CHAPTER 1

Introduction
1. INTRODUCTION

1.1 Introduction to bronchial asthma

1.1.1 Definition:

Asthma is a complex syndrome with many clinical phenotypes in both children and adults. Its major characteristics include a variable degree of airflow obstruction, bronchial hyperresponsiveness, and airway inflammation. For many patients, the disease has its roots in infancy, and both genetic factors (atopy)\(^1\) and environmental factors (viruses, allergens and occupational exposure)\(^2-4\) contribute to its inception and evolution.

According to the definition utilized by many physicians, asthma is not a disease but rather a syndrome, which unlike a disease cannot be attributed to one specific cause, but rather to several causes. The other way of defining asthma is where there is a recurrent ‘reversible’ obstruction of the airways in response to stimuli which are not in themselves noxious and which do not affect non-asthmatic subjects. According to guidelines of The National Asthma Education and Prevention Program (NAEPP) of heart, lung and blood institute, the current definition of asthma is, “a chronic inflammatory disorder of airways in which many cells and cellular elements play a role, in particular, mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells”. According to the Global Initiative for Asthma (GINA), the definition of asthma is “a chronic inflammatory disorder inflammation causes an associated increase in airway hyper responsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night or in the early morning.” These episodes are usually associated with widespread but variable airflow obstruction that is often reversible either spontaneously or with treatment. Thus it is a complex disease characterized by Bronchial hyper responsiveness (BHR), Airway remodeling and Excessive Mucus production. (Figure 1.1)
Figure 1.1: A. shows the location of the lungs and airways in the body. B. shows a cross-section of a normal airway. C. shows a cross-section of an airway during asthma symptoms.

It is an episodic disease manifested clinically by paroxysms of dyspnoea, allergic rhinitis and wheezing without cold. The net result is an increase in airway resistance, a decrease in forced expiratory volumes and flow rates, hyperinflation of the lungs and thorax, increased work of breathing, alterations in respiratory muscle function, changes in elastic recoil, abnormal distribution of both ventilation and pulmonary blood flow with mismatched ratios, and altered arterial blood gas concentrations. Thus although asthma is considered to be primarily a disease of airways, virtually all aspects of pulmonary function are compromised during an acute attack. [5]

1.1.2 Incidence and Prevalence of bronchial asthma

Respiratory diseases are second to cancer as the causes of death and disability to adults. Acute respiratory infection, tuberculosis and chronic obstructive pulmonary disease rank third, fourth and fifth respectively as per the global health situation. [6]

According to a survey in UK in the year 2008, respiratory diseases occupied 15% among all other diseases. Asthma is the commonest disease in children in
economically developed countries and it is also common in adults and it is increasing in prevalence and severity. Around 275 million people around the globe suffer from asthma and this number is rising worldwide, deaths from this condition have reached 18 million annually. The number of deaths from asthma also has risen in the United States. The WHO says about five thousand Americans die from asthma attacks each year. In the early 1980s, the yearly death rate from asthma in the United States was about half of that.

Asthma is a common and costly health condition. More than the 30 million people in the united state have asthma. More women than men suffer from asthma and have a much higher death rate. Every year, asthma is responsible for about 5,00,000 hospitalization and 5,000 death only in USA. [7] Around 8 % people of the Swiss population suffer from asthma. In Western Europe as a whole, asthma has doubled in ten years. There are about 3 million asthmatics in Japan of whom 70 % have severe and 30 % have moderate asthma. Around 30 million asthmatic in USA alone and number of asthmatics has leapt by over 60 % since the early 1980s. In Germany, there are an estimated 4 million asthmatics. In Australia, one child in six under the age of 16 is affected.

Figure 1.2: The prevalence of asthma in the world. [8]
Asthma is not just a public health problem of the developed countries but also is the health problem of developing countries too; the incidence of this disease has become alarming. In the Western Pacific Region of WHO, the incidence varies from over 50% among children in the Caroline Islands to virtually zero in Papua New Guinea. In Brazil, Costa Rica, Panama, Peru and Uruguay, prevalence of asthma symptoms in children varies from 20% to 30%. In Brazil, Peru and Panama, prevalence of asthma symptoms in children varies from 20-30%. In Kenya, it approaches 20%. Asthma accounts for 1-3% of all office visits, 5,00,000 hospital admission than any other single illness. In India rough estimates indicate a prevalence of between 10% and 15% in 5-11 yrs old children.

An alarming fact is that since 1980, asthma in children under age 5 has risen remarkably (2,283 per 100,000 among women and 2,640 among men per 100,000). In school age children, asthma has risen by 75%. Asthma is a common chronic disease of childhood, affecting an estimated 4.4 million children in the United States; the prevalence of asthma is slightly higher in boys than in girls under age 18. However, the most rapid increase in cases of asthma are occurring in children under five years old, with rates increasing over 160 percent between 1980 and 1994. (Figure 1.3)

![Figure 1.3 Estimated average annual rate of self-reported asthma during 12 months by various age groups in United States from the year 1980 to 1994.](image-url)
Economical burden of asthma is very important in reality. In spite of the fact that on average, up to 10% of the family budget will go towards meeting the costs of treatment for asthma sufferers. In 1998, the cost of asthma care was estimated to be US$ 11.3 billion in United State of America; nearly a double increase from US$ 6.2 billion was estimated in 1990.\textsuperscript{[9]}

According to study the estimated of total per-person annual costs of asthma average $4,912 in USA, with direct and indirect costs accounting for $3,180 (65%) and $1,732 (35%), respectively. The largest components within direct costs were:

- Pharmaceuticals [$1,605 (50%)]
- Hospital admission [$463 (15%)]
- Non emergency department ambulatory visits [$342 (11%)]

The mortality due to asthma is not comparable in size to the day to day effect of the disease. Although largely avoidance, asthma tends to occur in epidemic and affect young people. The human and economic burden associated with this condition is severe. But no clear data regarding economic burden are not available in Indian society. The cost of asthma to the society could be reduced to large extent through concentrated international and national action.
• Worldwide, the economic costs associated with asthma are estimated to exceed those of T.B. and HIV/AIDS combined.
• In the United State, for example, annual care costs exceed $ 12.7 billion.
• At present Britain spends about US $ 1.8 billion of health care of asthma.

1.1.3 Aetiology of bronchial asthma
Asthma can be triggered by just about all of the same things that trigger allergies. It also can be triggered by cold air, exercise, and other factors. Although the aetiology of asthma has not yet been fully delineated, it is generally believed that this disease results from exposure to environmental factors in genetically susceptible individuals. The strongest risk factors for developing asthma are wide range of provoking stimuli, which are listed in Table 1.1.

**Table 1.1: List of agents involve in events of triggering asthma.**

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>List of agents</th>
<th>Events triggering Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Respiratory Infection</td>
<td>Respiratory synsytial virus (RSV), rhinovirus, influenza, para influenza, mycoplasma pneumonia</td>
</tr>
<tr>
<td>2</td>
<td>Allergens</td>
<td>Air bone pollens (grass, trees, weeds), house-dust mites, animal dander, cockroaches, fungal spores</td>
</tr>
<tr>
<td>3</td>
<td>Environment</td>
<td>Cold air, ozone, sulfur dioxide, nitrogen, tobacco smoke, wood smoke</td>
</tr>
<tr>
<td>4</td>
<td>Emotions</td>
<td>Anxiety, stress, laughter</td>
</tr>
<tr>
<td>5</td>
<td>Exercise</td>
<td>Particularly in cold, dry climate</td>
</tr>
<tr>
<td>6</td>
<td>Drugs/preservatives</td>
<td>Aspirin, NSAIDs, sulfites, benzalkonium chloride, β blockers</td>
</tr>
<tr>
<td>7</td>
<td>Occupational stimuli</td>
<td>Bakers (flour dust), farmers (hay mold), printers, chemical workers (azodyes, ethylenediamines, toluene, PVC), plastics, rubber workers (formaldehydes, dimethylethanolamine)</td>
</tr>
<tr>
<td>8</td>
<td>Other individual triggers</td>
<td>Wood or grain dust, cotton dust, grain, weevils, mites</td>
</tr>
</tbody>
</table>
A key step in controlling asthma is to identify which of these triggers make your asthma worse, and then work to eliminate or avoid them. Sometimes it takes exposure to more than one of these factors before an asthma episode is triggered. There are some things, like cold viruses, that you can't completely control, but you can at least avoid being around others who are sick.

1.1.4 Classification of Asthma
Asthma can be classified on the basis of symptom relates to pathological indices of airway inflammation. Therefore asthma is classified according to the severity grades "intermittent", "mild persistent", "moderate persistent" and "severe persistent" (Grade 1 - Grade 4). This classification involving a stepwise approach facilitates decisions concerning the disease management at the initial assessment as well as during maintenance treatment. Before the start of treatment patients are classified according to symptoms and lung function only, during maintenance treatment classification needs to combine current treatment and symptoms present under medication.

Table 1.2: Classification of asthma severity by clinical features as per National Asthma Education and Prevention Program (NAEPP)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Symptoms description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intermittent</td>
<td>Symptoms less than once a week</td>
</tr>
<tr>
<td></td>
<td>Brief exacerbations</td>
</tr>
<tr>
<td></td>
<td>Nocturnal symptoms not more than twice a month</td>
</tr>
<tr>
<td></td>
<td>• FEV1 or PEF $\geq 80%$ predicted</td>
</tr>
<tr>
<td></td>
<td>• PEF or FEV1 variability $&lt; 20%$</td>
</tr>
<tr>
<td>Mild persistent</td>
<td>Symptoms more than once a week but less than once a day</td>
</tr>
<tr>
<td></td>
<td>Exacerbations may affect activity and sleep</td>
</tr>
<tr>
<td></td>
<td>Nocturnal symptoms more than twice a month</td>
</tr>
<tr>
<td></td>
<td>• FEV1 or PEF $\geq 80%$ predicted</td>
</tr>
<tr>
<td></td>
<td>• PEF or FEV1 variability 20–30%</td>
</tr>
</tbody>
</table>
CHAPTER-1

Introduction

<table>
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<tr>
<th>Moderate persistent</th>
<th>Severe persistent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms daily</td>
<td>Symptoms daily</td>
</tr>
<tr>
<td>Exacerbations may affect activity and sleep</td>
<td>Frequent exacerbations</td>
</tr>
<tr>
<td>Nocturnal symptoms more than once a week</td>
<td>Frequent nocturnal asthma symptoms</td>
</tr>
<tr>
<td>Daily use of inhaled short-acting β 2-agonist</td>
<td>Limitation of physical activities</td>
</tr>
<tr>
<td>● FEV 1 or PEF 60–80% predicted</td>
<td>● FEV 1 or PEFs 60% ≤ predicted</td>
</tr>
<tr>
<td>● PEF or FEV 1 variability 30%</td>
<td>● PEF or FEV 1 variability &gt; 30%</td>
</tr>
</tbody>
</table>

There are two general categories for classifying Asthma depending upon the types of stimuli that trigger attacks: Extrinsic asthma and Intrinsic asthma.

Asthma may also be classified depending upon the types of stimuli that trigger attacks or airflow limitation i.e. Extrinsic asthma and Intrinsic asthma. Although both approaches have to be considered in diagnosis and disease control classification of severity grades is more practicable.

Table 1.3: Classification of asthma based upon the stimuli initiating.

<table>
<thead>
<tr>
<th>Extrinsic asthma (allergic / atopic)</th>
<th>Intrinsic asthma (idiosyncratic/ non atopic)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset usually in childhood or early adult life.</td>
<td>Onset usually (but not invariable) in older adults. Begins after the age of 30 and tends to perennial and more severe. A status asthmatic is more common in this group.</td>
</tr>
<tr>
<td>Family history of multiple allergies (asthma, hay fever, eczema) common</td>
<td>Family history of multiple allergies less common (20%).</td>
</tr>
</tbody>
</table>
(50%) well defined allergic history to a variety of inhaled allergens (atopy).

<table>
<thead>
<tr>
<th>Known external allergens.</th>
<th>No known external allergens.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgE raised in 50-60% of subjects.</td>
<td>IgE normal or low.</td>
</tr>
<tr>
<td>Other allergies (hay fever and eczema) often present (54%).</td>
<td>Other allergies uncommon</td>
</tr>
<tr>
<td>Positive immediate skin tests.</td>
<td>Negative skin tests.</td>
</tr>
<tr>
<td>Intermittent asthma.</td>
<td>More continuous asthma.</td>
</tr>
</tbody>
</table>

Asthma can also be classified based on cause. Some of these definitions commonly used overlap and therefore are of limited value concerning a precise definition. Therefore at times multiple types of asthma can be present simultaneously. The most important categories are:

- **Extrinsic or allergic or atopic asthma**: In extrinsic asthma an increased responsiveness of the airways is caused by exposure to environmental trigger factors. Onset is usually in childhood and almost always before the age of thirty.

- **Intrinsic or non-allergic or non-atopic or idiosyncratic asthma**: In intrinsic asthma airways become hyper-reactive as a result of an imbalance between the parasympathetic and sympathetic responses of the airways. It is attributed to pathophysiological disturbances and not to environmental factors.

- **Mixed-type asthma**: as its name suggests, this is a mixture of intrinsic and extrinsic asthma. Patients react to some allergens but their asthma is also triggered by other factors.

- **Exercise-induced asthma**: is due to a narrowing of the airways occurring in moderate to heavy exercise.

- **Nocturnal asthma**: describes asthma, which suddenly worsens in the middle of the night, typically between 2 and 4am. Affected patients frequently feel totally exhausted during the day and need catnaps to keep functioning at an adequate level.

- **Occupational asthma**: develops from a few months to years after starting a new job. Symptoms usually improve while on holiday. Some common causes
include: sawdust, chemical fumes, constant dusty conditions, close contact with animals, glue fumes, etc.

- **Seasonal asthma:** can be triggered by particular allergens, i.e. pollens from trees, grasses or flowers, or by a particular climate.

### 1.1.5 Symptoms of Asthma:

**Early symptoms include:**
- Wheezing, Tightness in the chest
- A cough that won’t go away (day and night)
- Shortness of breath
- Poor response to medicines (bronchodilators)

**Late, severe symptoms include:**
- Severe wheezing (both when breathing in and out)
- Coughing that won’t stop
- Very rapid breathing
- Inability to catch your breath
- Chest pain or pressure
- Tightened neck and chest muscles (retractions)
- Difficulty talking
- Inability to perform a peak expiratory flow
- Feelings of anxiety or panic
- Pale, sweaty face
- Blue lips or fingernails

### 1.1.6 Pathophysiology of asthma

The pathogenesis of asthma involves both genetic and environmental factors, and the asthmatic attack itself consists, in many subjects, of two main phases: an immediate and a late (or delayed) phase.

Numerous cells and mediators play a part in the pathogenesis of asthma, and the full details of the complex events involved are still a matter of debate. The following
simplified account is intended to provide a basis for understanding the rational use of drugs in the treatment of asthma.

Asthmatics have activated T cells, with a Th2 profile of cytokine production, in their bronchial mucosa. How these cells are activated is not fully understood, but allergens are one mechanism. (Figure 1.5)

![Diagram of T lymphocytes in allergic asthma](image)

**Figure 1.5:** The part played by T lymphocytes in allergic asthma

In genetically susceptible individuals, allergen (green circle) interacts with dendritic cells and CD4+ T cells, leading to the development of Th0 helper lymphocytes, which give rise to a clone of helper Th2 lymphocytes. These then (i) generate a cytokine environment that switches B cells/plasma cells to the production and release of IgE; (ii) generate cytokines, such as interleukin (IL)-5, which promote differentiation and activation of eosinophils; and (iii) cytokines (e.g. IL-4 and IL-13) signals the switch from IgM to IgE antibodies. The cross linkage of two IgE molecules by allergen causes mast cells to degranulate, releasing histamine, leukotrienes, and other mediators that perpetuate the airway inflammation. They also enhance adhesion of eosinophils to endothelium.

Some asthmatics, in addition to these mechanisms, are also atopic-i.e. They make allergen-specific IgE that binds to mast cells in the airways. Inhaled allergen cross-links IgE molecules on mast cells, triggering degranulation with release of histamine and leukotriene B4, both of which are powerful bronchoconstrictors to which
asthmatics are especially sensitive because of their airways hyper-responsiveness. This provides a mechanism for acute exacerbation of asthma in atopic individuals exposed to allergen. The effectiveness of omalizumab (an anti-IgE antibody) serves to emphasise the importance of IgE in the pathogenesis of asthma as well as in other allergic diseases. Noxious gases (e.g. sulfur dioxide, ozone) and airway dehydration can also cause mast cell degranulation. Atopic asthma is termed as extrinsic asthma frequently and non-atopic asthma as 'intrinsic' asthma; but the terms allergic and non-allergic are more preferable.

The asthmatic subjects have intermittent attacks of dyspnoea, wheezing, and cough. In many subjects the asthmatic attack consists of two main phases as can be demonstrated by tests of FEV.

- The immediate phase
- The late phase

**The immediate phase**

Inhaled allergen challenge in allergic patients leads to an early allergic inflammatory reaction. Exposure to allergen leads to sensitization and formation of antibodies through differentiation of b- lymphocytes. It is initiated after activation of cells bearing allergen-specific IgE.\(^{[10]}\) Respiratory system, called as separate immune organ of the body gets primed and ready to give allergic reaction after second exposure.\(^{[11]}\) Interaction of allergen with mast cells fixed antibodies IgE release pro-inflammatory mediators histamine with LTC4 & LTD4, PGE2, and NK-4. Various chemokines and chemotoxines attracts inflammatory mediators cells particularly eosinophils, causing inflammation.\(^{[12-14]}\)

**The late Phase**

It is progressive inflammatory reaction that occurs at variable time interval time interval, generally 6-9 hrs after allergen provocations and may be nocturnal. Initiation of this phase occurs during first phase, the influx of Th2 lymphocytes is particular importance. Inflammation here differs from other inflammation reaction as it involves leakage of cells. It also infiltrates cytokines releasing Th2 cells, whose product causes damage and loss of epithelium.\(^{[15]}\) The other putative mediators of inflammatory...
process, in late phase are adenosine\textsuperscript{16}, neuropeptides\textsuperscript{17} and bradykinin.\textsuperscript{18} Growth factors released from inflammatory cell, as it secretes mediators- Eosinophilic Cationic Protein (ECP), Eosinophil Derived Neurotoxin (EDNT), GM-CSF, TNF, PG and cytokines which results in epithelial shedding, bronchoconstriction and promotion of inflammation in respiratory tract.\textsuperscript{12, 19} However, in some subject, only one of the phases may be obvious.

The gross pathology of asthmatic airways displays lung hyperinflation, smooth muscle hypertrophy, lamina reticularis thickening, mucosal edema, epithelial cell sloughing, cilia cell disruption, and mucus gland hypersecretion. Microscopically, asthma is characterized by the presence of increased numbers of eosinophils, neutrophils, lymphocytes, and plasma cells in the bronchial tissues, bronchial secretions, and mucus. Initially, there is recruitment of leukocytes from the bloodstream to the airway by activated CD4 T-lymphocytes.

The activated T-lymphocytes also direct the release of inflammatory mediators from eosinophils, mast cells, and lymphocytes. In addition, the subclass 2 helper T-lymphocytes subset of activated T-lymphocytes produces interleukin IL-4, IL-5, and IL-13. IL-4 in conjunction with IL-13 signals the switch from IgM to IgE antibodies. The crosslinkage of two IgE molecules by allergen causes mast cells to degranulate, releasing histamine, leukotrienes, and other mediators that perpetuate the airway inflammation.

IL-5 activates the recruitment and activation of eosinophils. The activated mast cells and eosinophils also generate their cytokines that help to perpetuate the inflammation.

Regardless of the triggers of asthma, the repeated cycles of inflammation in the lungs with injury to the pulmonary tissues followed by repair may produce long-term structural changes ("remodeling") of the airways.\textsuperscript{20}
Asthma has been described primarily as an inflammatory process in the last one decade. The inflammatory mediators are now considered to be an immunological initiated, mediator’s driven event. The three main components of the immune system are antibodies, inflammatory cells, and inflammatory mediators. Antibodies are specific proteins created by the immune system to identify and bind to foreign and potentially invading substances. Inflammatory cells circulate in the bloodstream and can sense the body surrounding or exposures. Inflammatory mediators are chemical substance that are secreted by immune cells to induce (or respond to) a going immune response generated against a specific exposure to the body.

1.1.7.1 Antibodies

An antibodies or an immunoglobulin is a small protein molecule created by the immune system to have a close structural fit to the surface of a foreign substance. The foreign substance is an antigen. The body manufactures five classes of antibodies, namely IgM, IgG, IgA, IgD, IgE. 

![Figure 1.6: Pathophysiology of Bronchial Asthma](image-url)
Immunoglobulin:
The antibodies class of allergic diseases including allergic asthma is Ig E. It is fundamental to the allergic immune response. Although the usual antibody response to an antigen is to generate Ig M or Ig G antibodies (or both), it is unclear why some antigens in some patients leads to generation of a specific Ig E antibody response. An antigen that stimulates an Ig E antibody response is more specifically termed an allergen. These IgE antibodies are generally directed against substances that are not harmful to the body, including pollens; fur from cats; certain foods; certain drugs; and (most commonly) droppings from microscopic dust mites.\textsuperscript{[22]}

After initial exposure of the patient to an allergen, the primary immune response is to generate unique IgE antibodies that become bound to the surface of mast cells. If the patients are later re- exposed to an allergen by inhalation, the allergen binds to the surface- bound IgE on mast cells in the bronchi. Bindings of at least two Ig E molecules, bridged by a single allergen molecule, is termed cross- linking. Crosslinking of IgE by allergen on the mast cell is the initial biologic event of an allergic reaction shown in figure 1.7.\textsuperscript{[22]}

![Figure 1.7: Cross linking of mast cell and immediate bronchoconstriction](image)

An allergic reaction can be technically referred to as an immediate hypersensitivity reaction; this term derives from the two key aspects of an allergic reaction:
The reaction occurs very quickly after exposure to the substance that stimulates the reaction (allergen). The reaction may occur (and may be life-threatening) 5-10 minutes or less after exposure to the allergen and thus is termed immediate.

The person having the allergic reaction is more sensitive (i.e., shows hypersensitivity) to the offending substance than one who is not allergic. A person without allergies would be expected to have absolutely no discernible reaction to the very same substance that could be fatal to one who has exquisite allergic sensitivity to that substance.

1.1.7.2 Inflammatory cells

Inflammatory cells can be of 3 types viz resident cells, which include mast cells and macrophages recruited cells compromising of eosinophills, lymphocytes and monocytes and structural cells, which include epithelium airway smooth muscle, fibroblasts.

Resident cells of the airway.

Airway smooth muscle is not only a target of the asthma response (by undergoing contraction to produce airflow obstruction) but also contributes to it (via the production of its own family of pro-inflammatory mediators). As a consequence of airway inflammation and the generation of growth factors, the airway smooth muscle cell can undergo proliferation, activation, contraction, and hypertrophy - events that can influence airway dysfunction of asthma.

Mast cells:

Mast cells are pivotal in the allergic response type 1 or the anaphylactic type a rapidly progressing chain reaction that causes sudden attack of asthma. Mast cells are ubiquitous and are found around blood vessels in the connective tissue, in the lining of the gut and importantly in the lining of the upper and lower respiratory tract. These are large mononuclear cells heavily granulated, with granules containing a host of pharmacologically active substances. The allergen (antigen) enters into the human body through the respiratory tract, skin and/or Gastro Intestinal Tract (GIT). After the exposure to antigens, antibodies directed against specific antigens (i.e. IgE) are
formed and are fixed to their respective receptors on the surface of the mast cells. This process is called sensitization of mast cells. Cross-linking of surface IgE by an allergen molecule triggers a rapid activation (<15 minutes) of the mast cell, which then releases numerous inflammatory mediators into the tissue surrounding the cell.

The granules, which are released from the mast cells, get ruptured and cause immediate release of histamine and TNF-α, proteases. These cause bronchoconstriction and vasodilation. This results in the acute attack of asthma. The rupture of lipid membrane of the mast cells causes release of mediators like prostaglandins, leukotrienes and platelet activating factor. They also cause bronchoconstriction, vasodilation and increase in vasopermeability. The cytoplasm of the mast cells causes production of no. of cytokines like IL-1,-3,-4,-5,-6,-8 and causes stimulation of neutrophils, eosinophils, macrophages and adhesion of molecules.

**Macrophages:**
The primary function of alveolar macrophages in the normal airway is to serve as “scavengers”, engulfing and digesting bacteria and other foreign materials. They are found in large and small airways, ideally located for affecting the asthmatic response. Mediators like PAF, LTB4, LTC4, and LTD4 are produced by these cells and they produce inflammation.\(^{[21]}\)

**Eosinophils:**
The eosinophils are the inflammatory cell most closely associated with asthma.\(^{[22]}\) Unlike mast cells that are fixed in various tissues throughout the body, eosinophils are very mobile. In association with asthma, elevated numbers of eosinophils have been identified in various tissue compartments, including:

- Circulating in the peripheral blood. The increase in peripheral blood eosinophils in asthma is probably due to inflammatory cells or mediators coming from the lungs to cause increased production of eosinophils by the bone marrow.
- In biopsies of lung tissue, particularly in the bronchial wall of patients with asthma.
In fluid specimens obtained from the lung using a bronchoscope. With this method, fluid that “washes out” the bronchi and alveoli-termed bronchoalveolar lavage (BAL) fluid is obtained by inserting a fiberoptic scope down the air passages and into the lungs.

In secretions or sputum of patients with asthma. A sputum specimen is basically a coughed-up sample of the mucus that is coating the airway lining.

The mobility of eosinophils indicates that they can be stimulated to leaves the bloodstream and enter in the tissues. In asthma, eosinophils move from the blood into the bronchi (as documented in bronchial biopsies and in BAL fluid) and onto the surface of the airway lining (as documented in sputum). Eosinophils migrate from their origin in the bone marrow to the airways by cell rolling through interaction with selectin and eventually adhere to the endothelium through the binding of integrines to adhere proteins [vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1)]. Cell rolling activates eosinophils and requires the participation of the β₁ and β₂ classes of integrins on the eosinophils surface.

Eosinophils and lymphocytes express the β₁ integrin α₄ β₁ integrin (also referred to as very late antigen 4, or VLA4), which binds to its ligand, VCAM-1. Adhesion of the eosinophils to VCAM-1 decreases the threshold for activation of the cell by mediators. The interactions between the β₂ integrins on eosinophils and ICAM-1 on vascular tissue appear to be important for the transendothelial migration of eosinophils. [23]

Eosinophils release inflammatory mediators such as leukotrienes, particularly the cysteinyl leukotrience 4, which contracts airway smooth muscle, increase vascular permeability and may recruit more eosinophils to the airways. Challenge of the airway with allergen increase the local concentration of interleukin-5, which correlated directly with the degree of airway eosinophilia [24] shown in figure 1.8.
Lymphocytes:
Lymphocytes lack granules and manufacture other kinds of proteins that are involved in the inflammatory process. B-lymphocytes have the important function of manufacturing antibodies. T-lymphocytes play an essential role in events that lead to airway inflammatory process.

There are two types of T-helpers CD4+ cells. Type 1 T-helper (Th1) cells produce IL-2 and interferon Y, both essential for cellular defence mechanism. Th2 cells produce cytokines (IL-4, 5, 6, 9 and 13) that mediate allergic inflammation. CD8+ T cells may also be classified in a similar fashion according to their cytokine profiles. [25]

Neutrophils:
Neutrophils may play a pivotal role in the disease process, at least in the sudden onset fatal cases as high numbers of neutrophils have been reported to be present in the airways of patients who died from sudden onset fatal asthma. Neutrophils are source for a variety of mediator’s like PAF, PGs, TXs, LTs causing bronchial Hyperresponsiveness and airway inflammation. [26]
T cells:
T cell is often considered to play a crucial role in maintaining and regulating the allergic immune response in asthmatics in humans. Upon antigen presentation and activation, CD8$^+$ T cells proliferate and differentiate into cytotoxic T cells, and CD4$^+$ cells into either T helper type 1 (Th1) or Th2 effector cells.

NKT cells:
Study analyzing sputum from severe asthmatics found that NKT cells were increased as compared with normal controls. Most peripheral blood NKT cells express receptors for chemokines, which mediate homing to extra-lymphoid tissue or sites of inflammation, whereas few NKT cells express lymphoid tissue-homing chemokine receptors. Interestingly, NKT cells from asthmatics have more T-cell expression of IL-4 and IL-13, whereas NKT cells derived from healthy donors have more IFN-γ expression.

Basophils:
Basophils possess high levels of the FcεRI receptor and are capable of an immediate response to allergen. Basophils have been reported in the sputum of patients with symptomatic asthma. During the late response to allergen challenge, large numbers of basophils have appeared in BAL specimens after segmental allergen challenge (SAC) and have been noted in airway tissue after inhalation bronchoprovocation. Like mast cells, basophils release histamine on activation; unlike mast cells, however, they do not produce PGD2.

Epithelial cells:
Bronchial epithelial cells traditionally have been considered as a barrier, participating in mucociliary clearance and removal of noxious agents. IgE dependent mechanisms, viruses, pollutants, or histamines can activate epithelial cells. In fatal asthma, extensive epithelial shedding occurs.

Adhesion molecules:
Adhesion molecules help adhesion of the various cells to each other and the tissue matrix to facilitate infiltration and migration of these cells to the site of inflammation.
Adhesion molecules are found on a variety of cells, such as neutrophils, monocytes, lymphocytes, basophils, eosinophils, granulocytes, platelets, endothelial cells, and epithelial cells, and can be active by the many inflammatory mediators present in asthma. A major role of adhesion molecules is in the recruitment of leukocytes from the vascular lumen to tissues. Finally, firm adhesion of the leukocytes to endothelial cells surfaces allows diapedesis between endothelial cells and migration of leukocytes into the extracellular matrix.\[15\]

1.1.7.3 Inflammatory mediators
The immunologic cascade and the ensuing inflammatory reaction comprise activation of specific inflammatory cells that also release various inflammatory mediators, such as histamine, mast cell tryptase, leukotrienes, prostaglandins, eosinophils cationic protein, and cytokines. However, these represent a small subset of the several inflammatory mediators that have been identified and studied worldwide.

**Histamine:**
Histamine is stored in granules within mast cells and basophils and can be released under immunological condition following the cross linking of antigen to high affinity IgE receptors present on the surface of mast cells and basophils or by non immunological stimuli (e.g. compound 48/80, calcium ionophore, substance P and hypo-osmolar solutions).\[10\] Acute release of histamine following an allergic or non allergic insult may lead to bronchoconstriction, which can be attenuated by selective H1-receptor antagonists.\[27\] These are evidence that histamine may also stimulate sensitized afferent nerves.\[28\] With regard to inflammatory cells, histamine has been shown to activate eosinophils.\[29\]

**Prostanoids:**
Immediately following acute antigen challenge of asthmatic subjects, increased levels of prostaglandin (PG) F2, PGD2 and thromboxane (TX) B2 are detected in bronchoalveolar lavage fluid \[10\], which are derived from macrophages, airway epithelium and activation of mast cells. When inhaled, these prostanoids cause bronchoconstriction \[30\] and increase airway responsiveness to spasmogen unrelated to
alterations in airway calibre\textsuperscript{[31]} which suggests that prostanoids may play a greater role in modulating airway responsiveness.

Prostanoids are synthesized by cyclooxygenase (COX). Various pro-inflammatory cytokines stimulate the induction of COX2 in human airway epithelium\textsuperscript{[32]} and smooth muscle\textsuperscript{[33]} in culture suggesting that during inflammation, COX2 expression may be augmented.

Some of the studies have shown that inhaled PGE2 inhibits the development of the late asthmatic response unrelated to functional antagonism of airway smooth muscle contraction and attenuates the attendant increase in sputum eosinophilia.\textsuperscript{[34]} Which other studies have shown that PGE2 may have pro-inflammatory properties, which might play a role in the development of the allergic response by down regulating IFN-γ and IL-12 production from T-lymphocytes and monocytes, respectively, there by promoting T helper 2 lymphocytes development.\textsuperscript{[35]}

\textbf{Leukotrienes:}
Leukotrienes are responsible for activation and recruitment of inflammatory cells and are very potent spasmogens of human airway smooth muscle.\textsuperscript{[36]} Cysteinyl leukotrienes are known to activate two receptors, CysLT1 and CysLT2, and the biological activity of the cysteinyl leukotriene is mediated via activation of CysLT1, LTC4, LTD4 and LTE4 increase the sensitivity of the air ways to inhaled histamine which may because the leukotriene may alter the excitability of afferent nerve\textsuperscript{[37]} there by increasing the sensitivity of the airways to indirect acting stimuli.

\textbf{Bradykinin:}
The release of kinin within the airways could lead to the activation of bradykinin receptors including β1 receptors whose expression is regulated by inflammatory mediators, cytokines. β2 receptors present on various cells within the airway wall including vascular endothelium, airway smooth muscle, sub mucosal glands, nerves and airway epithelium.\textsuperscript{[38]} Clinical study suggests that bradykinin induces bronchoconstriction via sub population of afferent nerves, C-fibres\textsuperscript{[39]} and mediates the release of sensory neuropeptides from these.\textsuperscript{[40]} The increase in airway
responsiveness to bradykinin correlates with the number of eosinophils in BAL, bronchial biopsies and sputum. \[41\]

**Sensory Neuropeptides:**

The preprotackykinin-1 (PPT-1) gene Encodes substance P and neurokinin-A while PPT-2 encodes neurokinin-B. \[42\] Alternate splicing of the PPT-1 gene results in the information of three mRNAs. Post translation processing of these mRNAs yields substance P. Studies have related that neutral endopeptidase which is localized in the epithelium of the lung of guinea pig \[43\] and man \[44\] is responsible for the degradation of tackykinins. \[22\] Three different types of tackykinin receptors i.e. NK1, NK2 and NK3 exist in the ileum of various animal species. The effect of the tackykinins induced by activation of NK1 receptors in the lung includes mucus secretion, micro vascular permeability and inflammatory cell recruitment and activation \[45\], tackykinin contract human isolated bronchial via NK2 receptors. \[46\] In human studies, neuropeptides including substance P, calcitonin gene related peptide (CGRP), neuropeptides-γ and vasointestinal peptide (VIP) have been detected in the lung. \[47\] A common pathway by which various stimuli induce bronchial hyperresponsiveness might be the sensory nerves as it has been claimed that substance P containing nerves are more abundant in lungs obtained an autopsy from asthmatic as compared with healthy individuals. \[48\] An elevated level of substance P like immunoreactivity has also detected in the sputum of the patients with asthma or chronic bronchitis following hypertonic saline inhalation. \[49\]

**Endothelin:**

Endothelin were originally discovered as potent vasoconstrictor peptides, which are encoded by three distinct genes. \[50\] They are formed via the action of endothelin converting enzyme (ECE). The expression of mRNA for the endothelin and ECE has been documented in human in human bronchial epithelial cells. \[51\]

Endothelin is a potent contractile agonist of human airway smooth muscle \[52\] and augments cholinergic nerve mediated response in human airways in vitro \[53\], both effects mediated via the activation of ETB receptors. Few studies have examined the pro inflammatory action of endothelines in the airways \[54\] ETA but not ETB receptor
antagonists attenuated allergen induced recruitment of eosinophils in a murine model of inflammation \[55\] in part by increased production of IFN-γ from pulmonary lymphocytes. Endothelin cannot be stored and require de-novo synthesis, which may occur several hours after acute allergen challenge.

**Cytokines:**

Cytokines are extracellular signalling proteins produced by different cell types including immune cells like T-lymphocytes. They act on target cells to cause a wide array of cellular functions like activation, proliferation, chemotaxis, immunomodulation, release of inflammatory mediators, growth and cell differentiation and apoptosis. CD+4 lymphocytes are classified as T-helper (Th1) cells, which provide immunity to pathogens and as T-helper (Th2) cells, that gives rise to allergic inflammation. Th1 lymphocytes secrete IFN-γ, IL2 and tumor necrosis factor (TNF)-β, while Th2 lymphocytes secrete cytokines such as IL-3, IL-4, IL-5, IL-10, IL-13 and GM-CSF. \[56-57\] IL-4 and IL-13 switch on β cells to produce IgE. \[58\]

Allergic reaction in the airways are caused by IgE sensitized mast cells and CD+4 Th2, whose activation leads to the infiltration of inflammatory cells, notably eosinophils, leading to tissue damage. \[59\] Cytokines also exerts regulatory effect on expression of ICAM-1 and VACM-1, on epithelial cells of bronchial circulation and airway epithelial cells. \[60\]

**Chemokines:**

Chemokines are chemotactic cytokines, which are potent chemoattractant of eosinophils, basophils, monocytes and T-lymphocytes. They are classified into two major groups. (a) Chemokines, which the first two cysteine residues are separated by an amino acid and (b) Chemokines, in which the cysteine residues are adjacent to each other.

**Growth factors:**

A variety of growth factors are thought to play a role in altering the structure of the airways. A number of growth factors, including platelet derived growth factor (PDGF), transforming growth factor (TGF) and epidermal growth factor (EGF) has
been investigated in bronchial biopsies from asthmatic.\textsuperscript{[61]} A number of in vitro studies have shown that PDGF is a potent mitogen of human airway smooth muscle.\textsuperscript{[62]} TGF-β is a potent stimulant for fibroblast mitogenesis and is important in wound healing and fibrosis\textsuperscript{[63]} and inhibits proliferation of airway smooth muscle.\textsuperscript{[64]} Eosinophils, fibroblasts and epithelial cells are major sources of TGF-β\textsuperscript{[65]} and ET-1 potentiates EGF induced airway smooth muscle proliferation\textsuperscript{[66]} and ET-1 potentiates EGF induced airway smooth muscle proliferation.\textsuperscript{[67]}

**Protease:**

Tryptase which is a mast cell serine protease affect fibroblast proliferation, degrade fibrinogen, generate C3a\textsuperscript{[68]}, simulate mucus secretion\textsuperscript{[69]} and degrade sensory neuropeptides.\textsuperscript{[70]} Thus mast cell tryptase could play a role in regulation of haemostasis, mucus secretion and vascular permeability. Other protease like thrombin induces proliferation of human airway smooth muscle.\textsuperscript{[67]}

**Mucus Production:**

The mucociliary system is the lung’s primary defence mechanism against irritants and infectious agents. Mucus, compose of 95 % water and 5 % glycoprotein is produced by bronchial epithelial glands and goblet cells. Mucus either too viscous or too watery will not be transported optimally. Expectorant mucus from patients with asthma tends to have a high viscosity.

**1.1.8 Neural control / neurogenic inflammation**

The airway is innervated by parasympathetic, Sympathetic and non adrenergic inhibitory nerves. The normal resting tone of human airway smooth muscle is maintained by vagal efferent activity. These ending probably represent the irritant receptor of the airways; stimulation of these irritant receptors produces reflex bronchoconstriction. The airway smooth muscle contains β2 adrenergic receptors that produce bronchodilation.

**1.1.9 Airway remodelling**

Remodelling entails thickening of the airway walls, with increase in sub mucosal tissues, the adventitia and smooth muscle.\textsuperscript{[71-73]} Acute inflammation is a beneficial
non-specific response of tissues to injury and generally leads to repair and restoration of the normal structure and function. In contrast asthma represents a chronic inflammatory process of the airways followed by healing. The end result may be an altered structure referred to as a remodelling of the airways. In asthma the repair process can be followed by complete or altered restitution of airways structure and function, presenting as fibrosis and an increase in smooth muscle and mucus gland mass. These features differ in asthma and chronic obstructive pulmonary disease, in allergic and non allergic asthma, and with the severity of asthma. The precise mechanisms underlying the remodelling process are under intense study. Recent observation in children with asthma (age 5-12 years) suggest that preventing the progressive loss of lung function in childhood may require recognition and treatment of the disease during the first five years of life.

1.1.10 Genetic consideration
It has long been known that asthma and atopy run in families. Asthma, which begins in childhood generally, occurs in atopic individuals who produce significant amounts of IgE on exposure to small amounts of common antigens. This contrast with those patients who developed asthma in adult life and who are non atopic, so called intrinsic or late onset asthma. First degree relatives of asthmatics patients have a higher prevalence of asthma when compared to relatives of non asthmatic subjects. Chromosomes 6q contains region that are important in antigen presentation and mediation of the inflammatory response. Chromosome 6q contains two genes that could influence atopy and airway hyper responsiveness, including nitric oxide synthase.

1.1.11 Diagnosis of Asthma:
The diagnosis of asthma involves a thorough medical history, physical examination, and objective assessments of lung function (spirometry preferred) to confirm the diagnosis. Bronchoprovocation challenge testing and assessing for markers of airway inflammation may also be helpful for diagnosing the disease, particularly when objective measurements of lung function are normal despite the presence of asthma symptoms.
Medical history

• Assess for classic symptoms of asthma:
  – Wheezing
  – Breathlessness
  – Chest tightness
  – Cough (with or without sputum)
• Assess for symptom patterns suggestive of asthma:
  – Recurrent/episodic
  – Occur/worsen at night or early in the morning
  – Occur/worsen upon exposure to allergens (e.g., animal dander, pollen, dust mites) or irritants (e.g., exercise, cold air, tobacco smoke, infections)
  – Respond to appropriate asthma therapy
• Assess for family or personal history of atopic disease (particularly allergic rhinitis)

Physical Examination

• Examine for wheezing on auscultation
• Examine upper respiratory tract and skin for signs of other atopic conditions

Objective Measurements

• Perform spirometry (preferred) to confirm the diagnosis
  – Diagnostic criteria: FEV₁ ↑ (after bronchodilator): ≥ 12% and ≥ 200 mL
• Consider PEF as an alternative if spirometry is unavailable
  – Diagnostic criteria:
    PEF ↑ (after bronchodilator): ≥ 20% and 60 L/min
    Diurnal variation: >20%
• If spirometry (or PEF) is normal, but symptoms are present consider:
  – Challenge testing (e.g., methacholine, histamine, mannitol, exercise)
  – Non-invasive markers of airway inflammation (exhaled nitric oxide, sputum eosinophilia)
  – Trial of appropriate asthma therapy

Allergy testing

• Perform skin tests to assess allergic status and identify possible triggers
1.1.12 Pharmacotherapy of bronchial asthma

According to guidelines of the National Asthma Education and Prevention Program’s (NAEPP) guidelines for the diagnosis and management of asthma, the treatment should have following goals:

- Maintain normal activity levels, including exercise.
- Maintain normal or near normal pulmonary function.
- Prevent chronic and troublesome symptoms.
- Prevent recurrent exacerbations.
- Avoid adverse effects from medicines.

The pharmacological management of asthma depends upon frequency and severity of patient’s symptoms. The following categories of drugs are used in asthma:

I. Bronchodilators

Bronchodilator drugs have an anti-bronchoconstrictor effect that may be demonstrated directly in vitro by drug-induced relaxation of pre-contracted airways.

Bronchodilators promptly reverse airway obstruction in asthmatics. This action believed to be mediated by a direct affect on airway smooth muscle. Only three types of bronchodilators are in current clinical use: β-adrenergic agonist, methylxanthines, and anticholinergics.

(a) β-adrenergic agonists:

Epinephrine has been used to treat asthma since the beginning of the 20th century. β-adrenergic agonists are most widely used and effective bronchodilators for the treatment of asthma. Bronchodilation is mediated by β2 receptors; β2 selective drugs (Salmeterol and Formoterol) have been developed that have very long duration of effect. β-adrenergic agonists leads to relaxation of bronchial smooth muscle that promotes bronchodilation. Activation of adenylate cyclase increases the concentration of intracellular cyclic adenosine 3’, 5’-monophosphaste (cAMP), leading to activation of specific cAMP-dependent protein kinases that cause relaxation. Relaxation may also be due to inhibition of myosin phosphorylation. β-adrenergic agonists reverse
bronchoconstriction irrespective of the contractile agent. β-adrenergic agonists prevent release of mediators from a number of inflammatory cells in vitro.\(^\text{[80]}\) In addition, β adrenergic agonists increase mucus secretion from sub mucosal glands and ion transport across airway epithelium. These effects enhance mucociliary clearance caused by asthma.\(^\text{[81]}\)

β2 selective agents cause tachycardia and palpitation by reflex cardiac stimulation secondary to peripheral vasodilation. These side effects are common in patients with heart diseases; so in such patients these drugs use cautiously. Muscle tremor is caused by stimulation of β2 adrenergic receptors in skeletal muscle and is the primary adverse effect of albuterol and bitolterol. Transient hypokalemia may be induced by high dose of these agents.

**Drug interaction:**

Treatment of β-agonists along with corticosteroids increases risk of hyperkalemia at high dose of corticosteroids. β-agonists at high dose interact with theophylline therapy. It increases risk of hypokalemia.

(b) **Anticholinergics:**

Datura plants contain the muscarinic antagonist stramonium and were smoked for relief of asthma two centuries ago. Now a days, atropine and ipratropium bromide are the most commonly available anticholinergics.

Anticholinergics specifically antagonize muscarinic receptors. They inhibit reflex cholinergic bronchoconstriction and do not significantly block the direct effects of inflammatory mediators such as histamine and leukotrienes on bronchial smooth muscle and vessels. When given by inhalation, anticholinergics produce bronchodilation by competitively inhibiting cholinergic receptors in bronchial smooth muscle. This activity blocks acetylcholine with the net effect being a reduction in cyclic guanosine monophosphate (cGMP) that normally acts to constrict bronchial smooth muscle. Anticholinergic drugs usually are less effective as bronchodilators in asthmatic subjects than β-adrenergic agonists.\(^\text{[82]}\) Nevertheless, they may have an additive effect with β-adrenergic agonists.
Atropine reduces mucociliary clearance in normal subjects and in patients with asthma and chronic bronchitis, but the quaternary derivative, ipratropium bromide, even when given in high does, has no such detectable effect either on normal subjects or in patients with airway disease. [81]

Ipratropium bromide has been shown to decrease the effectiveness of voluntary cough on clearing mucus from the airways, which may affect its role in the treatment of patients who have excessive mucus production. Ipratropium has a slower onset of action and a more prolonged bronchodilator effect compared with standard β2-agonists and has been considered to be less suitable for use on an “as needed” basis for immediate relief of bronchospasm.

Tiotropium bromide is a long acting quaternary anticholinergic agent. Tiotropium in human lungs shows approximately 10 fold more potency than ipratropium and protects against cholinergic bronchoconstriction for greater than 24 hours.

**(c) Methylxanthines:**

Hyde Salter described the bronchodilator effect of strong coffee during the last century. Methylxanthines such as theophylline are related to caffeine and have been used to treat asthma since 1930. The methylxanthines may produce bronchodilation through numerous mechanisms, including:

- Inhibition of phosphodiesterase, thereby increasing cAMP levels
- Inhibition of calcium ion influx into smooth muscle
- Prostaglandin antagonism
- Stimulation of endogenous catecholamine
- Adenosine receptor antagonism
- Inhibition of release of mediators from mast cells and leukocytes.

Theophylline inhibits release of mediators from mast cells, increases mucociliary clearance, and prevents the development of micro vascular leakiness, as would an “anti-inflammatory” drug. [83]
For nocturnal asthma, a single dose of slow release theophylline at bedtime often is effective. This has been demonstrated to reduce overnight declines in FEV1 and morning respiratory symptoms. Taken alone it increases exercise tolerance without improving spirometry tests. Some derivates such as acepiphylline, diprophylline, and proxophylline, are less effective than theophylline. The most common adverse effects are headache, nausea and vomiting, abdominal discomfort, and restlessness.

**Drug interaction:**
- $\beta$-agonists at high dose interact with theophylline therapy. It increases risk of hypokalemia.
- Certain enzyme inhibitors i.e. cimetidine, ciprofloxacin, Verapamil may increase plasma levels of Theophylline.
- Certain enzyme inducers like barbiturates, phenytoin, carbamazepine smoking, and rifampicin may decrease plasma levels of theophylline.

II. Anti-inflammatory drugs
Although the type of inflammatory responses may differ among diseases, inflammation is a common denominator of several lung diseases. Anti-inflammatory drugs suppress the inflammatory response by inhibiting
- Infiltration and activation of inflammatory cells as well as their synthesis
- Release of mediators
- Effects of inflammatory mediators themselves.

(a) Corticosteroids:
Since asthma is viewed as a chronic inflammatory disease and inhaled corticosteroids are known to have low toxicity, they may be considered as first line therapy. Prednisolone and dexamethasone were effective when they were given systematically to treat asthma but they had no anti-asthmatic activity when they were given by inhalation. Corticosteroids potentially inhibit the accumulation of neutrophils, inhibit secretion of human pulmonary macrophages of leukotrienes and prostaglandins, inhibit formation of interleukins, inhibit degranulation and adherence of eosinophils, and reduce number of circulating T lymphocytes and formation of an IgE binding...
suppressive factor. Steroids prevent and reverse the increase in vascular permeability due to inflammatory mediators and may therefore lead to resolution of airway edema. [88]

**Inhaled Glucocorticoids**

Beclomethasone dipropionate (BDP), betamethasone and budesonide, were effective in treating asthma when given by inhalation. Asthmatic patients maintained on Inhaled Glucocorticoids show improvement in symptoms, improvement in lung function, reduction or elimination of need for systemic steroids and lowered requirement for rescue β- adrenoceptor agonists. Steroids potentiate the effects of β adrenergic agonists on bronchial smooth muscle. [87] Inhaled steroids have no proven value in the management of acute asthma. Patients with chronic bronchitis occasionally respond to steroids, possibly because some have an element of undiagnosed asthma.

Adverse effects of corticosteroids include fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract, and phychosis. [89] Two toxicities in elder patients are cataract and glaucoma at high dose.

**(b) Anti-leukotrienes:**

Leukotrienes possess potent pro-inflammatory actions resulting in increased vascular permeability, mucus secretion and bronchial hyper responsiveness. They are derived from the 5-lipoxygenase pathways in mast cells, eosinophils and macrophages. Antileukotrienes improve lung function and diminish symptoms, exacerbation rate and the need for rescue bronchodilator. These are drugs of choice in case of aspirin induced asthma, in which patients have high LTE4 levels in urine and nasal secretions and even higher after taking aspirin. [90] Leukotrienes modifiers are drugs that modify the response of these mediators of inflammation by one of the four ways.

**CysteinyL LT receptor inhibitors**

C-LTs promote eosinophils influx, bronchospasm and mucus hyper secretion, all are considered hallmark of asthma. C-LT receptor inhibitors antagonize or inhibit
leukotrienes predominantly LTD4. These agents inhibit phospholipases, prostaglandins, leukotrienes, and IL-1 synthesis. Probilukast and Iralukast belong to this class. [91-93]

5-lipoxygenase inhibitors
They prevent the formation of leukotrienes by blocking a 5-lipoxygenase pathway in their synthesis. Zileuton, ZD-2138, ABt-761 belongs to this class. [93]

5-lipoxygenase activating protein (FLAP) inhibitors
MK-0591 [94] and MK-886 [95] attenuated the early and late asthmatic response following antigen challenge but not the attendant increase in airway responsiveness to spasmogen.

Leukotrienes receptor antagonists
Montelukast, zafirlukast, pranlukast are selective and high affinity LT1 antagonists.

Zileuton has shown efficacy in exercise-induced asthma, aspirin induced bronchospasm and following chronic administration, an improvement in pulmonary function (FEV1) and a reduction in oral and inhaled corticosteroid use. [96] Furthermore, in a small study, zileuton attenuated both airway and blood eosinophilia in nocturnal asthmatics. [97] Zafirlukast has been demonstrated to attenuate the acute airway obstructive response to allergen and exercise challenge. [84, 98]

Montelukast has been shown to block the early and late response to allergen challenge following single dosing, to improve FEV1 in both children (6-14 years) and adults [99] and to protect against the development of exercise induced bronchoconstriction in both children and adults. [100-101] Tolerance to the bronchoprotective effects of montelukast in attenuating exercise induced bronchospasm does not develop following at least 12 weeks of therapy. [101] Pranlukast increases FEV1 within 1 hour of dosing, improves patient summary symptom and nighttime asthma scores and reduces the use of rescue bronchodilators.
(c) Mast Cell Stabilizer:

**Cromolyn Sodium**

Cromolyn Sodium (Sodium cromoglycate) is a derivative of khellin. Cromolyn inhibited the release of mediators by allergen in passively sensitized animal and human lung preparations. \[102\] Cromolyn was classified as mast cell stabilizer. In vivo chromolyn can block both the early response that may be mediated by mast cells to allergens and the late response. \[103\] It is the first choice anti-inflammatory drug for children because it has few adverse effects. \[104\] Nedocromil sodium has a similar pharmacologic profile of activity to cromolyn. \[105\] Ketotifen also is described as a drug to be used for prophylaxis against asthma.

**1.1.13 Newer target in asthma treatment**

The current pharmacotherapeutics approaches to asthma have several limitations. First there is no known asthma cure and little evidence that prevention is possible in susceptible persons. Hence, patients continue to be at risk of symptoms and exacerbations. Mortality remains a severe problem. Finally, the medications have adverse effects. New inhalation devices and new generation beta agonist are available. At the same time, new understanding of the molecular pathology of asthma has identified several novel therapeutics targets.

**1.1.13.1 TXA2 Inhibitors (Thromboxane A2 Synthesis Inhibitors):**

TXA2 is the potent bronchoconstrictor, mucus producer & blood vessel permeability inducer & causes airway hyper responsiveness. Serbians, Domitroban, Ozagrel are the example of these. \[106\] TXA2 synthetase inhibitor ozagrel reduced cough sensitivity to capsaicin \[107\] and bronchoconstriction to the acetaldehyde. TXA2 antagonist BAYu3405 produced a modest decrease in airway responsiveness to methacoline following 2 weeks treatment in asthmatics. \[108\]

**1.1.13.2 Tachykinin Receptor Antagonist:**

The effect of Tachykinin induced by activation of neurokinin-1 receptor in the lung, Muscarinic secretion, Micro vascular Permeability, Inflammatory cell recruitment and activation. The first nonpeptides tachykinin receptors antagonist was CP -96345,
which is a potent NK1 receptor antagonist. \cite{109} SR48968, 5R-144190, GR-159897 are selective non peptide NK2 receptor antagonist. \cite{110} 5R -142801, 5B-223412 are selective NK3 receptor antagonist.

### 1.1.13.3 Tryptase Inhibitor:
They inhibit both early and late reactions. APC-366 inhibited antigen induced late phase response and bronchial hyperresponsiveness to carbachol in sheep. \cite{111} Lactoferrin disrupts the quaternary structure of tryptase, also attenuates antigen induced late phase response and bronchial hyperresponsiveness in allergic sheep. \cite{112}

### 1.1.13.4 Cytokine Inhibitors:
One of the novel approaches for the treatment of asthma is to target cytokines and develop cytokine modulators as drug. Two humanized anti IL-5 monoclonal antibodies, Sch-55700 and SB-240563 reduces blood eosinophils count for several weeks and prevents eosinophils recruitment into airways after allergen challenge in asthmatics patients. \cite{113}

### 1.1.13.5 Chemokine Inhibitors:
A variety of chemokines, one of which is the chemoattractant eotaxin, are secreted by inflamed lung tissues thereby attracting eosinophils. Eotaxin receptor blockers are being investigated, as eosinophils are believed to be major contributors to the pulmonary damage seen in asthma. Monoclonal antibody (7B11) for human CCR3 has shown to completely block the binding and signalling of the known CCR3 ligands, thus blocking the chemotactic response of human eosinophils to all chemokines.

### 1.1.13.6 Adhesion Molecule Antagonist:
Interaction of eosinophils with intra cellular adhesion molecule-1 (ICAM-1) is thought to be necessary for eosinophils recruitment into airways. Antibodies to ICAM-1 blocked both eosinophils recruitment into the airway in the monkey model of asthma and importantly the increase in the airway reactivity associated with allergen challenge. \cite{114-115}
1.1.13.7 Phosphodiesterase Inhibitors:
Considerable interest has been generated in the potentially utility of iso enzyme selective inhibitors of cyclic nucleotide Phosphodiesterase (PDE) in the treatment of asthma and other inflammatory disorders. First, inhibition of PDE activity increase the cellular content of two key second messengers, cAMP and cGMP, thereby activating specific protein phosphorylation cascades that elicit a variety of functional responses.

Increase in cAMP content suppresses a broad array of functions in inflammatory and immune cells. SB 207499, V11294A, CP-220 and rolumilast are PDE4 inhibitors with less gastrointestinal side effects. [116-117]

1.1.13.8 Endothelin Modulators:
There are two approaches for ET-1 directed therapeutics- (1) Inhibitors of endothelin converting enzyme (ECE), which mediates the synthesis of ET-1 from its precursor [32] (2) Receptor antagonists of the effect of ET-1 at the end organ level. These agents reverse and/or prevent the increase in pulmonary artery pressure and vascular remodelling elicited by acute or chronic hypoxia. Examples are BQ-123, SB-217242 and boseatan. [118]

1.1.13.9 Immunotheraphy:
A conventional immunotherapy is associated with significant increase in mRNA for IL-12 and interferons, which are an important immunomodulator in converting Th2 response to Th1. New immunotherapy using anti-IgE aimed at specific action of IgE to its receptors on mast cells and basophills. In this way it is able to inhibit the early and late reactions in asthma, reduce sputum eosinophils and reduce methacholine sensitivity. It decrease serum IgE levels in allergic patients and maximum bronchoconstriction was reduced up to 60%. [119-120] A new form of immunotherapy is the use of gene vaccination with plasmid DNA. This modality induced a Th1 response that dominated over a Th2 response. [121]

1.1.14 Ayurvedic concept of asthma
The ancient ayurvedic system of medicine has an elaborate description of this disease from the earliest times. Shwasa word in normal terminology means respiration.
According to ayurveda, different types of Shwasa (Asthma) are Kshudra Shwasa, Maha Shwasa, Urdhva Shwasa, Chhinna Shwasa, and Tamak Shwasa.

**1.1.14.1 Kshudra Shwasa:**
Because of vititation of vayu in the alimentary tract, minor dyspnoea is caused. This condition does not give much pain; it does not interfere in the courses of food and breathing. It does not disturb the sensory organs. This condition is mainly because of excessive intake of ruksha eatable and excessive exercise.

**1.1.14.2 Maha Shwasa:**
This condition is caused because of disturbance in respiratory movement of Vayu. The patient feels great obstruction in respiration, breaths without break with a very loud and long stertore making a sound like intoxicated bull.

**1.1.14.3 Urdhva Shwasa:**
Under this condition the expiratory phase is prolonged and the inspiratory process is just insignificant as evident from the name of this condition. Month and the respiratory tract get obstructed with Kaph. His eyes are turned upword, he is almost obvious to his surroundings, and eyes are restless. Affected with severe pain, he enters into stupor. Such a condition can be found in pneumonia, abscesses of the lungs, gangrene or acute inflammation in the lungs and also in different types of epilepsy.

**1.1.14.4 Chhinna Shwasa:**
Under this condition, the whole of the breathing system is depressed. The patient has to breathe with full force and with great difficulty. He suffers from constipation, excessive sweating, repeated fainting, burning and retention of urine, having eyes full of tears and entering unconsciousness every now and then having dry mouth. The allopathic system of medicine groups such condition under interrupted respiratory dyspnoea (Cheyne-stoke’s respiration).

**1.1.14.5 Tamaka Shwasa:**
Acharya Sushruta has clearly defined Shwasa as condition in which Prana Vayu leaving its normal function moves upword associated with Kapha and thus producing
igasping and laboured breathing. Tamaka Shwasa is one of the five conditions of Shwasa discussed for its Nidan (etiology causative factors), Purva Roop (Prodornal symptoms), Roop (symptoms). Upshava (Diet, drugs and practices useful to the patient), Anupshaya (Diet drugs and practices harmful to the patient), Samprapti (Pathogenesis) and Chikitsa (treatment). Acharya classified Tamaka Shwasa in two conditions viz. Pratamaka Shwasa and Santamak Shwasa. Febrile dyspnoea appears in a patient with fever and fainting in Pratamaka Shwasa.

1.1.15 Herbs and Asthma

Over the recent decade, there has been an immense interest in drugs of natural origin, particularly from plant sources. Herbal medicine is the third most popular choice of both adults (11%) and children (6%) suffering from asthma. A survey by the National Asthma Campaign in Britain found that 60% of people with moderate asthma and 70% with severe asthma have used complementary and alternative medicine to treat their condition. The historical importance of herbal medicine in the treatment of asthma is indisputable. Four of the five classes of drugs currently used to treat asthma—namely, β2 agonists, anticholinergics, methylxanthines and cromones have origin in herbal treatments going back at least 5000 years. [122] There is a high prevalence of usage of complementary medicine for asthma.

<table>
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<tr>
<th>Sr. No</th>
<th>Name of plant</th>
<th>Part used/extract/fraction</th>
<th>Major chemical constituent(s)</th>
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<td>Alkaloids</td>
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<td>2</td>
<td><em>Albizia lebbeck</em></td>
<td>Stem bark/Aqueous</td>
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</tr>
<tr>
<td>3</td>
<td><em>Alstonia scholaris</em></td>
<td>Leaves/Ethanol</td>
<td>Ditamine, Echitamine</td>
</tr>
<tr>
<td>4</td>
<td><em>Artemisia caerulescens</em></td>
<td>Aerial parts/Butanol</td>
<td>Quercetin, isorhamnetin</td>
</tr>
<tr>
<td>5</td>
<td><em>Benincasa hispida</em></td>
<td>Fruits/Methanol</td>
<td>Triterpenes, Glycosides, Sterols</td>
</tr>
<tr>
<td>6</td>
<td><em>Clerodendron serratum</em></td>
<td>Stem bark/Aqueous</td>
<td>Phenolic glycoside</td>
</tr>
<tr>
<td>7</td>
<td><em>Elaeocarpus sphaericus</em></td>
<td>Fruits/Aqueous, Petether, Benzene</td>
<td>Glycoside, Steroids, Alkaloid,</td>
</tr>
</tbody>
</table>
### Table 1.5: Herbal Mast Cell Stabilizers \[122\]

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of plant</th>
<th>Part used/extract/fraction</th>
<th>Major chemical constituent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Achyranthes aspera</em></td>
<td>Aerial parts/Aqueous</td>
<td>Oleanolic acid</td>
</tr>
<tr>
<td>2</td>
<td><em>Allium cepa</em></td>
<td>Bulbs/Juice</td>
<td>α and β unsaturated Thiosulphinate</td>
</tr>
<tr>
<td>3</td>
<td><em>Aquillaria agallocha</em></td>
<td>Stem/Aqueous extract</td>
<td>Triterpenoids</td>
</tr>
<tr>
<td>4</td>
<td><em>Bacopa monniera</em></td>
<td>Leaves/Ethanol</td>
<td>Bacosides, Alkaloids</td>
</tr>
<tr>
<td>5</td>
<td><em>Cassia torosa</em></td>
<td>Seeds</td>
<td>Gentiobiosides</td>
</tr>
<tr>
<td>6</td>
<td><em>Citrus unshiu</em></td>
<td>Peels</td>
<td>Flavanoids</td>
</tr>
<tr>
<td>7</td>
<td><em>Coleus forskohlii</em></td>
<td>Roots</td>
<td>Forskolin (diterpenoid)</td>
</tr>
<tr>
<td>8</td>
<td><em>Crinum glaucum</em></td>
<td>Leaves/Aqueous</td>
<td>Alkaloids</td>
</tr>
<tr>
<td>9</td>
<td><em>Curcuma longa</em></td>
<td>Rhizome</td>
<td>Tumerones, curcuminoids</td>
</tr>
<tr>
<td>10</td>
<td><em>Mentha piperita</em></td>
<td>Leaves</td>
<td>Flavanoidal glycosides</td>
</tr>
<tr>
<td>11</td>
<td><em>Ocimum sanctum</em></td>
<td>Leaves/Aqueous</td>
<td>Myrcenol, Nerol, Eugenol</td>
</tr>
<tr>
<td>12</td>
<td><em>Morings Oleifera</em></td>
<td>Bark</td>
<td>B-sitosterol</td>
</tr>
<tr>
<td>13</td>
<td><em>Tinospora cordifolia</em></td>
<td>Stem/Aqueous</td>
<td>Tinosporin</td>
</tr>
<tr>
<td>14</td>
<td>Vitex negund</td>
<td>Leaves/Ethanol</td>
<td>Casticin, iso-orientin Chrysophenol D</td>
</tr>
<tr>
<td>15</td>
<td><em>Solanum xanthocarpum</em></td>
<td>Roots/Alkaloidal fraction</td>
<td>Solasodine</td>
</tr>
<tr>
<td>Sr. No</td>
<td>Name of plant</td>
<td>Part used/extract/fraction</td>
<td>Major chemical constituent(s)</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------</td>
<td>-----------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td>1</td>
<td>Adhatoda vasica</td>
<td>Leaves/Methanol</td>
<td>Vasicinol, vasicine</td>
</tr>
<tr>
<td>2</td>
<td>Albizzia lebbeck</td>
<td>Stem bark/Aqueous</td>
<td>Saponins</td>
</tr>
<tr>
<td>3</td>
<td>Aquillaria agallocha</td>
<td>Stem/Aqueous extract</td>
<td>Triterpenoids</td>
</tr>
<tr>
<td>4</td>
<td>Camellia sinensis</td>
<td>Leaves</td>
<td>Flavanoids</td>
</tr>
<tr>
<td>5</td>
<td>Citrus unshiu</td>
<td>Peels</td>
<td>Flavanoids</td>
</tr>
<tr>
<td>6</td>
<td>Cnidium monnieri</td>
<td>Fruits/Ethanol</td>
<td>Osthol</td>
</tr>
<tr>
<td>7</td>
<td>Curcuma longa</td>
<td>Rhizomes</td>
<td>Curcumin and Tetrahydrocurcumin</td>
</tr>
<tr>
<td>8</td>
<td>Dalbergia odorifera</td>
<td>Heart Wood</td>
<td>Flavanoids, Tannins</td>
</tr>
<tr>
<td>9</td>
<td>Ginkgo biloba</td>
<td>Leaves</td>
<td>Ginkgolides</td>
</tr>
<tr>
<td>10</td>
<td>Gleditsia sinensis</td>
<td>Fruits/Ethanol</td>
<td>Saponinsv</td>
</tr>
<tr>
<td>11</td>
<td>Hydrangea macrophylla</td>
<td>Leaves</td>
<td>Glycosides</td>
</tr>
<tr>
<td>12</td>
<td>Magnolia officinalis</td>
<td>Bark/Aqueous</td>
<td>Honokiol, Magnolol</td>
</tr>
<tr>
<td>13</td>
<td>Sarcostemma brevistigma</td>
<td>Twigs/Alkaloidal fraction</td>
<td>Bregenin</td>
</tr>
<tr>
<td>14</td>
<td>Solanum xanthocarpum</td>
<td>Roots/Alkaloidal fraction</td>
<td>Solasodine</td>
</tr>
<tr>
<td>15</td>
<td>Terminal chebula</td>
<td>Fruits/Aqueous</td>
<td>Ellagic acid, Tannins Chebulagic acid</td>
</tr>
<tr>
<td>16</td>
<td>Vitex negundo</td>
<td>Leaves/Ethanol</td>
<td>Casticin, isoorientin Chrysophenol D</td>
</tr>
</tbody>
</table>

**Table 1.6: Herbal Anti-Allergics** [122]

**Table 1.7: Herbal Anti-Inflammatory** [122]
<table>
<thead>
<tr>
<th>No.</th>
<th>Plant Name</th>
<th>Part Used</th>
<th>Solvent Used</th>
<th>Chemical Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Aloe vera Tourn.ex Linn. (Liliaceae)</td>
<td>Leaves/Aqueous, Chloroform and ethanol</td>
<td>Anthraquinons, sterols, saponins and carbohydrates</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Bryonia laciniosa</td>
<td>Leaves/chloroform Extract</td>
<td>Flavanoids</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Calotropis procera</td>
<td>Latex</td>
<td>α-amyrin, β-amyrin calotropin</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cinnamomum Zeylanicum</td>
<td>Oil</td>
<td>Eugenol, cinnamic aldehyde, α-terpeniol</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Curcuma longa</td>
<td>Rhizomes</td>
<td>Tumerones, curcuminoids</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>C. gigantia</td>
<td>Flower</td>
<td>Alpha and beta calotropeol, giganteol, Calotropin</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Dalbergia odorifera</td>
<td>Heart Wood</td>
<td>Flavanoids, tannins</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Elaeocarpus sphericus</td>
<td>Fruits/Aqueous, Petether, Benzene, Acetone and ethanol</td>
<td>Glycoside, Steroids, alkaloid, flavanoids</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Nelsonia canescens</td>
<td>Leaf/ Ethananol extract</td>
<td>Flavanoids</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Indigofera tinctoria</td>
<td>Whole plant/Methanol</td>
<td>Polyphenols</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Butea frondosa Koen.</td>
<td>Leaves/Aqueous</td>
<td>Flavanoid, glycosides, Proteins, aminoacids.</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Ocimum sanctum</td>
<td>Leaves/Aqueous</td>
<td>Myrcenol, Nerol, Eugenol</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Ophiopogon japonicas</td>
<td>Root/Aqueous extract</td>
<td>Ruscogenin and ophiopogonin D</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Pavetta crassipes</td>
<td>Leaves/Aqueous</td>
<td>Flavanoids, Tannins, Anthraquinones</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Tylophora asthmatica</td>
<td>Leaves/Alkaloidal</td>
<td>Tylophorine</td>
<td></td>
</tr>
</tbody>
</table>
### Table 1.8: Herbal Antispasmodic [122]

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of plant</th>
<th>Part used/extract/fraction</th>
<th>Major chemical constituent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aegle marmelos</td>
<td>Leaves/Ethanol</td>
<td>Aegelin, Aegelemine, Aegeline</td>
</tr>
<tr>
<td>2</td>
<td>Asiasarum sieboldi</td>
<td>Roots/Methanol</td>
<td>Methyleugenol 1, gamma-asarone, Elemicin,</td>
</tr>
<tr>
<td>3</td>
<td>Asystasia gangetica</td>
<td>Leaves/Methanol, Ethylacetate</td>
<td>Isoflavone glycoside, Dalhorin</td>
</tr>
<tr>
<td>4</td>
<td>Bacopa monniera</td>
<td>Leaves/Ethanolic</td>
<td>Bacosides, Alkaloids, Glycosides</td>
</tr>
<tr>
<td>5</td>
<td>Belamcanda chinensis</td>
<td>Leaves/Ethanol</td>
<td>Tectorigenin</td>
</tr>
<tr>
<td>6</td>
<td>Cissampelos glaberrina</td>
<td>Leaves, Root Bark/Aqueous</td>
<td>Warifteine, α-bisbenzylisoquinoline.</td>
</tr>
<tr>
<td>7</td>
<td>Drymis winteri</td>
<td>Bark</td>
<td>Terpene</td>
</tr>
<tr>
<td>8</td>
<td>Cnidium monnieri</td>
<td>Fruits/Ethanol</td>
<td>Osthol</td>
</tr>
<tr>
<td>9</td>
<td>Coleus forskohlii</td>
<td>Roots</td>
<td>Forskolin (diterpenoid)</td>
</tr>
<tr>
<td>10</td>
<td>Crinum glaucum</td>
<td>Leaves/Aqueous</td>
<td>Alkaloids, lycorine, Crinamine</td>
</tr>
<tr>
<td>11</td>
<td>Ferula ovina</td>
<td>Aerial parts/Ethanol</td>
<td>Carvacrol, alpha-pinene, geranyl isovalerate and geranyl propionate</td>
</tr>
<tr>
<td>12</td>
<td>Ferula sinica</td>
<td>Roots/Ethanol</td>
<td>Resins</td>
</tr>
<tr>
<td>13</td>
<td>Pavetta crassipes</td>
<td>Leaves/Aqueous</td>
<td>Flavanoids, Tannins, ant hr aquino nes</td>
</tr>
<tr>
<td>14</td>
<td>Saussurea leppa</td>
<td>Alkaloidal fraction</td>
<td>Sesquiterpene lactone, terpenoids</td>
</tr>
<tr>
<td>15</td>
<td>Thymus vulgaris</td>
<td>Ethanol</td>
<td>Flavanones</td>
</tr>
<tr>
<td>16</td>
<td>Tylophora asthmatica</td>
<td>Leaves/Alkaloidal fraction</td>
<td>Tylophorine</td>
</tr>
</tbody>
</table>
### Table 1.9: Immunomodulatory activity [122]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of plant</th>
<th>Part used/extract/fraction</th>
<th>Major chemical constituent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Trichilia glabra</em></td>
<td>Leaf/Aqueous</td>
<td>Polysaccharides</td>
</tr>
<tr>
<td>2</td>
<td><em>Ipomoea carnea</em></td>
<td>Leaf/Aqueous</td>
<td>Nortropane alkaloids, calystegines β2</td>
</tr>
<tr>
<td>3</td>
<td><em>Clausena exauata</em></td>
<td>Wood/Aqueous</td>
<td>Phenolic compounds, furanocoumarins, flavanoids and</td>
</tr>
<tr>
<td>4</td>
<td><em>Magnifera indica</em></td>
<td>Ether, alcoholic</td>
<td>Magniferin</td>
</tr>
<tr>
<td>5</td>
<td><em>Cleome viscose</em></td>
<td>Aerial parts/Aqueous,</td>
<td>Alkaloids, Saponins</td>
</tr>
<tr>
<td>6</td>
<td><em>Plantago ovate</em></td>
<td>Seeds/Aqueous</td>
<td>Polysaccharides glycosides</td>
</tr>
<tr>
<td>7</td>
<td><em>Angelica sinensis</em></td>
<td>Roots/Aqueous and Ethanolic</td>
<td>Polysaccharides</td>
</tr>
</tbody>
</table>

### Table 1.10: Anaphylactic drug [122]

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of plant</th>
<th>Part used/extract/fraction</th>
<th>Major chemical constituent(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Lycopus lucidus</em></td>
<td>Whole plant/Aqueous</td>
<td>Betulinic acid, Triterpenes</td>
</tr>
<tr>
<td>2</td>
<td><em>Poncirus trifoliate</em></td>
<td>Fruit/Aqueous</td>
<td>Flavanoids</td>
</tr>
<tr>
<td>3</td>
<td><em>Trichopus zeylanicus</em></td>
<td>Leaves/Butanol</td>
<td>Lipoprotein/Glycolipoprotein</td>
</tr>
<tr>
<td>4</td>
<td><em>Cryptotympana atrata</em></td>
<td>Whole plant/Aqueous</td>
<td>oleanolic acid</td>
</tr>
<tr>
<td>5</td>
<td><em>Striga orobanchioides</em></td>
<td>Whole plant/Aqueous,</td>
<td>Flavonoids, apigenin and luteolin</td>
</tr>
<tr>
<td>6</td>
<td><em>Crinum glaucum</em></td>
<td>Bulbs/Aqueous</td>
<td>Alkaloids</td>
</tr>
<tr>
<td>7</td>
<td><em>Acanthopanax senticosus</em></td>
<td>Stem/Aqueous</td>
<td>Acanthoside A, B &amp; C, Chisanoside, Saponin, flavones,</td>
</tr>
<tr>
<td>8</td>
<td><em>Syzygium aromaticum</em></td>
<td>Flower bud/Aqueous</td>
<td>Phenols</td>
</tr>
</tbody>
</table>
1.3.16 Models for evaluation of asthma

Some models used to evaluate antiasthmatic activity preclinically are enlisted below

A. Bronchodilatory activity and other respiratory parameters

a. Invivo models for acute asthma in conscious animals
   i. Histamine and acetylcholine induced bronchospasm in conscious guinea pigs
   ii. Antigen inhalation induced bronchospasm in passively sensitized guinea pigs

b. Invivo models for acute asthma in anaesthetized animals
   i. Bronchospasmoalytic activity in anaesthetized guinea pigs (Konzett Rossler method)
   ii. Pneumotachography in anaesthetized guinea pigs
   iii. Body plethysmography and respiratory parameters after histamine induced bronchospasm in anaesthetized guinea pigs
   iv. Anaphylectic cronchoconstriction in actively sensitized guinea pigs
   v. Bronchodilator effects on anaesthetized dog’s respiration and blood pressure

c. Invivo models for chronic asthma in conscious animals
   Tryspin and egg albumin induced bronchospasm in conscious guinea pigs

d. Invitromodels
   i. Histamine/ acetylcholine induced contraction on isolated guinea pig tracheal chain preparation
   ii. Histamine/ acetylcholine induced contraction on isolated guinea pig tracheal strip preparation
   iii. Cshultz-dale’s reaction in tracheal strips of passively sensitized guinea pigs
   iv. Spasmolytic activity in isolated guinea pig lung strips

<table>
<thead>
<tr>
<th></th>
<th>Terminalia chebula</th>
<th>Fruit/Aqueous</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td><em>Vitex rotundifolia</em></td>
<td>Fruit/Aqueous</td>
<td>Flavanoids</td>
</tr>
</tbody>
</table>
v. Calcium induced contraction on isolated guinea pig Tania coli preparation
vi. Histamine/ Acetacholine induced contraction on isolated guinea pig ileum
vii. Histamine induced contraction on isolated goat tracheal chain
viii. Spasmolytic activity on isolated rat uterus
ix. Bronchial perfusion of isolated lungs
x. Microscopic examination of lung section of rabbits Isolated guinea pig right atrium preparation

B. Models for anti allergic activity
   a. Atabilization of mast cells using rats sensitized by horse serum and bordetella pertusis
   b. Passive paw anaphylaxix in rats
   c. Heterologus passive cutaneous anaphylaxis model in mice

C. Models for mast cell stabilizing activity
   a. Degranulation of rat peritoneal mast cells
      i. Immunological preparation of peritoneal mast cell suspension
      ii. Non immunological (compound 48/80 or clonidine induced)
   b. Rat mesenteric mast cell degranulation (by compound 48/80)
   c. Rat mesenteric mast cell degranulation (by egg albumine)

D. Models for anti-eosinophilic activity
   a. Bronchoalveolar lavage
   b. Milk induced leucocytosis and eosinophilia

E. Models for detecting indirect antihistaminic activity
   a. Clonidine/haloperidol induced catalepsy

F. Models for occupational asthma
   a. Toludine Di-isocyanate induced occupational asthma model in rats
G. Enzyme assay models
   a. Phosphodiesterase inhibitory activity
   b. Invitro enzyme assay of TXA2 synthatase

H. Receptor assay models
   a. Histamine(H1) receptor binding
   b. Adenosine receptor binding assay

I. Method to find out mechanism of action
   a. Effects on thromboxone B2 production using intact platalates and whole blood

J. Models for anti inflammatory activity
   As bronchial asthma is described primarily as an inflammatory disease which is immunologically initiated and mediator driven event. One should also go for testing test drugs anti-inflammatory activity.
   a. In vitro models
      i. \(^3\)H-Bradykinin receptor binding
      ii. \(^3\)H-Substance P receptor binding
      iii. Assay of PMN leukocytes chemotaxis
      iv. PMN leukocytes aggregation by FMLP
      v. Constitutive & inducible cellular arachidonic acid metabolism
      vi. Induced release of cytokines
      vii. TNF-a antagonism
   b. In vivo models:
      i. For acute and sub acute phase of inflammation
         1. Carrageenan induced paw edema in rats
         2. Ultraviolet erythema in guinea pigs
         3. Croton oil ear edema in rats and mice
         4. Oxazolone induced ear edema in mice
         5. Carrageenan induced pleurisy in rats
         6. Granuloma pouch in rats
ii. For proliferative phase of inflammation
   1. Cotton wool granuloma in rats
   2. Sponge implantation in rats
   3. Glass rod granuloma in rats

c. Airway microvascular leakage

K. Models for immunomodulatory activity
   a. Determination of phagocytic index
   b. Delays type hypersensitivity reaction using SRBC as an antigen
   c. Humoral antibody response to SRBC in mice (Parameters: Plaque forming cell assay, blood lymphocytes count, splenic lymphocytes count)
   d. Neutrophil adhesion test

Some important models from above are described in detail as follows.

1.1.16.1 Histamine and Acetylcholine induced bronchospasm in conscious guinea pigs [123]

   Rationale: Symptoms like asphyctic convulsions resembling bronchial asthma in patients can be induced by inhalation of histamine or other bronchospasm inducing agents in guinea pigs. The first symptoms are increased breathing frequency, forced inspiration, and finally asphyctic convulsions. The occurrence of these symptoms can be delayed by antagonistic drugs. Pre-convulsion time (PCT), i.e. time until asphyctic convulsions, can be measured.

   Method:

   Hartley strain guinea pigs of either sex weighing 350-500g are selected. The animals are kept in a closed chamber and exposed to an aerosol of 0.5% histamine hydrochloride and time for preconvulsion time (PCT) (The time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions) noted. As soon as PCT commenced, animals are removed from the chamber and placed in fresh air to recover. This time for PCT is taken as basal value. After 15 days of wash out period the same animals are randomly divided into groups each containing six animals. The following schedule of treatment is administered:
Group I  Control (vehicle)
Group II  Standard (Ketotifen, 1 mg/kg)
Group III Test

Two hours after the respective drug treatment, animals are exposed to histamine aerosol and PCT is noted. The effect of drug was calculated by the following formula:

\[ \% \text{ Increase in PCT} = \left(1 - \frac{T_1}{T_2}\right) \times 100 \]

where, \( T_1 \) = PCT on day 0
\( T_2 \) = PCT on day 15

Similar procedure can be repeated by exposure of aerosol of 0.5% Ach in another four groups of animals. The “guinea pig asthma” has been applied as useful method in various modifications by many laboratories.

1.1.16.2 Trypsin and egg albumin induced bronchospasm in conscious guinea pigs [124]

**Rationale:** Here, trypsin & egg albumin are used as sensitizer (antigen).

**Method:**
Guinea pigs, weighing around 400 g are selected. On day zero, the animals are examined for the following parameters.

- Pre convulsion time to histamine
- Serum pCO2 and pO2
- Serum bicarbonate level
- Respiratory volumes
- Eosinophils and macrophages count in the broncho alveolar fluid
- Histopathological changes in the ling tissues

The animals are exposed once daily to aerosol of trypsin (1mg/ml, 1ml/min) for 5 min. followed by a rest of 2 hrs and then exposed to egg albumin (1% solution, 1ml/min, 10 min.). This procedure is repeated for 10 days. Later on egg albumin aerosol is discontinued where as trypsin exposure was continued till 21st day. On 22nd
day the animals are examine for the above-mentioned parameters. Then guinea pigs are grouped as

Group I Control  
Group II Asthmatic control  
Group III Std (dexamethasone 4 mg/kg)  
Group IV Test

And given respective drug treatment from day 22\textsuperscript{nd} to 35\textsuperscript{th}, then on 35\textsuperscript{th} day again observe for the aforementioned parameters.

Difference in results between Day 0 to 21\textsuperscript{st} – induction of asthma  
Day 22\textsuperscript{nd} to 35\textsuperscript{th} – drug treatment

1.1.16.3 Histamine/Acetylcholine induced contractions on isolated guinea pig tracheal chain preparation \cite{125}

\textbf{Rationale:} To detect bronchodilator ($\beta_2$), antihistaminic, antispasmodic, parasympatholytic activity, LT receptor blocking, bradykinin antagonistic activity.

\textbf{Method:}  
An adult guinea pig of either sex, weighing around 400 g, is killed by a blow on the head and the trachea is removed with the aid of a pair of scissors. Trachea is sectioned into 12 rings of about the same width kept moist with Ringer’s solution and connected by means of short loop of silk thread. The chain of rings is suspended in a tissue bath containing van Dyke-Hastings solution saturated with carbogen and a layer of liquid petrolatum. Keeping the bath temperature at 37±1 °C, a writing lever with 12-fold magnification is used to record contraction or relaxation of the tracheal chain. A tension of 0.5 g is exerted on the liver. To study spasmolytic effects, the test compound is first added alone and washed out. Then a spasmogen added, and at the height of the contraction, the spasmolytic agent added again which if effective, causes relaxation. Histamine, acetylcholine, carbachol, leukotriene D\textsubscript{4} and Barium chloride.
Determination of mechanism of action (testing for β-sympathomimetic effect)

After obtaining the initial carbachol (cholinergic agonist) induced spasm, propranolol is administered 5 min before the addition of the test drug. Three minutes later, the tissue is challenged by carbachol administration.

The percent inhibition of carbachol or other spasmogen-induced contractions is calculated. From dose-response curves $ED_{50}$ values can be calculated.

Advantage: The preparation is free of spontaneous contractions and active for 12 h. Effects quite different from those of intestine. e.g., aminophylline are ineffective in antagonizing histamine contraction in intestine, but quite active in antagonizing that in trachea.

The isolated guinea pig trachea has been proven to be a useful tool for several purposes, e.g., screening procedures and studies on mode of action, e.g., of potassium channel openers.

1.16.4 Bronchial perfusion of isolated lungs

Rationale: Bronchial perfusion of the isolated lung was described by Sollmann T and von Oettingen WF as a simple method for studying pharmacological reactions of bronchiolar muscle. The method consists in perfusing fluid down the trachea through the bronchi, and allowing it to escape from the alveoli through scratches on the surface of the lungs. Bronchoconstriction results in a reduced rate of flow; bronchodilatation is indicated by an increased flow. The method has been used to evaluate sympathomimetic drugs by Tainter ML et al. and by Luduena FP et al.

Method:

Guinea pigs weighing about 200 g are sacrificed by a head blow. The chest is opened, the trachea cut at the upper end and removed with the lung. The trachea is attached to the cannula of a perfusion apparatus. Only one lung is perfused, the other being tied off. The lower part of the lower lobe is cut off and the rest of the lung surface is scratched deeply assuring maximal pre-medication flow.
The perfusion fluid has the following composition in percentage of anhydrous salts: NaCl 0.659, NaHCO$_3$ 0.252, KCl 0.046, CaCl$_2$ 0.005, MgCl$_2$ 0.0135, NaH$_2$PO$_4$ 0.01, Na$_2$HPO$_4$ 0.008, glucose 5%, pH 8.0. The temperature of the perfusion medium is 37.5 °C and the lung is enclosed in a glass cylinder to be protected from variations in the environmental temperature. The trachea is attached to the cannula of a perfusion apparatus which pumps the solution at a constant rate into a manometric tube connected with the perfused organ. Resistance to the flow (bronchoconstriction) results in an increase in the height of the column of fluid in the manometer. The intensity of bronchodilator effect is measured by the fall of the column in the manometer. After the lung is attached to a T-shaped cannula, the pump is set in motion and the fluid, after filling the lung, flows out of the system through the third opening of the cannula. By gentle pressure air bubbles are forced out of the lung into the overflow. The lung then treated in the aforesaid manner, and the upper outlet of the cannula closed. Histamine HCl is added in a concentration of 1: 2 500 000 as soon as the perfusion starts and the flow is adjusted to obtain a constant progressive increase in pressure. The drugs are injected near the cannula when the perfusion pressure reaches a level of 500–650 ml of water. The volume injected is always 0.1 ml. Each drug is tested for bronchodilating activity against the bronchoconstriction induced by histamine in parallel with l-arterenol following a Latin square, including three doses of each drug and three doses of l-arterenol graded at 0.5 log intervals. Activity ratios of bronchodilating agents versus the standard can be calculated with a 3 + 3 point assay including confidence limits.

1.1.16.5 Degranulation of rat peritoneal mast cells

This involves microscopic examination of rat mast cells on exposure to the test compound and STD. Degranulation of rat peritoneal mast cell can be induced in vitro by two different stimuli:

1) **Immunological (Egg albumin induced)**

**Preparation of peritoneal mast cell suspension:**

Normal saline containing 5 units /ml of heparin is injected in the peritoneal cavity of male rats lightly anaesthetized with ether. After a gentle abdominal massage, the peritoneal fluid containing mast cells is collected in centrifuge
tubes placed over ice. Peritoneal fluid of 4-5 rats is collected and pooled and centrifuged at 2000 rpm for 5 min. Supernatant solution is discarded and the cells are washed twice with saline and resuspended in 1 ml of saline/tyrode’s solution.

2) **Non-immunological (Compound 48/80 or Clonidine induced)**

0.1 ml of the peritoneal cell suspension is transferred to 6 test tubes and is treated as follows.

- Test tube no.1 & 2 - Saline
- Test tube no.3, 4 - 0.1 ml of 10μg/ml of Ketotifen fumarate
- Test tube no.5 - 0.1 ml of test agent in Saline

Each test tube is incubated for 15 min at 37°C and then Compound 48/80 (0.1 ml, 10μg/ml) is added to each test tube except test tube no. 1. After further incubation for 10 min. at 37°C, the cells are stained with 0.1% toluidine blue solution made in distilled water and examined under the high power of light microscope. Percent protection of the mast cells in the control group and the treated groups is calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted.

**Immunological (Egg albumin induced)**

Rats are sensitized by administering three doses of 350 μg of egg albumin adsorbed on 60 mg of aluminum hydroxide gel, the doses being given on the first, third and fifth day subcutaneously. The mast cells are collected on the tenth day of sensitization. The study is conducted in the same manner as above and the sensitized cells were degranulated using egg albumin (1mg/ml). Percent protection of the mast cells in the control group and the treated groups are calculated by counting the number of degranulated mast cells from total of at least 100 mast cells counted. Control group consisted of positive control group in which egg albumin is added without addition of test agent and a negative control group in which neither egg albumin nor the test agent is added to correct for spontaneous degranulation of mast cells without any degranulating agent.
1.1.16.6 Airway microvascular leakage\textsuperscript{[129]}

**Rationale:** Plasma exudation in guinea-pig airways \textit{in vivo} can be determined by Evans Blue dye and is fairly correlated with radiolabelled albumin.

**Method:**
Female Dunkin-Hartley guinea pigs weighing 380–600 g are anesthetized with an initial dose of 1.5-g/kg urethane injected i.p. Additional urethane is given i.v. 30 min later to achieve an appropriate level of anesthesia. A tracheal cannula is inserted into the lumen of the cervical trachea, a polyethylene catheter into the left carotid artery to monitor blood pressure and heart rate and another polyethylene catheter into the external jugular vein for administration of drugs. The animals are connected to a constant volume mechanical ventilator and then given an injection of 1.0–1.5 mg/kg suxamethonium i.v. To prevent interference with spontaneous respiration. A tidal volume of 10 ml/kg and a frequency of 60 strokes/min are used.

Lung resistance is measured as an index of airway function and monitored throughout the experiment. Transpulmonary pressure is measured with a pressure transducer with one side attached to a catheter inserted into the right pleural cavity and the other side attached to the side port of the intratracheal cannula. Airflow is measured by a pneumotachograph connected to a pressure transducer. The signals of the transducers are used for instantaneous calculation of lung resistance by an appropriate computer program. The test compound (bradykinin receptor antagonist) is given intravenously. Ten min later, Evans Blue dye (20 mg/ml) is injected i.v. for 1 min. After 1 min, bronchoconstriction and microvascular leakage is induced by injection of bradykinin or by inhalation of bradykinin or PAF or vagal stimulation. Six min after induction of leakage, the thoracic cavity is opened, and a cannula is inserted into the aorta through a ventriculotomy. Perfusion is performed with 100–150 ml 0.9% saline at a pressure of 100–120 mmHg in order to remove the intravascular dye from the systematic circulation. Blood and perfusion liquid are expelled through an incision in the right and left atrium. Subsequently, the right ventricle is opened, and the pulmonary circulation is perfused with 30 ml of 0.9% saline. The lungs are then removed, and the connective tissue, vasculature, and parenchyma are gently scraped.
The airways are divided into 4 components: lower part of the trachea, main bronchi, the proximal 5 mm portion, and the distal intrapulmonary airways.

The tissues are blotted dry, and then weighed. Evans Blue dye is extracted in 2 ml of formamide at 40 °C for 24 h, and measured in a spectrophotometer at 620 nm.

Evans Blue dye concentration, expressed as ng/mg tissue, as well as lung resistance is compared by statistical means (unpaired Student’s *t*-test or Mann-Whitney U test) between treated groups and controls receiving the challenge only.

This method can be used to study the antagonism against bradykinin- and platelet-activating factor-induced airway microvascular leakage and vagal stimulation-induced airway responses.

1.2 Introduction to Phytochemistry:

Phytochemistry is in the strict sense of the word the study of phytochemicals. These are chemicals derived from plants. In a narrower sense the terms are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant diseases. They also exhibit a number of protective functions for human consumers.

Identification of herb is based on macroscopical and microscopical features. Macroscopical feature involves odour, taste, colour, size shape and special feature of plant and microscopically involves leaf content, trichome, stomata etc. Botanical evaluation- sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements etc.

Evaluation of drugs means confirmation of its identity and determination of its quality and purity and detection of adulteration. This is done by certain microscopical and chemical tests.

Standardization describes study from birth of plant to its clinical application. It’s also include the herbal drugs preparation to a define content of a constituent or a group of
substance with known therapeutic activity respectively by addition of excipients or by mixing herbal drugs preparation. In other words it’s ensuring that every packet of medicine has correct ingredient in correct amount and will induce intended therapeutic effect. Various parameters for identification, evaluation and standardization of herbs are summarise in table 1.11. Examples of some herbs used for various diseases are given in table 1.12.\textsuperscript{[130]}

### Table 1.11: The various parameters for identification, evaluation and standardization\textsuperscript{[130]}

<table>
<thead>
<tr>
<th>Evaluations</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Authentication</td>
<td>A. Parts of plants collect like leaf, flower, root, stolen</td>
</tr>
<tr>
<td></td>
<td>B. Regional status</td>
</tr>
<tr>
<td></td>
<td>C. Family</td>
</tr>
<tr>
<td></td>
<td>D. Biological source</td>
</tr>
<tr>
<td></td>
<td>E. Chemical constituents</td>
</tr>
<tr>
<td>2. Morphology or Organoleptic</td>
<td>A. Odour</td>
</tr>
<tr>
<td></td>
<td>B. Taste</td>
</tr>
<tr>
<td></td>
<td>C. Size</td>
</tr>
<tr>
<td></td>
<td>D. Shape</td>
</tr>
<tr>
<td></td>
<td>E. Special feature</td>
</tr>
<tr>
<td>3. Microscopy evaluation</td>
<td>A. Leaf content</td>
</tr>
<tr>
<td></td>
<td>B. Trichomes</td>
</tr>
<tr>
<td></td>
<td>C. Stomata</td>
</tr>
<tr>
<td></td>
<td>D. Quantitative microscopy</td>
</tr>
<tr>
<td>4. Chemical evaluation</td>
<td>A. Chemical test</td>
</tr>
<tr>
<td></td>
<td>B. Chemical assay</td>
</tr>
<tr>
<td></td>
<td>C. Phytochemical screening</td>
</tr>
<tr>
<td>5. Physical evaluation</td>
<td>A. Moisture content</td>
</tr>
<tr>
<td></td>
<td>B. Viscosity</td>
</tr>
<tr>
<td></td>
<td>C. Melting point</td>
</tr>
<tr>
<td></td>
<td>D. Solubility</td>
</tr>
</tbody>
</table>
### 6. Biological evaluation

<table>
<thead>
<tr>
<th>Plants name</th>
<th>Biological name (family)</th>
<th>Part used</th>
<th>Chemical constituents</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nux-vomica</td>
<td><em>Strychnos nux vomica</em> (Loganiaceae)</td>
<td>Seeds</td>
<td>Strychnine, Brucine</td>
<td>CNS stimulants, bitter stomachic and tonic</td>
</tr>
<tr>
<td>Opium</td>
<td><em>Papaver somniferum</em> (Papaveraceae)</td>
<td>Dried latex from capsules</td>
<td>Narcotine, Papaverine</td>
<td>Narcotic analgesic, Diarrhoea</td>
</tr>
<tr>
<td>Rauwolfia</td>
<td><em>Rauwolfia serpentina</em> (Apocyanaceae)</td>
<td>Root</td>
<td>Reserpine</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>Pecac</td>
<td><em>Cephalelis ipecacuanha</em> (Rubiaceae)</td>
<td>Rhizomes and roots</td>
<td>Emetine, Cephahline</td>
<td>Antiamoebic, emetic And expectorant</td>
</tr>
</tbody>
</table>
1.2.1 Classes of Phytoconstituents:

Alkaloids

These are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen. The compounds have basic properties and are alkaline in reaction, turning red litmus paper blue. In fact, one or more nitrogen atoms that are present in an alkaloid, typically as 1°, 2° or 3° amines, contribute to the basicity of the alkaloid. The degree of basicity varies considerably, depending on the structure of the molecule, and presence and location of the functional groups. They react with acids to form crystalline salts without the production of water.

Majority of alkaloids exist in solid such as atropine, some as liquids containing carbon, hydrogen, and nitrogen. Most alkaloids are readily soluble in alcohol and though they are sparingly soluble in water, their salts of are usually soluble. The solutions of alkaloids are intensely bitter. These nitrogenous compounds function in the defence of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent biological activities. In nature the alkaloids exist in large proportions in the seeds and roots of plants and often in combination with vegetable acids.

Alkaloids have pharmacological applications as anesthetics and CNS stimulants. More than 12000 alkaloids are known to exist in about 20% of plant species and only few have been exploited for medicinal purposes. The name alkaloid ends with the suffix-ine an plant-derived alkaloids in clinical use include the analgesics morphine and codeine, the muscle relaxant (+) -tubocurarine, the antibiotics sanguinafine and berberine, the anticancer agent vinblastine, the antiarrhythmic ajmaline, the pupil dilator atropine, and the sedative scopolamine. Other important alkaloids of plant origin include the addictive stimulants caffeine, nicotine, codeine, atropine, morphine, ergotamine, cocaine, nicotine and ephedrine. Amino acids act as precursors for biosynthesis of alkaloids with ornithine and lysine commonly used as starting materials.
Glycosides
Glycosides in general, are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxy (occasionally thiol) compounds (invariably monohydrate in character), in such a manner that the hemiacetal entity of the carbohydrate must essentially take part in the condensation. Glycosides are colourless, crystalline carbon, hydrogen and oxygen-containing (some contain nitrogen and sulfur) water-soluble phytoconstituents, found in the cell sap. Chemically, glycosides contain a carbohydrate (glucose) and a non-carbohydrate part (aglycone or genin). Alcohol, glycerol or phenol represents aglycones. Glycosides are neutral in reaction and can be readily hydrolyzed into its components with ferments or mineral acids. Glycosides are classified on the basis of type of sugar component, chemical nature of aglycone or pharmacological action. The rather older or trivial names of glycosides usually as suffix ‘in’ and the names essentially included the source of the glycoside, for instance: strophanthinidin from Strophanthus, digitoxin from Digitalis, barbaloin from Aloes, salicin from Salix, cantharidin from Cantharides, and prunasin from Prunus. However, the systematic names are invariably coined by replacing the ‘ose’ suffix of the parent sugar with ‘oside’. This group of drugs are usually administered in order to promote appetite and aid digestion.

Glycosides are purely bitter principles that are commonly found in plants of the Genitiiaceae family and though they are chemically unrelated but possess the common property of an intensely bitter taste. The bitters act on gustatory nerves, which results in increased flow of saliva and gastric juices. Chemically, the bitter principles contain the lactone group that may be diterpene lactones (e.g. andrographolide) or triterpenoids (e.g. amarogentin). Some of the bitter principles are either used as astringents due to the presence of tannic acid, as antiprotozoan, or to reduce thyroxine and metabolism. Examples include cardiac glycosides (acts on the heart), anthracene glycosides (purging, and for treatment of skin diseases), chalcone glycoside (anticancer), amarogentin, gentiopicrin, andrographolide, ailanthone and polygalin reported that extracts of plants that contain cyanogenic glycosides are used as flavouring agents in many pharmaceutical preparations. Amygdalin has been
used in the treatment of cancer (HCN liberated in stomach kills malignant cells), and also as a cough suppressant in various preparations. Excessive ingestion of cyanogenic glycosides can be fatal. Some foodstuffs containing cyanogenic glycosides can cause poisoning (severe gastric irritations and damage) if not properly handled. To test for O-glycosides, the plant samples are boiled with HCl/H2O to hydrolyse the anthraquinone glycosides to respective aglycones, and an aqueous base, e. g. NaOH or NH4OH solution, is added to it. For C-glycosides, the plant samples are hydrolysed using FeCl3/HCl, and an aqueous base, e. g. NaOH or NH4OH solution, is added to it. In both cases a pink or violet colour in the base layer after addition of the aqueous base indicates the presence of glycosides in the plant sample.

**Flavonoids**

Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15 aromatic compounds) and numerous reports support their use as antioxidants or free radical scavengers. The compounds are derived from parent compounds known as flavans. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavonoids present in nearly 70% of plants. Other group of flavonoids include flavones, dihydroflavones, flavanflavonols, anthocyanidins, proanthocyanidins, calchones and catechin and leuco anthocyanidins.

**Phenolics**

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural colour pigments responsible for the colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections. They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavonones, flavones, xanthones and catechins) and (iii) non-flavonoid polyphenolics. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by
chlorogenic acid known to cause allergic dermatitis among humans. Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Other examples include flavones, rutin, naringin, hesperidin and chlorogenic.

Saponins
The term saponin is derived from Saponaria vaccaria (Quillaja saponaria), a plant, which abounds in saponins and was once used as soap. Saponins therefore possess ‘soaplike’ behaviour in water, i.e. they produce foam. On hydrolysis, an aglycone is produced, which is called sapogenin. There are two types of sapogenin: steroidal and triterpenoidal. Usually, the sugar is attached at C-3 in saponins, because in most sapogenins there is a hydroxyl group at C-3. Quillaja saponaria is known to contain toxic glycosides quillajic acid and the sapogenin senegen. Quillajic acid is strenutatory and senegen is toxic. Senegen is also present in Polygala senega. Saponins are regarded as high molecular weight compounds in which, a sugar molecule is combined with triterpene or steroid aglycone.

There are two major groups of saponins and these include: steroidal saponins and triterpene saponins. Saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis, they give aglycones. Saponins are extremely poisonous, as they cause haemolysis of blood and are known to cause cattle poisoning. They possess a bitter and acid taste, besides causing irritation to mucous membranes. They are mostly amorphous in nature, soluble in alcohol and water, but insoluble in non-polar organic solvents like benzene and n-hexane. Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for activity of cardiac glycosides. The two major types of steroidal sapogenin are diosgenin and hecogenin. Steroidal saponins are used in the commercial production of sex hormones for clinical use. For example, progesterone is derived from diosgenin. The most abundant starting material for the synthesis of progesterone is diosgenin isolated from Dioscorea species, formerly supplied from Mexico, and now from China. Other steroidal hormones, e.g. cortisone and
hydrocortisone, can be prepared from the starting material hecogenin, which can be isolated from Sisal leaves found extensively in East Africa.

**Tannins**

These are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannins are soluble in water and alcohol and are found in the root, bark, stem and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e. to convert things into leather. They are acidic in reaction and the acidic reaction is attributed to the presence of phenolics or carboxylic group. They form complexes with proteins, carbohydrates, gelatin and alkaloids. Tannins are divided into hydrolysable tannins and condensed tannins. Hydrolysable tannins, upon hydrolysis, produce gallic acid and ellagic acid and depending on the type of acid produced, the hydrolysable tannins are called gallotannins or egallitannins. On heating, they form pyrogallic acid. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. Common examples of hydrolysable tannins include theaflavins (from tea), daidezein, genistein and glycitein. Tannin rich medicinal plants are used as healing agents in a number of diseases. In Ayurveda formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhea.

**Terpenes**

Terpenes are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins or oleo-resins. Terpenoids includes hydrocarbons of plant origin of general formula (C5H8)n and are classified as mono-, di-, tri- and sesquiterpenoids depending on the number of carbon atoms. Examples of commonly important monoterpenes include terpinen-4-ol, thujone, camphor, eugenol and menthol. Diterpenes (C20) are classically considered to be resins and taxol, the anticancer agent, is the common example. The triterpenes (C30) include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes: amyrins, ursolic acid and oleanic acid sesquiterpene (C15) like monoterpenes, are major components of many essential oils. The sesquiterpene acts as irritants when applied externally and when consumed
internally their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated and broadly they have antimicrobial (particularly antiprotozoal) and neurotoxic action. The sesquiterpene lactone, palasonin, isolated from *Butea monosperma* has anthelmintic activity, inhibits glucose uptake and depletes the glycogen content in *Ascaridia galli*. Terpenoids are classified according to the number of isoprene units involved in the formation of these compounds.

**Anthraquinones**

These are derivatives of phenolic and glycosidic compounds. They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols. Other derivatives such as chrysophanol, aloe-emodin, rhein, salinosporamide, luteolin and emodin have in common a double hydroxylation at positions C-1 and C-8. To test for free anthraquinones, powdered plant material is mixed with organic solvent and filtered, and an aqueous base, e. g. NaOH or NH4OH solution, is added to it. A pink or violet colour in the base layer indicates the presence of an- thraquinones in the plant sample.\footnote{132}

**Essential oils**

Essential oils are the odorous and volatile products of various plant and animal species. Essential oils have a tendency to evaporate on exposure to air even at ambient conditions and are so referred to as volatile oils or ethereal oils. They mostly contribute to the odoriferous constituents or ‘essences’ of the aromatic plants that are used abundantly in enhancing the aroma of some spices. Essential oils are either secreted either directly by the plant protoplasm or by the hydrolysis of some glycosides and structures such as directly Plant structures associated with the secretion of essential oils include: Glandular hairs (*Lamiaceae* e. g. *Lavandula angustifolia*), Oil tubes (or vittae) (*Apiaceae* eg. *Foeniculum vulgare*, and *Pimpinella anismum* (Aniseed), modified parenchymal cells (*Piperaceae* e. g. *Piper nigrum* - Black pepper); Schizogenous or lysigenum passages (*Rutaceae* e. g. *Pinus palustris* - Pine oil. Essential oils have been associated with different plant parts including leaves, stems, flowers, roots or rhizomes. Chemically, a single volatile oil comprises of more than 200 different chemical components, and mostly the trace constituents
are solely responsible for attributing its characteristic flavour and odour.\textsuperscript{[134]} Essential oils can be prepared from various plant sources either by direct steam distillation, expression, extraction or by enzymatic hydrolysis. Direct steam distillation involves the boiling of plant part in a distillation flask and passing the generated steam and volatile oil through a water condenser and subsequently collecting the oil in florentine flasks. Depending on the nature of the plant source the distillation process can be either water distillation, water and steam distillation or direct distillation. Expression or extrusion of volatile oils is accomplished by either by sponge method, scarification, rasping or by a mechanical process. In the sponge method, the washed plant part e. g. citrus fruit (\textit{e. g.}, orange, lemon, grape fruit, bergamot) is cut into halves to remove the juice completely, rind turned inside out by hand and squeezed when the secretary glands rupture. The oozed volatile oil is collected by means of the sponge and subsequently squeezed in a vessel. The oil floating on the surface is separated. For the scarification process the apparatus Ecuelle a Piquer (a large bowl meant for pricking the outer surface of citrus fruits) is used. It is a large funnel made of copper having its inner layer tinned properly. The inner layer has numerous pointed metal needles just long enough to penetrate the epidermis. The lower stem of the apparatus serve two purposes; \textit{first}, as a receiver for the oil; and \textit{secondly}, as a handle.

Now, the freshly washed lemons are placed in the bowl and rotated repeatedly when the oil glands are punctured (scarified) thereby discharging the oil right into the handle. The liquid, thus collected, is transferred to another vessel, where on keeping the clear oil may be decanted and filtered. For the rasping process, the outer surface of the peel of citrus fruits containing the oil gland is skilfully removed by a grater. Initially, the liquid has a turbid appearance but on allowing it to stand the oil separates out which may be decanted and filtered subsequently. The mechanical process involves the use of heavy duty centrifugal devices so as to ease the separation of oil/water emulsions invariably formed and with the advent of modern mechanical devices the oil output has increased impressively.

The extraction processes can be carried out with either volatile solvent (\textit{e. g.} hexane, petroleum ether or benzene) resulting into the production of ‘floral concretes’\textsuperscript{-} oils with solid consistency and partly soluble in 95\% alcohol, or non
volatile solvents (tallow, lard or olive oil) which results in the production of perfumes. Examples of volatile oils include amygdaline (volatile oil of bitter almond), sinigrin (volatile oil of black mustard), and eugenol occurring as gein (volatile oil of Geum urbanum).

**Steroids**

Plant steroids (or steroid glycosides) also referred to as ‘cardiac glycosides’ are one of the most naturally occurring plant phytoconstituents that have found therapeutically applications as arrow poisons or cardiac drugs. The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man or animal. Steroids (anabolic steroids) have been observed to promote nitrogen retention in osteoporosis and in animals with wasting illness. Caution should be taken when using steroidal glycosides as small amounts would exhibit the much needed stimulation on a diseased heart, whereas excessive dose may cause even death. Diosgenin and cecadine (from Veratrum veride) are examples of plant steroids.

Chemical Evaluation includes chemical test, assay, isolation, purification and identification of active constituents are chemical methods of evaluation. Various chemical tests are used to identify chemical constituents present in herbs which may be responsible for its biological activity. Such chemical tests are summarised in table 1.13.

**Table 1.13: Chemical tests**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Reagents used</th>
<th>Color/precipitates formed</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mayer’s test</td>
<td>Potassium mercuric iodide solution</td>
<td>Cream precipitates</td>
<td>Alkaloids may be present</td>
</tr>
<tr>
<td>Wagner’s test</td>
<td>Potassium iodide solution</td>
<td>Brown precipitates</td>
<td>Alkaloids may be present</td>
</tr>
</tbody>
</table>
### CHAPTER-1

**Introduction**

<table>
<thead>
<tr>
<th>Test</th>
<th>Reagent/Condition</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hager’s test</td>
<td>Saturated solution of picric acid</td>
<td>Yellow colour</td>
<td>Alkaloids may present</td>
</tr>
<tr>
<td>Dragendorff’s solution</td>
<td>Ptassium bismuth iodide solution</td>
<td>Reddish Brown precipitates</td>
<td>Alkaloids may present</td>
</tr>
<tr>
<td><strong>Tests for amino acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Millon’s test</td>
<td>Millon’s white reagent</td>
<td>White precipitates</td>
<td>Amino acids may present</td>
</tr>
<tr>
<td>Ninhydrine test</td>
<td>Ninhydrin solution</td>
<td>Violet colour</td>
<td>Amino acids may present</td>
</tr>
<tr>
<td><strong>Tests for carbohydrates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Molisch’s tests</td>
<td>Alcoholic a-naphthol+sulphuric acid</td>
<td>Purple to violet colour rings</td>
<td>Carbohydrates may present</td>
</tr>
<tr>
<td>Barfoed’s tests</td>
<td>Barfoed reagents</td>
<td>Red colour (monosaccharide) after 10 min. colour form (disaccharide)</td>
<td>Monosaccharides or disaccharides may present</td>
</tr>
<tr>
<td>Selivanoff’s tests</td>
<td>Selivanoff’s reagents</td>
<td>Rose colour (keton)</td>
<td>Fructose like keto carbohydrates may present</td>
</tr>
<tr>
<td>Tests for pentoses</td>
<td>Hydrochloric acids + phloroglucinol</td>
<td>Red colour</td>
<td>Pentones may present</td>
</tr>
</tbody>
</table>

1.2.2 Extraction techniques for preparation of herbal plant extracts

**Ultrasonication-Assisted Extraction:**

The procedure involves the use of ultrasound waves, which have frequencies higher than 20 kHz, have great effects on extraction yield and kinetics. Unlike electromagnetic waves, sound waves must travel in a matter and they involve expansion and compression cycles during travel in the medium. Expansion pulls molecules apart and compression pushes them together. The expansion can create bubbles in a liquid and
produce negative pressure. The bubbles form, grow and finally collapse. UAE involves ultrasonic effects of acoustic cavitations. Under ultrasonic action solid and liquid particles are vibrated and accelerated and, because of that solute quickly diffuses out from solid phase to solvent.

**Microwave-Assisted Extraction**

Microwaves are electromagnetic fields in the frequency range of 300 MHz to 300 GHz or between wavelengths of 1cm and 1m. Microwave-assisted extraction (MAE) offers a rapid delivery of energy to a total volume of solvent and solid plant matrix with subsequent heating of the solvent and solid matrix, efficiently and homogeneously. Components of the sample absorb microwave energy in accordance to their dielectric constants. When plant material is immersed inside a microwave transparent solvent, the heat of microwave radiation directly reaches to the solid without being absorbed by the solvent, resulting in instantaneous heating of the residual moisture in the solid. Heating causes the moisture to evaporate and creates a high vapour pressure that breaks the cell wall of substrate and releases the content into solvent. The extracting selectivity and the ability of the solvent to interact with microwaves can be modulated by using mixtures of solvents. One of the most commonly used mixtures is hexane-acetone. During extraction the solvent volume must be sufficient to ensure that the solid matrix is entirely immersed. Generally, a higher ratio of solvent volume to solid matrix mass in conventional extraction techniques can increase the recovery. The main advantages of this technique are reduction in solvent consumption, shorter operational time, moderately high recoveries, good reproducibility and minimal sample manipulation for extraction process. This technique is used for extraction of nutraceuticals from plant source, in extracting anti-oxidative phenolic compounds from tomato, for extraction of phenols such as chlorogenic acids from green coffee beans.

**Accelerated Solvent Extraction**

It is a form of Pressurized Solvent Extraction technique in which solid–liquid extraction process performed at elevated temperatures, usually between 50 and 200 °C and at pressures between 10 and 15 MPa. Increased temperature accelerates the extraction kinetics and elevated pressure keeps the solvent in the
liquid state, thus achieving safe and rapid extraction. Also, pressure allows the extraction cell to be filled faster and helps to force liquid into the solid matrix. Elevated temperatures enhance diffusivity of the solvent resulting in increased extraction kinetics.\cite{139} The solvent used in ASE is usually organic solvents, sample is solid or semisolid. Compared with traditional Soxhlet extraction, there is a dramatic decrease in the amount of solvent and the extraction time for ASE.\cite{140} This technique performed with high extraction temperature may cause degradation of thermolabile compounds used for the extraction of high-temperature stable organic pollutants from environmental matrices, quantitative extraction of the flavonolignans, for rapid extraction of cocaine and benzoylcegonine from coca leaves.

**Counter-Current Extraction**

It is a liquid-liquid extraction process in which the solvent and the process stream in contact with each other flow in opposite directions. This method is continuous. Screw extractors and carousel extractors are the two type of equipments used for counter-current extraction.\cite{141} In this process, the material to be extracted is moved in one direction (generally in the form of fine slurry) within a cylindrical extractor where it comes in contact with extraction solvent. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end. This technique is done at room temperature, which spares the thermolabile constituents from exposure to heat which is employed in most other techniques.\cite{142} This technique is used in DNA purification, in food industry to isolate or eliminate particular flavours.

**Phytonics Process: An Extraction Methodology**

Advanced Phytonics Limited (Manchester, UK) has developed this patented technology termed “phytonics process”. The products mostly extracted by this process are fragrant components of essential oils and biological or phytopharmacological extracts which can be used directly without further physical or chemical treatment. The properties of the new generation of fluorocarbon solvents have been applied to the extraction of plant materials.
The core of The solvent used is 1, 1, 2,2- tetrafluoroethane, known as hydrofluorocarbon-134a (HFC-134a) having boiling point -25 °C, is non inflammable, nontoxic and does not deplete the ozone layer so eco friendly. By cosomizing solvent, the process can be made more selective specific class of phytoconstituents. The biological products made by this process have extremely low residual solvent. The residuals are invariably less than 20 parts per billion and are frequently below levels of detection. These solvents are neither acidic nor alkaline and, therefore, have only minimal potential reaction effects on the botanical materials. The processing plant is totally sealed so that the solvents are continually recycled and fully recovered at the end of each production cycle. The phytonics process can be used for extraction in biotechnology (e.g. for the production of antibiotics), in the herbal drug industry, in the food, essential oil and flavour industries, and in the production of other pharmacologically active products used in the production of top quality pharmaceutical-grade extracts, pharmacologically active intermediates.

There are some modern instrumental techniques available for identification, evaluation and standardization of herbs and herbal products. Content or assay is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeias. The choice of the extracting solvent depends on the nature of the compounds involved, and might be deduced from the traditional uses. Several chromatographic techniques, such as high-performance liquid chromatography (HPLC), gas chromatography (GC), capillary electrophoresis (CE) and thin layer chromatography (TLC), can be applied as quality assessment parameters.

**Chromatography and chemical fingerprints of herbal medicines**

The concept of phytoequivalence was developed in Germany in order to ensure consistency of herbal products. According to this concept, a chemical profile, such as a chromatographic fingerprint, for an herbal product should be constructed and compared with the profile of a clinically proven reference product.
Several problems influence the quality of herbal drugs:

- Herbal drugs are usually mixtures of many constituents.
- The active principle(s) is (are), in most cases unknown.
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable.
- Chemo-varieties and chemo cultivars exist.
- The source and quality of the raw material are variable.
- The methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) have an effect.

It is suggested that with the help of chromatographic fingerprints obtained, the authentication and identification of herbal medicines can be accurately conducted even if the amount and/or concentration of the chemically characteristic constituents are not exactly the same for different samples of drug. or, the chromatographic fingerprints could demonstrate both the “sameness” and “differences” between various samples successfully.

**Thin layer chromatography (TLC)**

TLC was the most common, versatile method of choice for herbal analysis before instrumental chromatography methods like GC and HPLC were established. Even nowadays, TLC is still frequently used for the analysis of herbal medicines since various pharmacopoeias such as Indian herbal pharmacopoeia, Ayurvedic pharmacopoeia; American Herbal Pharmacopoeia (AHP), etc. TLC is used as an easier method of initial screening with a semi quantitative evaluation. Thin-layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support,
its preparation and its use with different solvent. Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Under the usual experimental conditions employed in TLC, both the stationary phase and mobile phase remain ill defined and exist in the state of flux with vapor phase, all of which may change continuously during the separation process.

Contact with vapors, both prior to and during the chromatographic process, modifies the property of stationary phase. For adsorbents, the amount of activation and deliberate or accidental deactivation changes their chromatographic behavior.

The porosity of stationary phase changes and with it the apparent speed of the mobile phase. Capillary forces are stronger in the narrow inter particle channels, leading to more rapid advancement of the mobile phase. Larger pore below the solvent front are filled at a slower rate and result in increased thickness of the mobile phase layer. The fundamental parameter used to characterize the position of a spot in a TLC chromatogram is the Retardation factor or Rf value. It represents the ratio and its value ranges from 0 to 1 but ideal values are from 0.3 to 0.8.

TLC has the advantages of many-fold possibilities of detection in analyzing herbal medicines. In addition, TLC is rather simple and can be employed for multiple sample analysis. For each plate, more than 30 spots of samples can be studied simultaneously in one time. Thus, the use of TLC to analyze the herbal medicines is still popular.

It is most flexible, reliable and cost efficient separation technique. The advantage of automation, scanning, full optimization, selective detection principle, minimum sample preparation, hypenation, and so on enable it to be powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, food stuffs, and so on. For example, a TLC method is
developed to analyze the total saponin content, also referred to as the aescin content, in an herbal medicinal product containing two dry extract in capsules.

**High Performance Thin Layer Chromatography (HPTLC):**

HPTLC- High Performance Thin Layer Chromatography is a sophisticated and automated form of TLC. It is form of liquid chromatography in which the stationary phase is supported on a planar surface rather than a column. Separation in planar chromatography occurs because of differential migration velocities through the sorbet layer in a fixed separation time. HPTLC has developed to an extent that separation and quantitation can provide results that are comparable with another analytical method such as HPLC. HPTLC is used for standardization of herbs and herbal product. It can analyze the drug qualitatively as well as quantitatively in nanogram quantity and so now a days being a very popular method for standardization of marketed herbal formulation.

The features that distinguish chromatography from most other physical and chemical separation techniques are that two mutually immiscible phases are brought into contact where in one phase is stationary phase and other is mobile phase. The sample containing mixture of degradation product and drug substance is introduce into mobile phase and then it undergoes series of partitions many times between stationary phase and mobile phase as it being carried through the system by mobile phase.

It works on the same principle as of TLC. HPTLC layer thickness is typically 0.2 or 0.25 mm. Mean particle size is about 12 distances traveled by the sample compared to that traveled by the solvent front. Salient features of HPTLC are

- Simultaneous processing of sample and standard - better analytical precision and accuracy less need for Internal Standard
- Several analysts work simultaneously
- Lower analysis time and less cost per analysis
- Low maintenance cost
- Simple sample preparation - handle samples of divergent nature
- No prior treatment for solvents like filtration and degassing
Low mobile phase consumption per sample
No interference from previous analysis - fresh stationary and mobile phases for each analysis - no contamination
Visual detection possible - open system
Non UV absorbing compounds detected by post-chromatographic derivatization.

Preparation of standard and sample \[\begin{array}{c}
\text{Selection of chromatographic layer} \\
\text{Layer pre-washing} \\
\text{Layer pre-conditioning} \\
\text{Application of sample and standard} \\
\text{Chromatographic development} \\
\text{Detection of spots, scanning and documentation}
\end{array}\]

Schematic procedure for HPTLC

HPTLC instrument consist of following important components:
- Micro litre Syringe
- Spotting Device (Auto spotter)
- Development Chamber
- Scanner
- Software (proquant)

Steps involved in HPTLC
1. Selection of chromatographic layer
2. Sample and standard preparation
3. Layer pre-washing
4. Layer pre-conditioning
5. Application of sample and standard
6. Chromatographic development
7. Detection of spots
8. Scanning
9. Documentation of chromatic plate

Parameters that are affected by the changes in chromatographic conditions are:

1. Retention factor \( (R_f) \),
2. Peak purity.

1. **Retention factor \( (R_f) \):** Retention factor \( (R_f) \) is defined as the amount of separation due to the solvent migration through the sorbent layer as shown in the formula. It depends on time of development and velocity coefficient or solvent front velocity.

\[
R_f = \frac{\text{Migration distance of substance}}{\text{Migration distance of solvent front from origin}}
\]

2. **Peak purity:** The null hypothesis “these spectra are identical” can in this case (purity) with two sided significance. During the purity test the spectrum taken at the first peak slope is correlated with the spectrum of peak maximum \([r (s, m)]\) and the correlation of the spectra taken at the peak maximum with the one from the down slope or peak end\([r (m, e)]\) which is used as a reference spectra for statistical calculation. An error probability of 1 % only be rejected if the test value is greater than or equal to 2.576.

**Gas chromatography (GC)**

Gas chromatography (GC), also known as gas liquid chromatography (GLC), is a technique for separation of mixtures into components by a process which depends on the redistribution of the components between a stationary phase or support material in the form of a liquid, solid or combination of both and a gaseous mobile phase. It is well-known that many pharmacologically active components in herbal medicines are volatile chemical compounds. Thus, the analysis of volatile compounds by gas chromatography is very important in the analysis of herbal medicines. The GC analysis of the volatile oils has a number of advantages. Firstly, the GC of the volatile oil gives a reasonable “fingerprint” which can be used to identify the plant. The composition and relative concentration of the organic compounds in the volatile oil
are characteristic of the particular plant and the presence of impurities in the volatile oil can be readily detected. The extraction of the volatile oil is relatively straightforward and can be standardized and the components can be readily identified using GC-MS analysis. The relative quantities of the components can be used to monitor or assess certain characteristics of the herbal medicines. Changes in composition of the volatile oil may also be used as indicators of oxidation, enzymatic changes or microbial fermentation.

The advantages of GC clearly lie in its high sensitivity of detection for almost all the volatile chemical compounds. This is especially true for the usual FID detection and GC-MS. However, the most serious disadvantage of GC is that it is not convenient for its analysis of the samples of polar and non-volatile compounds. For this, it is necessary to use tedious sample work-up which may include derivatization.

**High-performance liquid chromatography (HPLC)**

High performance liquid chromatography (HPLC), also known as high pressure liquid chromatography, is essentially a form of column chromatography in which the stationary phase consists of small particle (3-50μm) packing contained in a column with a small bore (2-5mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high performance liquid chromatography most often used are ion exchange, partition and adsorption.

HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyze almost all the compounds in the herbal medicines. Thus, over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines.

It is necessary to notice that the optimal separation condition for the HPLC involves many factors, such as the different compositions of the mobile phases, their pH adjustment, pump pressures, etc. In order to obtain better separation, some new techniques have been recently developed in research field of liquid chromatography.
These are micellar electrokinetic capillary chromatography (MECC), high-speed counter-current chromatography (HSCCC), low-pressure size-exclusion chromatography (SEC), reversed-phase ion-pairing HPLC (RPIPC-HPLC), and strong anion-exchange HPLC (SAX-HPLC). They will provide new opportunities for good separation for some specific extracts of some herbal medicines.

On the other hand, the advantages of HPLC lie in its versatility for the analysis of the chemical compounds in herbal medicines, however, the commonly used detector in HPLC, say single wavelength UV detector, seems to be unable to fulfill the task, since lots of chemical compounds in herbal medicines are non-chromophoric compounds. Consequently, a marked increase in the use of HPLC analysis coupled with evaporative light scattering detection (ELSD) in a recent decade demonstrated that ELSD is an excellent detection method for the analysis of non-chromophoric compounds. This new detector provides a possibility for the direct HPLC analysis of many pharmacologically active components in herbal medicines, since the response of ELSD depends only on the size, shape, and number of eluate particles rather than the analysis structure and/or chromophore of analytes as UV detector do. Especially, this technique is quite suitable for the construction of the fingerprints of the herbal medicines. Moreover, the qualitative analysis or structure elucidation of the chemical components in herbal drug by simple HPLC is not possible, as they rely on the application of techniques using hyphenated HPLC, such as HPLC-IR, HPLC-MS, HPLC-NMR, for the analysis of herbal medicines. [143]

**Electrophoretic methods**

Capillary electrophoresis was introduced in early 1980s as a powerful analytical and separation technique and has since been developed almost explosively. It allows an efficient way to document the purity/complexity of a sample and can handle virtually every kind of charged sample components ranging from simple inorganic ions to DNA. The more or less explosive development of capillary electrophoresis since its introduction has to a great extent paralleled that of liquid chromatography. Most of the used techniques are capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE) and capillary isoelectric focusing (CIEF).
CE is promising for the separation and analysis of active ingredients in herbal medicines, since it needs only small amounts of standards and can analyze samples rapidly with very good separation ability. Also, it is a good tool for producing the chemical fingerprints of the herbal medicines, since it has similar technical characteristics of liquid chromatography. CE is a versatile and powerful separation tool with high separation efficiency and selectivity when analyzing mixtures of low-molecular-mass components.

**Hyphenation procedures**

For most (trace-level) analytical problems in the research field of herbal medicines, the combination of column liquid chromatography or capillary gas chromatography with a UV-VIS or a mass spectrometer (HPLC-DAD, CE-DAD, GC-MS and LC-MS, respectively) becomes the preferred approach for the analysis of herbal medicines. The additional and/or complementary information required in number of cases can be provided by, for example, atomic emission, Fourier-transform infrared (FTIR), fluorescence emission (FE), or nuclear magnetic resonance (NMR) spectrometry. It is demonstrated that, from a practical point of view, rewarding results can be obtained, since we need much more information to deal with the most complex analytical systems such as those samples from herbal medicines. With the help of chemometrics, a rather new discipline developed both in chemistry and statistics in the later part of the 1970s, we will definitely get more chance to deal with the difficult problems in the analysis of herbal medicines and also the problems in quality control of herbal medicines.

Thin layer chromatography which is particularly valuable for the qualitative determination. TLC is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of liquid. The adsorbent is relatively thin uniform layer of dry finely powdered material, applied to a glass, plate are the most communally used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption depending on the particular types of support its preparation and its use with different solvent system. A TLC method is developed to analyze the total saponin content, also
referred to as the aescin content, in an herbal medicinal product containing two dry extract in capsules.

HPTLC is used for standardization of herbs and herbal product. It can analyze the drug qualitatively as well as quantitatively in nanogram quantity and so now a days being a very popular method for standardization of marketed herbal formulation.

Other various Chemical and chromatographic techniques may be used to aid in identification of an herbal material or extract. Chromatographic technique such as HPLC, TLC, GC and capillary electrophoresis and spectroscopic methods such as IR, NMR, and UV-may also is used for fingerprinting. DNA fingerprinting has been widely used in many species, e.g. DNA fingerprinting of *Panax* species and their adulterants. Marker compounds may be used to help identify herbal materials, set specifications for raw materials, standardize botanical preparations during all aspects of manufacturing processes and obtain stability profiles.

Techniques commonly used in the field of phytochemistry are extraction, isolation and structural elucidation (MS, 1D and 2D NMR) of natural products, as well as various chromatography techniques. The list of simple elements of which plants are primarily constructed carbon, oxygen, hydrogen, calcium, phosphorus, etc. is not different from similar lists for animals, fungi, or even bacteria. The fundamental atomic components of plants are the same as for all life; only the details of the way in which they are assembled differs.

Phytochemistry is widely used in the field of Chinese medicine especially in the field of herbal medicine. Phytochemical technique mainly applies to the quality control of Chinese medicine, Ayurvedic medicine (Indian traditional medicine) or herbal medicine of various chemical components, such as saponins, alkaloids, volatile oils, flavonoids and anthraquinones. In the development of rapid and reproducible analytical techniques, the combination of HPLC with different detectors, such as diode array detector (DAD), refractive index detector (RID), evaporative light scattering detector (ELSD) and mass spectrometric detector (MSD), has been widely developed.
In most cases, biologically active compounds in Chinese medicine, Ayurveda, or herbal medicine have not been determined. Therefore, it is important to use the phytochemical methods to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. Modern pharmacological studies indicate that binding to receptors or ion channels on cell membranes is the first step of some drug actions. A new method in phytochemistry called biochromatography has been developed. This method combines human red cell membrane extraction and high performance liquid chromatography to screen potential active components in Chinese medicine.

1.3 Introduction to Phytopharmacology:
Phytopharmacology is a term coined by the Russian scientist David Macht in the 1930s. Macht used the term for the field of study of the effects of drugs on plants. The term has since changed its meaning to become an established field of drug research, where the active substances come from plants (a field Macht would have called zoopharmacology where the drugs are applied to humans or animals). The advantages of seeking medicines from plants are due both to the millions of years of co-evolution between plants and animals which has led to interactions between their constituent chemicals developing, and the nature of enzyme driven synthesis leading to optically pure chiral molecules whose reactions in the mammalian body can be very specific.

Many pharmacological preparations currently in use are derived from naturally occurring plant basis. Digoxin and aspirin are two of the earliest commercially refined plant preparations still available.

1.4 Introduction to plant: *C. occidentalis*

1.4.1 Vernacular Name: 
- Assamese: Hant-thenga
- Bengali: Kalkashunda
1.4.2 General Description: \cite{146}

*Cassia occidentalis* is an unarmed slender upright and short-lived (annual or biennial) shrub, 0.5-2.5 m tall, distinguished by foetid odour. Its once-compound leaves consist of 3-7 pairs of leaflets (2-10 cm long and 2-3 cm wide) that have pointed tips. There is a conspicuous dark-coloured gland near the base of the stalk of each leaf. Its flowers (2-3 cm across) have five yellow petals and are borne in small clusters in the upper leaf forks. Its fruit is a somewhat flattened, straight or slightly sickle-shaped, pod (7.5-13 cm long and 8-10 mm wide). This species gives off a foul odour when damaged.

1.4.3 Botanical Classification: \cite{130}

- Kingdom: Plantae
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Fabales
- Family: Fabaceae (alt. Leguminosae)
- Subfamily: Caesalpinioideae or Caesalpiniaceae
- Tribe: Cassieae
- Subtribe: Cassiinae
• Genus: Cassia
• Species: C. occidentalis
• Life cycle: Annu
• Biological name: Cassia occidentalis Linn.

1.4.5 Geographical distribution:
It is common weed scattered from foothills of Himalayas to West Bengal, South India, Burma and Shri Lanka. Indigenous to Brazil, it is also found in warmer climates and tropical areas of South, Central, and North America including the Amazon. The Cassia genus comprises some 600 species of trees, shrubs, vines, and herbs, with numerous species growing in the South American rainforests and tropics.

1.4.6 Cultivation and collection of the plant:
The plant requires dry and warm climate, bright sunshine, and occasional drizzle for good growth. It can grow in places where the average minimum and maximum temperatures fluctuate between 10 °C and 42 °C. A rainfall of 60-70 cm per year is sufficient for a good crop; however, 25-40 cm of rainfall was reported to be sufficient in dry areas. The plant is stripped three times during the season, first picking is in March and others before May; more picking can be taken later in October and December.

Immediately after picking the leaves are dried in sun. Quick drying ensures excellent green colour. The method of drying affects the percentage of sennosides in the leaves. In sun drying-sennosides are 2.98%; moisture, 70.60% and in oven drying (40 °C ± 2 °C) - sennosides are 3.03%; moisture 72.80%.

Ethnobotanical use of Cassia Occidentalis around the world

<table>
<thead>
<tr>
<th>Location</th>
<th>Ailment treated / Properties and Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>for abscesses, bile complaints, birth control, bronchitis, bruises, cataracts, childbirth, constipation, dysentery, edema, erysipelas, eye infections, fainting, fever, gonorrhea, guinea</td>
</tr>
</tbody>
</table>
worns, headache, hematuria, hemorrhages (pregnancy), hernia, increasing perspiration, inflammation, itch, jaundice, kidney infections, leprosy, malaria, pain (kidney), menstrual disorders, rheumatism, ringworms, scabies, skin diseases, skin parasites, sore throat, stomach ulcers, stomachache, swelling, syphilis, tetanus, worms, water retention, wounds

<table>
<thead>
<tr>
<th>Location</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amazonia</td>
<td>for abdominal pain, birth control, bile insufficiency, malaria</td>
</tr>
<tr>
<td>Brazil</td>
<td>for anemia, constipation, edema, fatigue, fever, gonorrhea,</td>
</tr>
<tr>
<td></td>
<td>liver disorders, malaria, menstrual disorders, skin problems,</td>
</tr>
<tr>
<td></td>
<td>tuberculosis, urinary disorders, water retention, weakness</td>
</tr>
<tr>
<td>Central America</td>
<td>for abortions, antifungal, athlete's foot, birth control,</td>
</tr>
<tr>
<td></td>
<td>constipation, diarrhea, fungal infections, headache,</td>
</tr>
<tr>
<td></td>
<td>menstrual disorders, menstrual pain, pain, respiratory infections,</td>
</tr>
<tr>
<td></td>
<td>ringworm, spasms, uterine pain, urinary tract infections, urinary</td>
</tr>
<tr>
<td></td>
<td>insufficiency, worms</td>
</tr>
<tr>
<td>Haiti</td>
<td>for acne, asthma, burns, colic, constipation, dropsy, eye infections,</td>
</tr>
<tr>
<td></td>
<td>gonorrhea, headache, malaria, rheumatism, skin rashes and infections,</td>
</tr>
<tr>
<td></td>
<td>and to increase perspiration</td>
</tr>
<tr>
<td>India</td>
<td>for abscesses, bites (scorpion), constipation, diabetes, edema,</td>
</tr>
<tr>
<td></td>
<td>fever, inflammation, itch, liver diseases, liver support, rheumatism,</td>
</tr>
<tr>
<td></td>
<td>ringworm, scabies, skin diseases, snakebite, wounds</td>
</tr>
<tr>
<td>Mexico</td>
<td>for chills, digestive sluggishness, dyspepsia, earache, eczema,</td>
</tr>
<tr>
<td></td>
<td>edema, fatigue, fever, headache, inflammation (skin), laxative,</td>
</tr>
<tr>
<td></td>
<td>leprosy, nausea, pain, rash, rheumatism, ringworms, skin problems,</td>
</tr>
<tr>
<td></td>
<td>sores, stomachache, swelling, tumors, ulcers, venereal disease,</td>
</tr>
<tr>
<td></td>
<td>water retention, worms, yellow fever</td>
</tr>
<tr>
<td>Panama</td>
<td>for colic, inflammation, spasms, stomach problems, worms,</td>
</tr>
</tbody>
</table>
and as an antiseptic

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<thead>
<tr>
<th>Country</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peru</td>
<td>for asthma, bronchitis, fever, liver problems, urinary insufficiency</td>
</tr>
<tr>
<td>Trinidad</td>
<td>for abortions, childbirth, colds, constipation, heart problems, inflammation, liver problems, palpitations</td>
</tr>
<tr>
<td>Venezuela</td>
<td>for asthma, colds, fever, intestinal gas, malaria, menstrual difficulties, skin problems, water retention</td>
</tr>
<tr>
<td>Elsewhere</td>
<td>for abdominal pain, abortions, bile insufficiency, birth control, bites (scorpion), childbirth, constipation, dermatosis, digestive problems, eczema, edema, eye infections, fevers, gonorrhea, headache, hemoglobin disorders, hemorrhage, hypertension, laxative, lice, liver, malaria, menstrual disorders, pain, parasites, rheumatism, ringworms, scabies, skin disorders, snakebite, spasms, urinary insufficiency, worms, yellow fever</td>
</tr>
</tbody>
</table>

1.4.7 Pharmacognostical studies:

1.4.7.1 Organoleptic Characters: [147]

The plant is annual shrub or undershrub, erect having 0.6 to 1.5 meter height, much branched. Branches are subglabrous, furrowed, and often purplish.

Leaves: Leaves are 15 to 20 cm long, arranged alternately along the stem, pitiolate, stipulate, peripinnate; Petiole with a distinct spherical gland that is 3-5 mm at its asymmetric base, 3 to 5 pairs of leaflets short stalked, ovate oblong to ovate lanceolate, acute or acuminate, glabrous, 4.5 to 10.5 cm length and 3 to 4.5 cm breadth. Leaves are green in colour, foetid in odour and slightly bitter in taste. They have slippery and papery texture, entire margin, pubescent surface and reticulate venations.
Figure 1.9: *C. occidentalis* Leaf

**Stem:** Erect, single purplish stem having glabrous surface and sparse branching. Young stems are four-angled, becoming rounded with age. The crushed foliage has an unpleasant odour.

Figure 1.10 *C. occidentalis* Stem

**Flowers:** Inflorescences are few-flowered axillary racemes with yellow-petaled flowers about 2 cm across. Flowers are with short peduncle, axillary and forming a terminal penicle, pedicels are 5 mm long elongating 1.3 cm in fruit, calyx is 1 cm long, petals are 5, subequal, 1.3 cm long, ovate – oblong, obtuse, yellow, faintly veined with orange, stamens. The species has 2n = 26, 28 chromosomes.
Fruit: Fruits are pod (legume) type having green color when unripe and brown when ripe colored, 5 mm thick, glabrous to slight pubescent, 7 to 12 cm long, straight to slightly curved.

Seed: Seeds are 40 or more in each pod which are ovoid, compressed at one end and rounded at the other, 6 mm long, 4 mm broad, hard, smooth, shining, dark olive green or pale brown in color.
Roots: Taproot with subroots

1.4.7.2 Microscopic Study:

Stem: Transverse section of stem shows a single layered epidermis composed of thin-walled cells covered externally by a thin cuticle. The cortex is composed of 8-14 layers of collenchymatous cells followed by 2 to 6 layers of parenchymatous cells. Endodermis is single layered, parenchymatous and found encircling the pericycle. Prismatic as well as rosette crystals of calcium oxalate are present in many cortical cells including endodermis, which shows the presence of only prismatic crystals. Each vascular bundle is capped by pericycle, which is represented in early stages by parenchymatous cells. Later many of these cells become thick walled and lignified and give rise to fibers and stone cells.

Leaf: The leaflet is dorsiventral in structure, the mesophyll being differentiated into palisade and spongy tissue. The upper epidermis is covered externally with moderately thick cuticle having horn like unicellular trichomes. The cells of the lower epidermis are somewhat rectangular in shape and arched outside and smaller than those of the upper epidermis. Stomata of paracytic type are present on both surfaces, but they are less abundant on the upper surface than the lower one. Anisocytic stomata also present in lower surface. Chloroplasts are present in abundance in the mesophyll cells.

Figure 1.14: T.S. of C. occidentalis leaf
1.4.8 Chemical constituents of the plant: [148]

The Cassia plants are well known for a group of chemicals with strong laxative actions called anthraquinones and a bianthraquinone. The ethanolic extract of C. occidentalis leaves yielded the flavonoid glycosides matteucinol 7-rhamnoside (I) and jaceidin 7-rhamnoside (II). The plant contains total alkaloids 0.13%.

The main plant chemicals in fedegoso include: achrosine, aloe-emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chrysobutusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol, emodin, essential oils, funiculosin, galactopyranosyl, helminthosporin, islandicin, kaempferol, lignoceric acid, linoleic acid, linolenic acid, mannotol, mannopyranosyl, matteucinol, obtusifolin, obtusin, oleic acid, physcion, quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, tannins, and xanthorin.

Chrysophanol and emodin, both free and their glycosides, and free physcion were found in the leaves, both free and glycosides of rhein and aloe emodin in the roots, and both free and glycosides of chrysophanol and physcion in the seeds of C. occidentalis. Total anthraquinones were higher in this species than in other Cassia species. Anthraquinone contents were higher in the seeds than roots and leaves.

Roots of C. occidentalis contains pinselin and 1,7-dihydroxy-3-methylxanthone in this plant may possibly be attributed to further utilization of the abundantly available 1,8-dihydroxyanthraquinone precursor chrysophanol via the intermediate 1,4,5-trihydroxyanthraquinone (helminthosporin).

Two new bis (tetrahydro) anthracene derivatives, occidentalol-I (III, R1 = Me and R2 = H) and occidentalol-II (III, R1 = R2 = H) were isolated from the roots of C. occidentalis alongue with chrysophanol, emodin, pinselin, questin, germichrysone, methylgermitorosone and singueanol-I (I, R1 = R2 = Me). The structures were established on the basis of spectral evidence.
Three C-glycosidic flavonoids, cassiaoccidentalins A, B and C, were isolated from aerial parts of C. occidentalis and their structures with a 3-keto sugar were established on the basis of spectroscopic and chemical evidence. While C. occidentalis does contain a small amount of these anthraquinones, it was shown in a rat study not to have the same strong purgative and laxative effects as others Senna. The main plant chemicals in C. occidentalis include: achrosin, aloe-emodin, emodin, anthraquinones, anthrones, apigenin, aurantiobtusin, campesterol, cassiollin, chryso-obtusin, chrysophanic acid, chrysarobin, chrysophanol, chrysoeriol, emodin, essential oils, funiculosin, galactopyranosyl, helminthosporin, islandicin, kaempferol, lignoceric acid, linoleic acid, linolenic acid, mannitol, mannopyranosyl, matteucinol, obtusifolin, obtusin, oleic acid, physcion, quercetin, rhamnosides, rhein, rubrofusarin, sitosterols, tannins, and xanthorin.

1.4.9 Medicinal Properties of the plant:
Plant pacifies vitiated vata, kapha, cough, bronchitis, allergy, asthma, fever constipation, diabetes, skin diseases, wounds and ulcers. Ayurveda indicated plant as Purgative, diuretic, febrifugal, expectorant, and stomachic. Leaves have been used internally and externally in scabies, ringworm and other skin diseases. A hot decoction is given as an antiperiodic. Seeds have been used for cough, whooping cough and convulsions. Roasted seeds (roasting destroys the purgative property) are mixed with coffee for strength.
Cassia occidentalis has been used as natural medicine in the rainforest and other tropical areas for centuries. Its roots, leaves, flowers, and seeds have been employed in herbal medicine around the world. The root is useful in ringworm, elephantiasis, and scorpion sting, and also used to cure snake bite. Fresh juice is useful in ringworm, heals wounds and cures ascites. The root is mostly used as a diuretic. The diuretic properties of the root are well recognized in India including many nations. In La Reunion, the root is considered bitter, tonic, and stomachic. Further, the root is said to be beneficial in obstructions of the stomach and in incipient dropsy. In Brazil, an infusion of the root-bark is used as a tonic and diuretic. The bark of the roots is used as quinine to cure fever. The washed and pounded bark mixed with black pepper and juice extracted after putting in a cloth is used to cure headache. Roots are considered a diuretic, tonic they are used for fevers, menstrual problems, tuberculosis, anaemia, liver complaints, and a decoction is made for fevers. The seeds are brewed into a coffee-like beverage for asthma, and a flower infusion is used for bronchitis. The plant can also be used for treatment of menstrual problems, tuberculosis, anaemia, constipation in babies.
The fruits of the plant are a cure for scorpion-sting. The seeds are bitter and considered to be a blood tonic and excellent diuretic. Seeds are also useful in cough and whooping cough. In the Konkan, the seeds are also used to treat seizures in children. The roasted and ground seeds are used as a blood tonic. The decoction of the powdered seeds in doses of half to two ounces, acts as a mild purgative.

The leaves are tasty; aphrodisiac, Protective against infection or poison (alexeteric), cure cough, hiccough, asthma, "kapha" and "vata"; sweetish, bitter, stomachic; cure "tridosha" fevers; good for sore throat and biliousness.

The leaves are boiled and the liquor drunk by children to cure worms. Further, the leaves have been reported to have prophylactic values in leucorrhoea, while the lotion made from its leaves is used as an eye-wash in tetanus. Paste made from its leaves with calcium hydroxide is abscesses and for pus clearance. The leaves also have therapeutic value in bone fracture. The leaves taken internally and applied externally are used to treat itch and other cutaneous diseases. Leaves are used in gonorrhoea, fever, edema, urinary tract disorders and menstrual problems, in Stomach colic, to remove worms and parasites from intestine, externally used for skin disorders, wounds, skin fungus, parasitic skin diseases, abscesses, and as a topical analgesic and antiinflammatory natural medicine. The whole plant is purgative, tonic, and febrifuge, and considered as a cure for sore eyes. In Madagascar, the whole plant is considered febrifuge. The constituent extracted from whole plant is used to make ointments used for various skin diseases.\(^{[150]}\)

Health practitioners today are employing Cassia Occidentalis in their practices much the same way it has been in traditional medicine for many years. It is an excellent natural remedy for bacterial and fungal infections and now is clinically shown to boost immune function simultaneously. As a liver tonic, science supports its beneficial action and use in various liver conditions including anemia, hepatitis, and liver damage (drug- or alcohol-induced). New research suggests, with its antimitogenic actions, Cassia Occidentalis could possibly help keep damaged liver cells from turning into cancerous ones, as often happens with chronic hepatitis B and C infections.
Although the seeds of *Cassia occidentalis* are used in herbal medicine in small amounts (and even roasted and brewed as a coffee substitute in some countries), several clinical studies have demonstrated the toxicity of the fresh and/or dried/roasted seeds. Ingestion of large amounts of the seeds by grazing animals has been reported to cause toxicity problems and even death in cows, horses, and goats. Due to the well-known and well-documented toxicity of these seeds, they are best avoided altogether. Toxicity studies on the aerial parts, leaves, and roots of *Cassia Occidentalis* have been published by several research groups. These studies reported that various leaf, seed and root extracts given to mice (administered orally and injected at up to 500 mg/kg) did not demonstrate any toxic effect or cause mortality.

1.4.10 Contraindications: [145]

*Cassia occidentalis* leaf extracts have demonstrated weak uterine stimulant activity and smooth-muscle relaxant actions in rats. As such, the use of this plant is contraindicated during pregnancy. *Cassia occidentalis* has demonstrated hypotensive activity in dogs and, as such, is probably contraindicated in people with low blood pressure. Individuals taking medications to lower their blood pressure should check with their doctor first before taking *Cassia occidentalis* (and monitor their blood pressure accordingly, as medications may need to be adjusted).

1.4.11 Drug Interactions: [145]

It may potentiate the effects of antihypertensive drugs. *Cassia Occidentalis* has demonstrated significant antihepatotoxic (liver protective), hepatotonic (liver tonic), and hepatic detoxification (liver detoxifying) effects in animal and human studies. As such, the use of this plant might interfere with the metabolism of some drugs in the liver by increasing the clearance of them and/or reducing their half-life (which may reduce the effects of those drugs that require metabolization in the liver).

1.5 Formulation of fast dissolving tablets:

Now-a-days, fast dissolving drug delivery systems are extensively used to improve bioavailability and patient compliance. Over the past three decades, Fast dissolving tablets (FDTs) have gained considerable attention as a preferred alternative to
conventional tablets and capsules due to better patient compliance, improved solubility and stability profiles. FDTs are solid dosage forms containing medicinal substances which disintegrate rapidly, usually in a matter of seconds, when placed on the tongue or placed in water or suitable solvent. New FDT technologies address many pharmaceutical and patient needs, ranging from enhanced life-cycle management to convenient dosing for paediatric, geriatric, and psychiatric patients with dysphagia. This has encouraged both academia and industry to generate new Fast dissolving formulations and technological approaches in this field.\textsuperscript{151}

Fast dissolving tablets dissolve or disintegrate instantly on the patient tongue or buccal mucosa. It is suited for tablets undergoing high first pass metabolism and is used for improving bioavailability with reducing dosing frequency to minimize side effect and make it more cost effective. This study was aimed, which can disintegrate or dissolve rapidly once placed in the oral cavity.\textsuperscript{151}

Herbal drugs comprise of a major share of all the officially recognised systems of health in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy. More than 70% of India’s 1 billion populations still use these herbal systems of medicine. Currently, there is no separate category of herbal drugs or dietary supplements, as per the Indian Drugs Act. However, there is a vast experiential-evidence base for many of the natural drugs. This offers enormous opportunities for Observational Therapeutics and Reverse Pharmacology. Evidence-based herbals are widely used in the diverse systems and manufactured, as per the pharmacopoeial guidelines, by a well-organised industry. Significant basic and clinical research has been carried out on the medicinal plants and their formulations, with the state-of-the-art methods in a number of Institutes/Universities.\textsuperscript{152} Indian medicinal plants also provide a rich source for antioxidants that are known to prevent/delay different diseased states. The antioxidant protection is observed at different levels. The medicinal plants also contain other beneficial compounds like ingredients for functional foods.\textsuperscript{152}

The most popular solid dosage forms are being tablets and capsules; one important drawback of this dosage forms for some patients, is the difficulty to swallow.
Drinking water plays an important role in the swallowing of oral dosage forms. Often times people experience inconvenience in swallowing conventional dosage forms such as tablet for example in the case of the motion sickness (kinetosis) and sudden episodes of coughing during the common cold, allergic condition and bronchitis. For these reason, tablets that can rapidly dissolve or disintegrate in the oral cavity or in water have attracted a great deal of attention.\textsuperscript{153} Fast dissolving tablets are also called as mouth-dissolving tablets, melt-in mouth tablets, Orodispersible tablets, rapimelts, porous tablets, quick dissolving etc. Fast dissolving tablets are those when put on tongue disintegrate instantaneously releasing the drug which dissolve or disperses in the saliva.\textsuperscript{154} The faster the drug into solution, quicker the absorption and onset of clinical effect. Some drugs are absorbed from the mouth, pharynx and esophagus as the saliva passes down into the stomach. In such cases, bioavailability of drug is significantly greater than those observed from conventional tablets dosage form.

The basic approach in development of FDT is the use of superdisintegrants like cross linked carboxymethyl cellulose (croscarmellose), sodium starch glycolate (primogel, explotab), polyvinylpyrrolidone (polyplasdone) etc, which provide instantaneous disintegration of tablet after putting on tongue, their by release the drug in saliva. The technologies used for manufacturing fast-dissolving tablets are freeze-drying, spray-drying, tablet molding, sublimation, sugar-based excipients, tablet compression, and disintegration addition. As a result of increased life expectancy, the elderly constitute a large portion of the worldwide population today. These people eventually will experience deterioration of their physiological and physical abilities.

\textbf{1.5.1 Criteria for Fast dissolving Drug Delivery System:}

The tablets should

- Not require water to swallow, but it should dissolve or disintegrate in the mouth in seconds.
- Be compatible with taste masking.
- Be portable without fragility concern.
- Have a pleasant mouth feel.
- Leave minimum or no residue in the mouth after oral administration.
Exhibit low sensitive to environmental condition as temperature and humidity.
- Allow the manufacture of the tablet using conventional processing and packaging equipments at low cost.

1.5.2 Salient Feature of Fast Dissolving Drug Delivery System:
- Ease of Administration to the patient who can not swallow, such as the elderly, stroke victims, bedridden patients, patient affected by renal failure and patient who refuse to swallow such as pediatric, geriatric & psychiatric patients.
- No need of water to swallow the dosage form, which is highly convenient feature for patients who are traveling and do not have immediate access to water.
- Rapid dissolution and absorption of the drug, which will produce quick onset of action.
- Some drugs are absorbed from the mouth, pharynx and esophagus as the saliva passes down into the stomach. In such cases bioavailability of drug is increased.
- Pregastric absorption can result in improved bioavailability and as a result of reduced dosage; improve clinical performance through a reduction of unwanted effects.
- Good mouth feel property helps to change the perception of medication as bitter pill particularly in pediatric patient.
- The risk of chocking or suffocation during oral administration of conventional formulation due to physical obstruction is avoided, thus providing improved safety.
- New business opportunity like product differentiation, product promotion, patent extensions and life cycle management.
- Beneficial in cases such as motion sickness, sudden episodes of allergic attack or coughing, where an ultra rapid on set of action required.
- An increased bioavailability, particularly in cases of insoluble and hydrophobic drugs, due to rapid disintegration and dissolution of these tablets.
- Stability for longer duration of time, since the drug remains in solid dosage form till it is consumed. So, it combines advantage of solid dosage form in terms of stability and liquid dosage form in terms of bioavailability.
1.5.3 Benefits of fast dissolving tablets

- Administered without water, anywhere, any time.
- Suitability for geriatric and pediatric patients, who experience difficulties in swallowing and for the other groups that may experience problems using conventional oral dosage form, due to being mentally ill, the developmentally disable and the patients who are un-cooperative, or are on reduced liquid intake plans or are nauseated.
- Beneficial in cases such as motion sickness, suede episodes of allergic attack or coughing, where an ultra rapid on set of action required.
- An increased bioavailability, particularly in cases of insoluble and hydrophobic drugs, due to rapid disintegration and dissolution of these tablets.
- Stability for longer duration of time, since the drug remains in solid dosage form till it is consumed. So, it combines advantage of solid dosage form in terms of stability and liquid dosage form in terms of bioavailability.

1.5.4 Limitations of Mouth Dissolving Tablets

- The tablets usually have insufficient mechanical strength. Hence, careful handling is required.
- The tablets may leave unpleasant taste and/or grittiness in mouth if not formulated properly.

1.5.5 Techniques for Preparing Fast dissolving Tablets

Many techniques have been reported for the formulation of Fast dissolving tablets or orodispensible tablets.

(i) Freeze drying / lyophilization
(ii) Tablet Moulding
(iii) Spray drying
(iv) Sublimation
(v) Direct compression
(vi) Mass extrusion
(i) Freeze-Drying or Lyophilization:
Freeze drying is the process in which water is sublimed from the product after it is frozen. This technique creates an amorphous porous structure that can dissolve rapidly. A typical procedure involved in the manufacturing of ODT using this technique is mentioned here. The active drug is dissolved or dispersed in an aqueous solution of a carrier/polymer. The mixture is done by weight and poured in the walls of the preformed blister packs. The trays holding the blister packs are passed through liquid nitrogen freezing tunnel to freeze the drug solution or dispersion. Then the frozen blister packs are placed in refrigerated cabinets to continue the freeze-drying. After freeze-drying the aluminum foil backing is applied on a blister-sealing machine. Finally the blisters are packaged and shipped. The freeze-drying technique has demonstrated improved absorption and increase in bioavailability. The major disadvantages of lyophilization technique are that it is expensive and time consuming; fragility makes conventional packaging unsuitable for these products and poor stability under stressed conditions.

(ii) Tablet Moulding:
Molding process is of two types i.e. solvent method and heat method. Solvent method involves moistening the powder blend with a hydro alcoholic solvent followed by compression at low pressures in molded plates to form a wetted mass (compression molding). The solvent is then removed by air-drying. The tablets manufactured in this manner are less compact than compressed tablets and posses a porous structure that hastens dissolution. The heat molding process involves preparation of a suspension that contains a drug, agar and sugar (e.g. mannitol or lactose) and pouring the suspension in the blister packaging wells, solidifying the agar at the room temperature to form a jelly and drying at 30°C under vacuum.

The mechanical strength of molded tablets is a matter of great concern. Binding agents, which increase the mechanical strength of the tablets, need to be incorporated. Taste masking is an added problem to this technology. The taste masked drug particles were prepared by spray congealing a molten mixture of hydrogenated cottonseed oil, sodium carbonate, lecithin, polyethylene glycol and an active ingredient into a lactose based tablet triturate form. Compared to the lyophilization
technique, tablets produced by the molding technique are easier to scale up for industrial manufacture.

(iii) Spray Drying:
In this technique, gelatin can be used as a supporting agent and as a matrix, mannitol as a bulking agent and sodium starch glycolate or croscarmellose or crospovidone are used as superdisintegrants. Tablets manufactured from the spray-dried powder have been reported to disintegrate in less than 20 seconds in aqueous medium. The formulation contained bulking agent like mannitol and lactose, a superdisintegrant like sodium starch glycolate & croscarmellose sodium and acidic ingredient (citric acid) and/or alkaline ingredients (e.g. sodium bicarbonate). This spray-dried powder, which compressed into tablets showed rapid disintegration and enhanced dissolution.

(iv) Sublimation:
To generate a porous matrix, volatile ingredients are incorporated in the formulation that is later subjected to a process of sublimation. Highly volatile ingredients like ammonium bicarbonate, ammonium carbonate, benzoic acid, camphor, naphthalene, urea, urethane and phthalic anhydride may be compressed along with other excipients into a tablet. This volatile material is then removed by sublimation leaving behind a highly porous matrix. Tablets manufactured by this technique have reported to usually disintegrate in 10-20 sec. Even solvents like cyclohexane; benzene can be used as pore forming agents.

(v) Direct Compression:
Direct compression represents the simplest and most cost effective tablet manufacturing technique. This technique can now be applied to preparation of ODT because of the availability of improved excipients especially superdisintegants and sugar based excipients.

(a) Superdisintegrants:
In many orally disintegrating tablet technologies based on direct compression, the addition of superdisintegrants principally affects the rate of disintegration and hence
the dissolution. The presence of other formulation ingredients such as water-soluble excipients and effervescent agents further hastens the process of disintegration.

(b) Sugar Based Excipients:
This is another approach to manufacture ODT by direct compression. The use of sugar based excipients especially bulking agents like dextrose, fructose, isomalt, lactitol, maltitol, maltose, mannitol, sorbitol, starch hydrolysate, polydextrose and xylitol, which display high aqueous solubility and sweetness, and hence impart taste masking property and a pleasing mouthfeel. Mizumito et al have classified sugar-based excipients into two types on the basis of molding and dissolution rate.

Type 1 saccharides (lactose and mannitol) exhibit low mouldability but high dissolution rate. Type 2 saccharides (maltose and maltitol) exhibit high mouldability and low dissolution rate.

(vi) Mass-Extrusion:
This technology involves softening the active blend using the solvent mixture of water-soluble polyethylene glycol and methanol and subsequent expulsion of softened mass through the extruder or syringe to get a cylinder of the product into even segments using heated blade to form tablet. The dried cylinder can also be used to coat granules for bitter drugs and thereby achieve taste masking.

1.5.6 Important Patented Technologies for Fast Dissolving Tablets

(i) Zydis Technology:
Zydis formulation is a unique freeze dried tablet in which drug is physically entrapped or dissolved within the matrix of fast dissolving carrier material. When zydis units are put into the mouth, the freeze-dried structure disintegrates instantaneously and does not require water to aid swallowing. The zydis matrix is composed of many material designed to achieve a number of objectives. To impart strength and resilience during handling, polymers such as gelatin, dextran or alginites are corporated. These form a glossy amorphous structure, which imparts strength.
To obtain crystallinity, elegance and hardness, saccharides such as mannitol or sorbitol are incorporated. Water is used in the manufacturing process to ensure production of porous units to achieve rapid disintegration while various gums are used to prevent sedimentation of dispersed drug particles in the manufacturing process. Collapse protectants such as glycine prevent the shrinkage of zydis units during freeze-drying process or long-term storage. Zydix products are packed in blister packs to protect the formulation from moisture in the environment.

(ii) Durasolv Technology:
Durasolv is the patented technology of CIMA labs. The tablets made by this technology consist of drug, filler and a lubricant. Tablets are prepared by using conventional tabletting equipment and have good rigidity. These can be packaged into conventional packaging system like blisters. Durasolv is an appropriate technology for product requiring low amounts of active ingredients. CIMA labs have developed Orasolv Technology. In this system active medicament is taste masked. It also contains effervescent disintegrating agent. Tablets are made by direct compression technique at low compression force in order to minimize oral dissolution time. Conventional blenders and tablet machine is used to produce the tablets. The tablets produced are soft and friable.

(iii) Flash Dose Technology:
Flash dose technology has been patented by fuisz. Nurofen meltlet, a new form of ibuprofen as melt in mouth tablets prepared using flash dose technology is the first commercial product launched by biovail corporation. Flash dose tablets consist of self-binding shear form matrix termed as “floss”. Shear form matrices are prepared by flash heat processing.

(iv) Wow tab Technology:
Wow tab technology is patented by Yamanouchi Pharmaceutical Co. WOW means “Without Water”. In this process, combination of low mouldability saccharides and high mouldability saccharides is used to obtain a rapidly melting strong tablet. The active ingredient is mixed with a low mouldability saccharide (eg. lactose, glucose,
and mannitol) and granulated with a high mouldability saccharide (eg. Maltose, oligosaccharides) and compressed into table.

(v) Flash tab Technology:
Prographarm laboratories have patented the Flash tab technology. Tablet prepared by this system consists of an active ingredient in the form of micro crystals. Drug micro granules may be prepared by using the conventional techniques like coacervation, micro encapsulation and extrusion spheronisation. All the processing utilized conventional tableting technology.

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Mechanism of Superdisintegrants:
There are four major mechanisms for tablets disintegration as follows.

(i) Swelling:
Perhaps the most widely accepted general mechanism of action for tablet disintegration is swelling. Tablets with high porosity show poor disintegration due to
lack of adequate swelling force. On the other hand, sufficient swelling force is exerted in the tablet with low porosity. It is worthwhile to note that if the packing fraction is very high, fluid is unable to penetrate in the tablet and disintegration is again slows down.

(ii) Porosity and capillary action (Wicking):
Disintegration by capillary action is always the first step. When we put the tablet into suitable aqueous medium, the medium penetrates into the tablet and replaces the air adsorbed on the particles, which weakens the intermolecular bond and breaks the tablet into fine particles. Water uptake by tablet depends upon hydrophilicity of the drug/excipient and on tableting conditions. For these types of disintegrants maintenance of porous structure and low interfacial tension towards aqueous fluid is necessary which helps in disintegration by creating a hydrophilic network around the drug particles.

(iii) Due to disintegrating particle/particle repulsive forces
Another mechanism of disintegratin attempts to explain the swelling of tablet made with ‘nonswellable’ disintegrants. Guyot-Hermann has proposed a particle repulsion theory based on the observation that nonswelling particle also cause disintegration of tablets. The electric repulsive forces between particles are the mechanism of
disintegration and water is required for it. Researchers found that repulsion is secondary to wicking.

**iv) Due to deformation**

During tablet compression, disintegrated particles get deformed and these deformed particles get into their normal structure when they come in contact with aqueous media or water. Occasionally, the swelling capacity of starch was improved when granules were extensively deformed during compression. This increase in size of the deformed particles produces a break up of the tablet. This may be a mechanism of starch and has only recently begun to be studied.

![Diagram of Deformation and Repulsion]

**Table 1.15: List of super disintegrants.**

<table>
<thead>
<tr>
<th>Super disintegrants</th>
<th>Example</th>
<th>Mechanism Of Action</th>
<th>Special comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crosscarmellose®</td>
<td>Crosslinked Cellulose</td>
<td>-Swells 4-8 folds in &lt; 10 seconds.</td>
<td>-Swells in two dimensions.</td>
</tr>
<tr>
<td>Ac-Di-Sol®</td>
<td></td>
<td>-Swelling and wicking both.</td>
<td>-Direct compression or granulation</td>
</tr>
<tr>
<td>Nymee ZSX®</td>
<td></td>
<td></td>
<td>-Starch free</td>
</tr>
<tr>
<td>Primellose®</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solutab®</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vivasol®L-HPC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crosspovidone</td>
<td>Crosslinked PVP</td>
<td>-Swells very little and returns to original size after compression but act by capillary action</td>
<td>-Water insoluble and spongy in nature so get porous tablet</td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>Crosspovidon M®</td>
<td>Crosslinked Starch</td>
<td>-Swells 7-12 folds in &lt; 30 seconds</td>
<td>-Swells in three dimensions and high level serve as sustain release matrix</td>
</tr>
<tr>
<td>Kollidon®</td>
<td>Primogel®</td>
<td>Crosslinked alginic acid</td>
<td>-Rapid swelling in aqueous medium or wicking action</td>
</tr>
<tr>
<td>Polyplasdone®</td>
<td>Sodium starch glycolate Explotab® Primogel®</td>
<td>Alginic acid NF Satialgine®</td>
<td>Calcium silicate</td>
</tr>
</tbody>
</table>