of TNF-α and IL-1β and strengths, its use as an agent to control airway mucus hypersecretion due to inhibition of cytokine suggest its long-term use to reduce the severity of asthma, sinusitis and chronic obstructive pulmonary disease (Juergens et al., 2004). In ovalbumin (OVA)-sensitized animals efficacy of the acute treatment in the guinea pig with or without pre-treatment with a single dose of inhaled 1,8-cineole assessed. The airway inflammatory parameters i.e. reduced levels of the pro-inflammatory cytokines TNF-α and IL-1β in bronchoalveolar lavage fluid (BALF), impairment of the OVA-induced increase of MPO activity in BALF, and preventive action in the reduction of the mucociliary clearance induced by the antigen presentation. The acute treatment using 1,8-cineole cause impairment in the development of airway hyperresponsiveness caused by carbachol (a cholinomimetic drug that binds and activates the acetylcholine receptor) in isolated tracheal

rings preparations (Bastos et al., 2011). 1,8-cineole in guinea pigs and Wistar rats reported broncodilatory activity compared to the effects of phenoterol, an adrenergic beta-2 agonist. 1,8-Cineole (1–30 mg/kg) and phenoterol exhibited similar efficacy in decreasing, in vivo, rat bronchial resistance (66.7 ± 3.2% vs. 72.1 ± 5.3%, respectively), showing a relaxant activity on airway smooth muscle by a nonspecific mechanism (Nascimento, et al., 2009). In double-blind, placebo-controlled multi-center-study (242 patients) with stable chronic obstructive pulmonary disease treatment with 200 mg of cineole or placebo three times for six months showed that 1,8-cineole reduced exacerbations as well as dyspnea and improved lung function and health status. Thus acting as a controller for airway inflammation in chronic obstructive pulmonary disease by interference in the pathophysiology of airway mucus membrane inflammation (Heinrich et al., 2009). 2,3,7,8-tetrachlorodibenzo- p-dioxin (TCDD environmental pollutant) has the toxic effects on the percentage of T-cell subsets and B-lymphocyte. The effectiveness of 1,8-cineole (100 mg/kg/day p.o.) and β-myrcene (100 and200 mg/kg/day p.o) were examined for 30 and 60 days. The blood samples analysis showed that TCDD significant percentage reduction of the of lymphocyte subsets of CD3+, CD4+, CD161+, CD45RA, CD4+CD25+ and total lymphocyte, but significantly increased the percentage of CD8+ cells where as in contrast 1,8-cineole and myrcene significantly decreased CD8+ cells levels but increased CD3+, CD4+, CD161+, CD45RA, CD4+CD25+ and total lymphocytes, indicating that these monoterpenes displayed immunomodulation and eliminated TCDD induced immune suppression (Ciftci et al., 2011). The oral administration of 1,8-cineole and intraperitoneal injection of peppermint oil, l-menthol, menthone and 1,8-cineole inhibited passive cutaneous anaphylaxis (PCA) of guinea pigs (Arakawa and Osawa, 2000). The comparison of the clinical efficacy of flavored and non-flavored chewing gums in allergic rhinitis (pollenosis) showed that the peppermint gums enriched with l-menthol, 1,8-cineole, as well as geraniol or citronellol, more effectively attenuated rhinitis symptoms (Arakawa and Osawa, 2000; Arakawa et al., 1992).

Borneol, Bornyl Acetate, and Terpineol

Borneol was found to exert inhibitory effects on the release of histamine from abdominal mast cells by 40.4% showing its mast cell membrane stabilizer that can be used as a type I

allergy inhibitor (Watanabe et al., 1994). Anti-inflammatory and analgesic effects evaluated in mouse hot-plate test, acetic-acid-induced twisting test, abdominal cavity capillary permeability increase test induced by acetic acid, and the rat pedal swelling test induced by carrageenan showed borneol significantly decreased the foot swelling of rats, increased pain threshold and inhibited twisting response of mice which indicated that the analgesic action of borneol was stronger than its anti-inflammatory action (Sun et al., 2007).

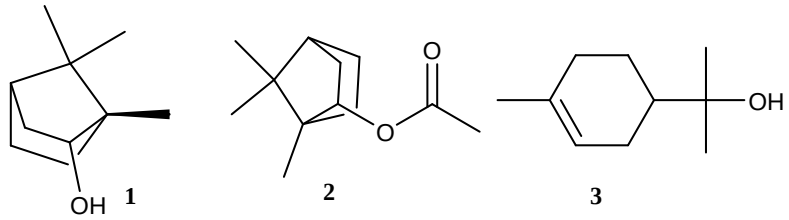


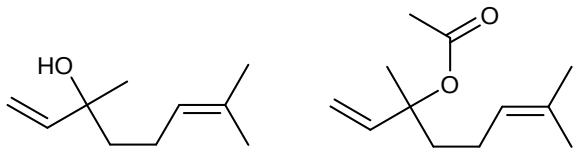
Figure 2.9: Structures of terpenoids 1. Borneol 2. Bornyl Acetate and 3. Terpineol

In-vitro ischemic model involving oxygen-glucose deprivation followed by reperfusion (OGD/R) showed borneol reversed OGD/R-induced neuronal injury, nuclear condensation, generation of intracellular reactive oxygen species (ROS), and dissipation of mitochondrial membrane potential. It reduced the nitric oxide (NO), the increase of iNOS enzymatic activity, the upregulation of iNOS expression, and inhibition of caspase-related apoptotic signaling pathway. Borneol blocked nuclear translocation of NF-κB p65 induced by OGD/R and inhibited the degradation of proinflammatory factor release and nuclear factor of kappa like polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα—a cellular proteins that function to inhibit the NF-κB transcription factor) indicating at borneol shows protection against cerebral ischemia/reperfusion injury through multifunctional protective pathways in cells, due to inhibition of IκBα-NF-κB and translocation signaling pathway (Liu et al., 2011). Borneol in inflammation of focal cerebral ischemia-reperfusion rats were also evaluated, followed by the detection of changes of neutrophils by immunohistochemistry stain of inter-cellular adhesion molecule-1 (ICAM-1), TNF-α and IL-1β showed positive results reporting fewer ICAM-1 positive vessels, IL-1β positive cells, TNF-α positive cells, and number of neutrophils, suggesting its anti-inflammatory property (He et al., 2006). Borneol has anti-fibrosis effect reported in anti-fibrosis

experimental model owing inhibitory action on fibroblasts mitosis, collagen and tissue inhibitors of metalloproteinase 1 (TIMP-1) production which suggesting use of borneol to treat oral submucous fibrosis (Dai et al., 2009). Isolated Borneol and terpineol (from Wu Hu Tang, a reputed Chinese formulation consisting of seven crude drugs used for the treatment of asthma for hundreds of years) evaluated for *in vitro* bronchoconstriction of guinea pig on isolated tracheal smooth muscle showed that both compounds prevented histamine-induced in vitro bronchoconstriction thus indicating antiasthmatic effect that could be the base of the antiasthmatic action of Wu Hu decoction (Chi et al., 2009). L-borneol and L-bornyl acetate main constituents of *Cinnamomum osmophloeum* Kaneh., the essential oil effect on NO and PGE2 production in LPS-activated RAW 264.7 macrophages evidenced that these exhibited excellent anti-inflammatory effects (Tung et al., 2008). Bornyl acetate, the major constituent of *Amomum villosum* volatile oil was evaluated for analgesic and anti-inflammatory potential showed to decrease writhing reaction induced by acetic acid and reducing the pain caused by the hot plate, and suppress ear swelling caused by dimethylbenzene, suggesting its anti-inflammatory action (Wu et al., 2004).

Linalool and Linalyl Acetate

(−)-Linalool effects on chronic inflammatory hypersensitivity was evaluated by inducing chronic inflammatory hypersensitivity on intraplantar injection of complete Freund's adjuvant (CFA) on adult female Swiss mice. Single intraperitoneal (i.p.) injection of (−)-linalool (50 or 200 mg/kg) or multiple treatments (twice daily for 10 days; 50 mg/kg, i.p.),



1 **2**

Figure 2.10: Structures of Terpenoids 1. Linalool and 2. Linalyl Acetate

both treatment protocols showed that (−)-linalool significantly reduced CFA-induced mechanical hypersensitivity and produced an effective reduction in CFA-induced paw

edema in acute treatment (Batista et al., 2010). (–) -Linalool, (±) -linalool racemic mixture and linalyl acetate (a monoterpene ester) by abdominal subcutaneous injection evaluated for the anti-inflammatory properties using the carrageenan-induced edema in male Wistar rats showed that 25 mg/kg, (–) -Linalool produced a delayed and more prolonged effect, while (±) -linalool significantly caused reduction of the edema only one hour after carrageenan administration. Higher doses (50 and 75 mg/kg) of (–)-linalool produced the maximum inhibitory effect against edema one hour after carrageenan injection (58% and 60%, respectively), whereas (±)-linalool (50 and 75 mg/kg) did not exert any anti-inflammatory activity one hour after carrageenan induction, but induced a significant effect after three (51% and 38%, respectively) and five hours (45% and 34%, respectively). In contrast, linalyl acetate was less effective and more delayed than (–)-linalool and (±)-linalool (Peana et al., 2002).

α- Terpinene, α-Terpineol, Terpinen-4-ol, α- β-Myrcene and Limonene

In experimental protocols α-terpinene, α-terpineol, α-carveol, menthone and pulegone. α-terpineol, showed selective inhibition of ovine COX-2 activity, for instance, showing higher COX-2 activity inhibition than aspirin (Kawata et al., 2008). Topical anti-inflammatory effect evaluated for *Zingiber cassumunar* essential oil and its monoterpenes showed that terpinene-4-ol and α-terpinene, but not γ-terpinene, effectively inhibited edema formation , the latter being the most active constituent and twice potent as the standard drug diclofenac (Pongprayoon et al 1997). The anti-inflammatory effect of *Melaleuca alternifolia* volatile oil and its three major constituents, i.e., terpinen-4-ol (42%), α-terpineol (3%) and 1,8-cineole (2%), was assessed for ability to reduce the production *in vitro* of TNF-α, IL-1β, IL-8, IL-10 and PGE2 by LPS-activated human peripheral blood monocytes. The oil was found to be toxic for monocytes at a concentration 0.016% v/v, whereas terpinen-4-ol significantly suppressed LPS-induced production of TNFα, IL-1β and IL-10 by 50% and PGE2 by 30%, after 40 h. The testing of the individual monoterpenes showed that only terpinen-4-ol suppressed the production of TNFα, IL-1β, IL-8, IL-10 and PGE2 by LPS activated monocytes (Hart et al., 2000). Limonene the inhibitory effects of on cytokines by measuring levels of TNF-α, IL-1β, and IL-6 in the cell supernatants of LPS stimulated macrophages by enzyme-linked immunosorbent assay

showed that limonene decreased their expression in a dose-dependent manner. Limonene effectively inhibited LPS-induced NO and PGE2 production that including dose-dependent reduction in the iNOS and COX-2 proteins expression.

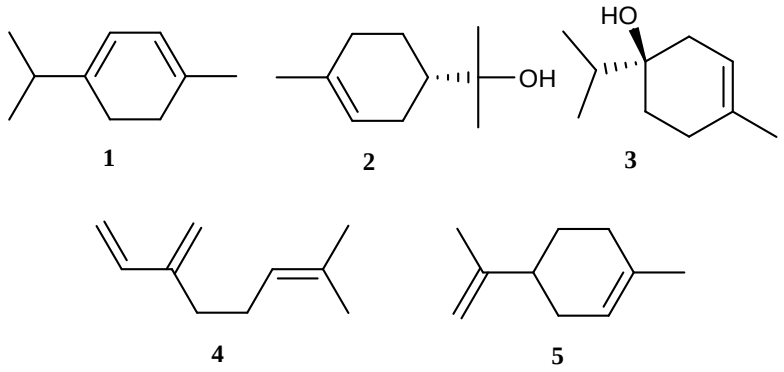


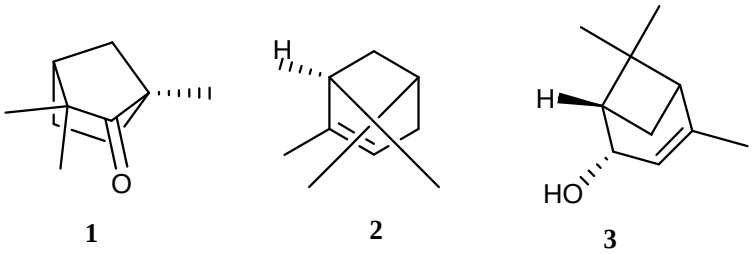
Figure 2.11: Structures of Terpenoids 1. α- Terpinene, 2. α-Terpineol, 3. Terpinen-4-ol, 4. Myrcene and 5. Limonene

In vitro anti-inflammatory evaluation of limonene on human eosinophilic leukemia HL-60 clone 15 cells by measuring the level of ROS, monocyte chemoattractant protein-1 (MCP-1), NF-κB, and p38 mitogen activated protein kinase (MAPK) was evaluated indicating that limonene (7.34 mmol/L) suppressed the synthesis of ROS for eotaxin-stimulated HL-60 clone 15 cells, also limonene (14.68 mmol/L) suppressed cell chemotaxis in a p38 MAPK and decreased MCP-1 production via NF-κB activation which can be comparable to the addition of the proteasomal inhibitor MG132. Thus limonene could be used as a potential anti-inflammatory agent for the treatment of bronchial asthma by suppressing cytokines, ROS production, and inactivating eosinophil migration (Hirota et al., 2010). Evaluation of pulmonary function in sensitized rats that inhaled either limonene or 1,8-cineole was carried out that showed limonene inhalation ant not 1,8-cineole, significantly prevented bronchial obstruction which suggests that unsaturated monoterpenes limonene could saturate the pulmonary membranes providing the airways with local chemical protection against ROS produce by either exogenous or endogenous ozone. The anti-inflammatory potential of limonene was also observed in pathological parameters, which exhibited reduced peribronchiolar and perivascular inflammatory infiltrates (Keinan et al., 2005). The effects of limonene on cell immune response in BALB/c mice sensitized and

challenged with 2,4-dinitrofluorobenzene (DNFB) macrophage in lymphoma-bearing mice. *In vitro* NO production and lymphocyte proliferation carried out with D-limonene, perillic acid, and perillyl alcohol. Limonene increased the survival for lymphoma bearing mice, delayed hypersensitivity reaction to DNFB, phagocytosis and microbicidal activity, and increased production of NO in peritoneal macrophages obtained from mice bearing tumor, this concluded the immunomodulatory effect of limonene with significant potential for clinical application (Del Toro-Arreola et al., 2005). The essential oils from *Conyza bonariensis* and *Porophyllum ruderale* (Asteraceae) were examined for anti-inflammatory activity in the mouse model of pleurisy which was induced by zymosan (500 µg/cavity) and LPS (250 µg/cavity). Limonene from *C. bonariensis* and β-myrcene from *P. ruderale* were also tested in the LPS-induced pleurisy model and assayed for immunoregulatory activity by measurement of NO inhibition and production of IFN-γ and IL-4. The oral administration of both oils suppressed the LPS-induced inflammation and cell migration in a similar way as that displayed by limonene. β-Myrcene and limonene both suppressed NO production effectively as well as the production of IFN-γ and IL-4 (Souza et al., 2003). The immunomodulatory effect and anti-inflammatory effects of orange juice (major constituents limonene, linalool and α-terpineol,) *in vitro* and *ex vivo* in epithelial buccal cells, reported in the suppression of IL-6 production and, an increase in IL-10 production. Further *ex vivo* experiments, on whole blood showed anti-inflammatory action in human macrophages after incubation with α-terpineol (Held et al., 2007). The intracellular formation of anti-inflammatory cytokine was evaluated for orange juice reduced formation of these cytokines orange juice, while linalool and limonene had no significant action, and α-terpineol exhibited a stimulating activity, showing for the first time the anti-inflammatory effect displayed by α-terpineol on cytokine production in buccal cells (Held and Somoza, 2008). The immunomodulatory evaluation of limonene, carvone and perillic acid in Balb/c mice showed an increase in the total white blood cell count, the total antibody production, and cells producing the antibody in spleen and bone marrow showing their potentiating effect on the immune system (Raphael and Kuttan, 2003).

Fenchone, α-Pinene and (S)-cis-Verbenol

The anti-inflammatory evaluation of fenchone (0.05, 0.10 and 0.20 ml/kg) exerted anti-inflammatory action in rats by reducing inflammation by 45.87%, 53.15% and 70.60%, respectively, as opposed to 95.70% reduction with the positive control drug indomethacin in the carrageenan- induced right hind-paw edema model (Ozbek, 2007). The anti-inflammatory of *Ugni myricoides* (Kunth) O. Berg essential oil and its major constituent, α-pinene (52.1%) evaluated for hypernociception using inflammatory and neuropathic models in mice and compared with those of indomethacin or gabapentin. The oil (5–50 mg/kg, p.o.) significantly prevented mechanical hypernociception induced by carrageenan or complete Freund's adjuvant (CFA) compared to indomethacin (5 or 10 mg/kg, p.o.), in mice. The treatment with the oil (5–25 mg/kg, p.o.), α-pinene (5–50 mg/kg, p.o.), or gabapentin (70 mg/kg, p.o.) abolished mechanical sensitization induced by CFA, indicating



1 **2** **3**

Figure 2.12: Structures of Terpenoids 1. Fenchone, 2.α-Pinene and 3. (S)-cis-Verbenol

that the effects displayed by *U. myricoides* essential oil are related to the presence of α-pinene, which shows its potential role in managing inflammation and neuropathic pain (Quintão et al., 2010). (S)-cis-Verbenol shown to have anti-ischemic activity, reducing cerebral ischemic injury caused by 1.5 hour occlusion of middle cerebral artery followed by 24-hour reperfusion. Also significant prevention against neuronal cell death caused by oxygen-glucose deprivation (OGD, 1 h) and subsequent re-oxygenation (5 h and diminished the intracellular level of ROS elevated by OGD/re-oxygenation), and decreased the of pro-inflammatory cytokines expression levels in ischemic brain and immunostimulated glial cells, suggesting that (S)-cis-verbenol is a useful therapeutic agent owing anti-oxidative and anti-inflammatory activities (Choi et al., 2010). Due to their properties of lipid organization disruption, (Cal et al., (2006)) studied the absorption

kinetics of four cyclic terpenes; alpha-pinene, beta- pinene, eucalyptol, and terpinen-4-ol. Each terpene varied in accumulation and elimination time with terpinen-4-ol showing the fastest penetration. To define the exact mechanism of phytoncide in asthma, further studies are required. In future, it is expected that volatile compounds like phytoncide are applicable to the patients with asthma as an additional therapeutic approach.

2.4.3 Vitamin C

Epithelial lining covering respiratory system, due to its large surface area, its role in gas exchange and host defense, is vulnerable to oxidant damage. Reactive oxygen species, produced from eosinophills, alveolar macrophages, and neutrophills play a major role in asthma by directly contracting airway smooth muscles; stimulating histamine release from mast cells and mucus hypersecretion. Oxidative-antioxidative imbalance should be maintained in the asthmatic response to antigen which triggers the inflammatory response during and after an asthma attack. Vitamin C investigated most extensively among antioxidants to a reduced risk of asthma. Lower plasma and leukocyte concentration of vitamin C is found in adults and in children with higher susceptibility to asthma, increased respiratory symptoms, reduced pulmonary functions and increased airway responsiveness. Vitamin C supplementation has been shown to decrease asthma severity and frequency, bronchospasm exercise induced asthma and airway responsiveness to methacholine. A 100 mg increase in daily vitamin C intake results in an approximately 10-50 ml increase in forced expiratory volume in one second (FEV1).

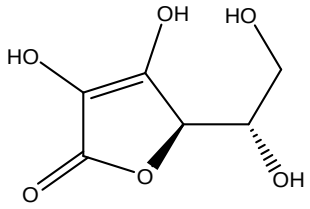


Figure 2.13: Structure of Vitamin C

Vitamin C being free radical scavenger in intracellular and extracellular lung fluids protects against both endogenous as well as exogenous oxidants. It is the most abundant antioxidant

present in the extracellular fluid in the lung and contributes to the regeneration of membrane bound oxidized vitamin E to function again. Additionally vitamin C is known for having general antihistamine effect and also inhibits the prostaglandin production. Mostly the exacerbations associated with childhood asthma occur due to upper respiratory viral infections, especially rhinovirus. Vitamin C supplementation may affect the susceptibility to common cold in subjects with low intake of vitamin-C, also reducing the duration of episodes and the severity of symptoms of the common cold (Gupta and Verma 2007). Ascorbic acid was evaluated in eight asthmatic subjects in a randomized, placebo controlled double-blind crossover trial where subjects were on their usual diet and were placed on either 2 weeks of ascorbic acid supplementation (1500 mg/day) or placebo, followed by a 1-week washout period, before crossing over to the alternative diet. Assessments were based on pre- and post-exercise pulmonary function, asthma symptom scores, fraction of exhaled nitric oxide (FENO), and urinary leukotriene (LT) C4–E4 and 9a, 11b-prostaglandin (PG) F2 at the beginning of the trial (usual diet) and at the end of each treatment period. The ascorbic acid diet caused significant reduction in the maximum fall in postexercise FEV1 compared to usual and placebo diet. Asthma symptom scores also improved significantly on the ascorbic acid diet compared to the placebo and usual diet. Post-exercise FENO, LTC4–E4 and 9a, 11b-PGF2 concentration was significantly lesser in the ascorbic acid diet compared to the placebo and usual diet (Tecklenburg et al., 2007).

2.4.2 Fatty acids

C-8 to C-24 containing saturated and unsaturated fatty acids were assessed for cyclooxygenase (COX-I and COX-II) inhibitory effect and antioxidant activities. The saturated fatty acids tested (60 μg mL^{-1}) caused an increase in antioxidant activity with increasing chain length from octanoic acid to myristic acid (C-8−C-14) and a decrease thereafter. Decanoic acid to lauric acid (C-3−C-5) at 100 μg mL-1 showed highest inhibitory activities among the saturated fatty acids tested on cyclooxygenase enzymes COX-I and COX-II. Among the unsaturated fatty acids tested, the highest activities were observed for cis-8, 11, 14-eicosatrienoic acid (C-25) and cis-13, 16-docosadienoic acid (C-27) at 100 μg mL-1 (Henry et., al 2002).

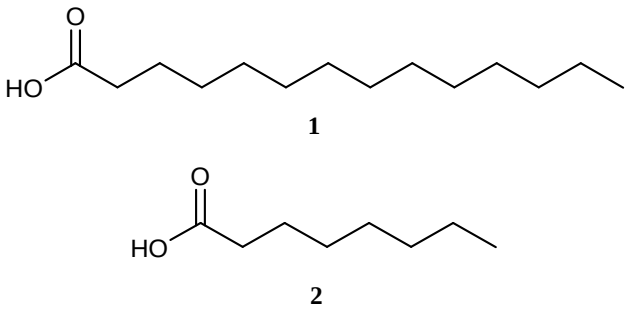


Figure 2.14: Structures of fatty acids 1. Myristic acid and 2. Octanoic acid

Most of the polyherbal formulations or ayurvedic or herbal products available in market incorporate various bioactive classes of compounds having high or moderate polarity like alkaloids, glycosides, flavonoids, phenols, tannins, triterpenoids and saponins, and some of which are admixed as active pharmaceutical ingredient to treat various respiratory disorders. The non-polar and volatile active constituents, mainly coumarins, terpenoids, vitamins and fatty acids in herbal preparations, of anti-asthmatic plants like *Ocimum basilicum*, *Melaleuca leucadendron*, *Eucalyptus globulus*, *Lavendula officinalis*, *Mentha piperita*, *Rosemarinus officinalis*, *Salvia sclarea*, *Pistacia integerrima*, *Hedychium spicatum* and *Cupressus sempervirens*, are not in existence perhaps these are reported as a promising candidate to treat and manage asthma.

Rationale for the research

- Current therapeutic regimens used in pharmacotherapy of asthma is not up to the mark for complete prevention of progression of various stages of complex pathophysiology of asthma and are associated with severe side effects and bear high cost.
- The authenticity, quality and purity of herbal drugs are the points of greatest concern which require attention for standardization of plant drugs with the reference given in pharmacopoeia.
- The rhizomes of *Hedychium spicatum var. acuminatum* and leaf galls of *Pistacia integerrima* J. L. Stewart ex Brandis in specific were selected as the plant parts are official in the Ayurvedic Pharmacopoeia of India (API, 1999a). More over Ayurveda and ethnobotanical literature further supports this selection.
- Both *H. spicatum* and *P. integerrima* are incorporated into several formulations used for oxidative, inflammatory, allergic disorders like asthma. Despite these have been suggested to be used for management of asthma in Ayurvedic and ethnobotanical literature still their pharmacologic/ therapeutic basis have not yet been clearly documented.
- Non polar herbal principles prove to provide symptomatic relief and assist in the inhibition of asthma. Phytomolecules like provide relief by arresting different pathological mechanism involved in progression of severe stages of asthma. Non polar herbal principles regained popularity for asthma, with better efficacy, safety and acceptability aspects supported by controlled clinical trials.
- Both the selected plant parts naturally possess volatile principles as part of their secondary metabolism. These nonpolar constituents of *H. spicatum* and *P. integerrima* as a whole has not been scientifically established and explored irrespective of their wide therapeutic uses. Moreover, the probable mechanisms of action needed to be unexplored though some pharmacologic reports supports their use.