INTRODUCTION

In the current scenario, use of different traditional medicinal systems of India has been used all over the world and it is continued to be used mainly as primary health care in India. In the previous decade, there has been reestablished consideration and enthusiasm for the utilization of customary prescription all around. In china, conventional pharmaceutical records for around 40% of all medicinal services conveyed. As well as in Chile 71%, in India 65% and in Colombia 40% of entire human beings are using Herbals.

Though, a pharmacist is responsible for the entire process of manufacturing the pharmaceutical products, isolation or extraction of active principles from crude drugs has been become a chemical or biochemical operation, rather than a pharmaceutical one. The crude drugs obtained from different natural sources are used in treatment of wide spectrum of diseases. Medicinal plants are of great importance in the field of medicine and cure of diseases.

Down to earth understanding and a few current researches considers have demonstrated that treatment utilizing plant is superior to utilizing manufactured chemicals. Traditional drugs which human beings used for treatment of different disorders are mainly known as ethno-medications (Like Ayurveda, Unani, Siddha, Homeopathy and naturopathy).

There is still large number of medicinal plant in which all active constituents have not yet been investigated even though their medicinal effect is established by folklore and traditional system of medicine. (Padma et.al 1998)

1.1 Ayurveda –Indian system of medicine:

Ayurveda is the traditional system of medicines which contains different concepts. These are required to explain to validate it with the help of scientific parameters for betterment of human beings and to increase its use.
From the different concepts following one is a concept mentioned in Sharangadhar Samhita as follows.

नवान्ये व हि योज्यानन्द द्रव्यार्थ्यखिरकरतम म।
वचनाऽवदेवगुरुणाभयं गुडधार्याज्यकार्कैः॥ (श.शृविम ्ण-२/३२)

This concept gives idea about the use of drugs. This shlok means all the herbal drugs used as a drug should be in fresh form, except few like Krishna, Makshika, Dhanya, Vidanga, Ajya and Guda should be used after storage in old form. The drugs used after storage has good quality as compare to fresh form. The quality of drug is important for its therapeutic efficacy. The drug is the important in treatment of disease. The herbal drug should possess suitability, easily availability in different forms.

Around 65% of populace in India is accounted Ayurvedic system to utilize and restorative herbals to complete their essential human needs and wellbeing of these energetic customs credited to utilize and printed references and upheld by logical examinations. Ayurved can describe as science which imparts knowledge about life.

“हितात्हितं सुखं सुखम् रजस्यात्तरतयो हितात्हितं माने च तत्त्वं रजोऽक्तं अनुवर्तेऽ स हेथयंते ||”

Ayurveda is the science which depicts the traverse of life and tells about valuable and unsafe, upbeat and despondent life and furthermore gives rules for what is advantageous and harmful to life.

“भवचनबच सत्त्वथ अर्थोऽक्तं, आत्मनत्व विकारप्रवाहानम् ||”

The point of ayurved is to keep up the strength of a solid individual and to cure the illnesses.

Ayurveda consider individual in general. It trusts that irregularity in the Dosha, Dhatus and Malas produced ailment and the rebuilding of adjust in these
takes out infection. The point of treatment is to cure the infection as well as to find the reason so it may not happen in future. The point of treatment is additionally to enhance the essentialness and to fortify the resistant framework.

A deeper study of Ayurveda can suggest the activities, which can be tested by modern methodologist. Natural products are contemplated the standard of efficiency and safety as applicable to any modern drug should be applied and if found acceptable, the only they become real ayurvedic remedies, which can be any modern medical practitioner of any healing art.

In these times of iatrogenic (drug induce) disease and drug price causing economic catastrophes in families and to the country, it is necessary to take resource to ayurvedic not merely herbal remedies for wider benefit of ailing humanity and living being the world. Common items assume a key part in social insurance and pharma inquire about, the same numbers of medicines are either regular items or subordinates thereof. It is assessed that 40% of all prescriptions are either characteristic items or their semi synthetic subsidiaries.

This may not be astounding as home grown drug has been a convention of human services since old circumstances and regular concentrates screening has been one of the underlying foundations of pharma inquire about, where penicillin, erythromycin and rifampicin (bacterial contaminations), quinine and artemisinin (jungle fever), paclitaxel, vinblastin and vincristin (tumor), salix corrosive and non addictive cocaine subordinates (torment alleviation), are a couple of surely understood common item based pharmaceuticals. For bacterial contaminations, around 80% of the meds in clinical utilize are either common items or their subsidiaries, while around 60% of all hostile to malignancy specialists are either normal items or subordinates thereof.
1.2 Drug discovery and development using plants:

Nature has been a wellspring of therapeutic operators for a huge number of decades and a noteworthy quantity of current medications has confined by characteristic origins, especially plants and animals; about their utilization from ancient time. With use of therapeutic science, and combination of biosynthetic and synthetic innovation, regular item will enhanced different premise of their organic exercises to form powerful bioactive and chemotherapeutic operators.

Since ancient times, people have used different plants for curing diseases, for protection and in wellbeing, for using as fragrance to different sustenance. Plants are used in India and China from 5000 yrs ago in different human services. And in Europe, plants were being used as major pharmaceuticals from around 50 years ago. Pharmaceutical developments of India, Greece, China and Arabic nations mainly took place due to their own unique pharmaceutical formulations. It might be the most unrivaled framework regarding importance of study Ayurvedic system of medicines.

The China has reported its traditional uses of drugs earlier from 5000 to 4000 B.C. different scientists gave their contribution in the study of natural drugs like Pythagorus, Theophrastus (370-287 B.C.), Aristotle (384-322 B.C.) and Hippocrates (460-370 B.C.) Has large no. of references of natural entities till today. In 77 B.C. “De material Medical” written by a Roman physician named Discords, described properties and nature of about 500 traditional herbals well known at that era. This literature was the most reliable and authentic during 16th centuries.

During dark years much improvement in the herbals does not takes place and medicinal uses of plants was restricted to the physicians and tribal communities only. In 15th century as invention of printing takes place in
Europe, many people’s printed their knowledge in form of books and published. These books consist of much more true and false details about the natural medications. During that era ‘Doctrine of Signs or signatures’ comes in force which indicates the identity of plants and its organs for use of that plant as medicines.

India contains abundant number of herbals with medicinal importance. Maximum medicines are used as it is included in ‘Rigveda’ which is Religious Grantha of India. In traditional system of Ayurveda different plants used as medicines are mainly collected from wild source instead of cultivated source. The knowledge of herbals in India having its own value in modern system of medicines as well as in the traditional system.

The Ayurvedic system has initiate existence around 6000 BC and it is one of most complete and established system of medicines. Traditional plants have consumed large part in old traditional system of medications in maximum countries. Due to vast use of these plants, there is requirement for documentation of standardisation of these traditional pharmaceuticals. These studies help in quality confirmation of different parts of the plant and whole plants also.

Plants have been utilized by man from ancient times for calming, enduring and curing diseased conditions. When a solders harmed in fight or when they had a damage to body part like cut, and flow of blood took place that time they were using anything by trial and mistake from forests to stop pain and blood flow. They observed that some plants were more useful than others for the treatment of these complications. Man has additionally collected such use of plants from his perception of forest animals which utilizes plants for treatment and curing their complications. Even now days, we found that residential animals like cat or dog when experience heartburn or some other illnesses, goes to field and bite some grasses or plants get rid of that.
Tribal people of have their own traditional plants as well as tribal formulations in large number of nations. Even today also they are mainly depending on their own home grown formulations. Today pharmaceutical study has included lot of practice for utilization of traditional plants by verifying their dynamic standards and their activities for wellbeing of human beings. All of these have contributed towards their acknowledgement in prescriptions and their incorporation in pharmacopeia’s of different countries. Indigenous frameworks of medication used in India are predominantly depending upon utilization of plants. In Charak Samhita (1000 BC 100 AD) we can get record of about 2000 plants and vegetable which are used in cure of different disorders.

It was observed that some therapeutic crude drugs which were used from ancient times still have their importance in present treatment of disorders and yet another alternative drug of treatment is not present for the same disorder. The medicinal importance of herbals depends upon the specific chemical constituents present in the herbals like glycosides, resins, steroids, tannins, alkaloids, gums, volatile oils etc. These constituents present in different parts of the plants like fruits, roots, woods, leaves, stems, bark, seeds etc.

They have their place in present day treatment. Like "Ephedra" is utilized as drug from last 4000 years back in china and, is still specified in current pharmacopeia’s as well as one of the useful medication, Sarpgandha (Rauwolfia serpentina) which was surely understood in India as solution for hypertension has now demonstrated that one of its constituents, reserpine, is miracle medicate today for treatment of hypertension.

Quinine, another important medication against malarial infection as a one of the part of advanced prescription, was isolated from cinchona plant. Medications should be given immediately at the start time of infection to minimise severity of infections and to treat them. Medications used as drug are either identified from nature or are of isolated source. Hence, use and traditional uses of medications are as old as mankind.
Today it is conceivable to utilize molecular systems for identification of hereditary variability and labeling wanted qualities and also winnowing out copies in promotions. The accessibility of high throughput screening has made the likelihood of changing over "hits" into lead mixes in nearly brief time. Drug development from plant sources utilizing quality/sub-atomic strategies is ending up progressively imperative.

As far as present day inquire about attempt sedate improvement frame plants should essentially infer a multi-disciplinary approach. Latest investigations of ethno medical data, but in particular ranges of natural action, have all things considered affirmed that plants are a reservoir of chemical entities with marked potential.

The objectives of utilizing plants as wellspring of remedial specialists are a) To separate bioactive moieties for use as medications.

e. g. vincristin, digitoxin, reserpine, vinblastin, digoxin, taxol and morphine.

b) To deliver bioactive compound of novel or referred to structures as lead mixes for semisynthesis to create patentable elements of higher effect and lower poisonous quality.

e. g. nabilone, teniposide, amiodarone, metformin, oxycodon, verapamil and taxotere.

c) To utilize operators as pharmacologic moieties.

e. g. lysergic acid, yohimbine, diethylamide.

d) To utilize the entire plant or part of it as a herbal cure.

e. g. Cranberry, feverfew, garlic, gingko biloba, Echinacea.

India is perhaps greatest maker of traditional medications and is rightly known as "Natural Garden of World". There are a lot of ancient herbs being utilized
from number of years, in some structure, under indigenous systems of
treatment like Ayurveda, Siddha and Unani. Since 1947, India has increased
huge ground in agro improvement, systematization, creative work, process
development and quality control.

India composed of thousands of homemade medicaments which plays an
important role in pharmaceutical business sector. India is a worldwide supplier
of natural items which fits with universal benchmarks. Now a day India’s
focuses have begun to look for patentable qualities and thus utilizing most
recent hereditary building systems. About more than 450 valuable plants
have been recognized and screening is continued recognize different plants.

Now efforts has been taken to isolate the natural medicaments from traditional
herbs like manufactured medicaments by different processes like Extraction
using different solvent systems, like water, ethyl acetate, chloroform, ethanol
and much more. Initially improvement in extraction process and later
innovations in it gives fast growth in last few decades for investigation,
isolation, characterisation and formulation with utilisation of preparing plants
to any medications and utilising plants for different formulations. (Chaudhari
1996)

Plants kingdom plays an important role for existence of human being on earth.
As plants have the vital role in life of humans. Plants were being used as a
source of medicine from the times immemorial as they were easily accessible
as well as inexpensive; they have provided the basis for the oldest medicinal
systems of human history. The earliest mention of medicinal use of plant is
found in Rigved. (Patwardhan and Hooper 1992)

Pharmacognosy is closely related to the botany and plant chemistry. The
utilization of modern separation methods and pharmacological testing
technique implies that new plant moiety generally finds their way into
Phytochemical and Pharmacological Study of Traditional Indian Plants for Antiasthmatic Activity.

Pharmaceutical as isolated substance instead of using as a ayurvedic medicaments.

Ethnobotanical Aspects: - The “ethnobotany” term was named by scientist J. W. harshberger in year 1895 for indication of plants to be used by different communities. ‘ethno’ means study of people and ‘botany’ means study of plants.

Ethnobotany is one of the branch of ethnobiology. It mainly deals with study as well as evaluation of human society and plant. Ethnobotanical evaluation of plants is related with the effects of the plants on different human beings. It includes medicinal uses, traditional purposes, economic importance and some other effects which directly or indirectly affect human beings.

Phytomedicines have huge history of valuable application are attracting expanding consideration pharmaceutical research. Additionally, while drug advancement is mainly works along with single constituent along with its most precisely physiological action. (Trease and Evans, 2005)

Natural formulations are generally used as a major source of medications from ancient time. In the whole world every country has a large no of natural remedies for treatment of different disorders and different unpleasant conditions in human beings and animals. Now a day research is mainly focused on the identification of natural resources for treatment without harmful effects with prominent usefulness. The presence or absence of chemical constituents differ the pharmacological effectiveness of the herbal drugs. As well as the quantity of chemical constituents also changes the pharmacological activity.

Plants are important sources of medicines and in United State at the present about 35% of pharmaceutical prescriptions contain at least one plant derived ingredient. A lot of numbers of species not less than 25,000 medicinal herbs
species has been identified in India in which about 700 traditional substances use more than 900 herbals for treatment of various diseases.

Herbals are having high traditional use in folk use of medicinal plants as a treatment due to its easy availability safety and less cost. Research on plants gives identification of different important drugs. The plants are recognized for their ability to produce large quantity of sec. metabolites, out of which many of them have biological and pharmacological activities, used as precursor for the formulation and invention of latest effective dosage forms.

The herbals obtained from wild source has imp. Source of phytochemicals, but the supply of these crude drugs is very difficult due to its limited availability from nature as well as economical and technical problems during cultivation and collection. Herbal industries increasing day by day in production of formulations; so their demand of herbals is increasing at every moment.

Right now 80% of the total population relies upon herbal medicines for first line of health care for humans since it has no adverse actions. Now a day, extensive consideration has been given to use natural plant-based items for the protective action and cure of various human disorders.

Because of the adverse effect of synthetic medications, the western countries are searching for natural treatment which is effective and protective. It is found that 75% of the total population has confidence in conventional prescription, especially plant drugs for their essential health.

It is for the most part evaluated that more than 9000 plants in India are being used in traditional, herbal and folk medicines, around 75% of the medicinal requirement of the underdeveloped nations. (Tylers 2000)

Phytomedicine is term, which comprise of specific chemical constituents present in different parts of plant having specific pharmacological activity on
living organisms. These phyto-medicines are mainly called as phyto-
constituents, which are mainly utilised for long time or hundreds of years in
different forms to those of typical therapeutic uses.

Research improvement in the area of phyto-medicines of phyto-therapy has
suffered from different issues, like absence of assurance of patent due to
quality differentiation. In these, generally small scale commercial industries
are included when compared with other pharmaceutical businesses. Despite
of fact that built up rules for surveying adequacy security of phyto-medicines
are practically insoluble and it could force phyto-medicines to permit for
impracticable monetary utilisation on phyto-medicines.

Use of traditional formulations in distinctive area is diverse and this all may
cause different problems for harmonization of financial control around the
world. For improvement in natural formulations there are two primary criteria
i.e. restorative viability clinical trials.

Natural cures which are prominent in traditional use, manufacturers are
allowed to show important bibliographic information as the proof for inspecting
their prior licenses of right. Otherwise it must be considered as concession by
permitting powers so as to audit the additional confirmation as per
requirement. Now a day, maximum consideration has been given towards
utilization of plant based bioactive items for treatment of different human
problems. Identification of organic mixes of specific phyto-constituents is
really important in plant based formulations. These specific constituents are
from optional metabolites. Complete phyto-chemical identification of
traditionally important herbs has not been yet done in this way.

Traditionally society has plants to treat various diseases and to solve different
health problems. The information about uses of traditional drugs was very
sharp and accurate from traditional era.
About 80% of people in developed countries yet depending on customary medications for their crucial human formulation requirements and higher plants are essential wellspring of their remedial effectiveness. It was thought that traditional herbs have lost a considerable measure of their importance.

Then studies co-ordinated in world in midst of 1958 and 1972 revealed that 43% of total arrangements distributed from gathering medication stores contained one or more consequences of regular commencement as accommodating administrators. Out of these, about 27% were from higher plants, 14% are from microorganisms and 3% from animal source. Remained 56% was identified with made solutions.

As per data from over all survey, it was found that about 145 plants were mainly utilised as a part of current practice. There are a couple of complications behind less utilisation of traditional plants to use as remedy. Maximum pharmaceutical industries, which act as funding agencies for evaluation as of new drugs, are located in highly developed countries and biodiversity mainly present in developing countries where information about traditional uses of plants is easily available.

Industries were therefore not willing to invest into procurement of plant materials from these countries for development of formulation. Different Many plant isolated constituents are biologically active, did not get approval as drugs in modern medicines as they do not pass through required clinical trials due to their toxicity.

**Reasons for Faster Extension of Traditional Drug Market:**

These are main reasons due to which now a day’s maximum industries investing maximum attention in development of formulations of natural origin.
1. Identification of possible harmful and side effects from synthetic drugs and formulations.
2. Unavailability of synthetic medications against some disorders.
3. Easily availability of plant origin drugs at low cost.
4. Western countries demanding herbal formulations.
5. Different pharmaceutical industries showing their attention towards isolation and characterisation of new molecules from natural sources.

Till today plants and natural sources provided a lot of important drugs to human beings for their treatment and health care and for other requirements. But for some disorders yet there is unavailability of complete and satisfactory solution. So the search is yet going on to complete the requirements. Hopefully, natural sources will help us in identification of solutions for different complications.
1.2 ASTHMA

Asthma is a inflammatory disease of respiratory system mainly in chronic form which is characterized by acute exacerbation of hacking, dyspnoea, and wheezing and chest tightness mainly at evening or in the early morning. Patients usually have decreased expiratory volume and airflow reduction. It also causes hyper-responsiveness of bronchi, irritation of respiratory track. These are non specific to asthma.

Bronchial Asthma is one of the parameters of Shwasa. From the different respiratory disorders Bronchial Asthma is most severe disease. The mortality & morbidity of it is increasing day by day in whole world. This continuous increase in number of asthmatic patients required much more scientific research on different drugs along with its treatment and mechanism of asthma.

Now a day’s asthma became most common disease in developing countries. The prevalence of asthma is mainly due to urbanization in these countries. The pharmacological evaluation is important to confirm pharmacological effectiveness, toxicity and potency of crude drug.

Its increased morbidity, prevalence and death rates have perceived the developing seriousness of asthma in the community in the last 25 years. From 1980 to 1987 the predominance rate of asthma in the United States expanded by 29 %. Asthma is also expanding in severity and is a main cause of mortality throughout the world. (Martin et al., 1997)

Asthma is generally identified as chronic disorder of lungs characterized with reversible broncho-constriction, eosinophils increase, raised basic tone of airway, lymphocyte deposition, submucosal fibrosis, dysfunction of activated epithelial cell, damage to smooth muscles, hypertrophy of submucosal gland, edema in airway wall, overproduction of mucus and non-specific hyper responsiveness of airway to produce spasmogens. (Peter et al, 2003)
The condition influence more than 10-15% of the population in industrialized nations. Most specialists concur that it is expanding in predominance and seriousness (Rang et al., 2003).

**Fig. No.1.1 Developments of Asthmatic Symptoms**

Risk factors for asthma:
1. Genetic factor (family history, atopy)
2. Environmental factors (air pollution, allergen, cigarette smoke, and viral infection).

Bronchial Inflammation

Bronchial Hyper-responsiveness

Bronchial Contraction

Asthmatic Symptoms, Wheeze, Shortness of breath, Chest tightness, cough etc.

Risk factor for exacerbation:
1. Respiratory infection
2. Exercise and hyperventilation
3. Weather changes
4. Foods (e.g. Milk, Egg, nuts)
5. Drugs (e.g. NSAIDs, carbamazepine),
Phytochemical and Pharmacological Study of Traditional Indian Plants for Antiasthmatic Activity.

Fig. No. 1.2 Anatomy of asthma
SYMPTOMS OF ASTHMA:

- Difficulty in breathing,
- Wheezing with noise, mainly at time of expiration,
- Difficulty in talking, as well as disturbance in lung function.
- Tightness in chest and Dyspnoea
- Neck Muscle Tightness
- formation of sputum and after physical activity coughing
- Irregular and frequent coughing
- Feeling Frightened, exhaustion
- Occasional chest pain.

Severity of Asthma:

In industrialized countries this condition affects about 5-10% of population. Most authorities agree that it is increasing in prevalence and severity. (Rang et al, 2003) Around the globe between 100 to 150 million people suffers from asthma. This aspect of the statistics indicates that the number of asthma patients are increasing worldwide and death from these conditions has reached over 190,000 annually.

India has estimated 15-20 millions asthmatics. Genetic predisposition is one of the factors in children’s for increased prevalence. Others factors like urbanizations, air pollutions and tobacco smoke contributes more significantly. Allergic respiratory disorders in particular asthma is global phenomenon and is increasing in prevalence. As estimated 55 to 65 millions of Americans now a day suffers from allergies and asthma.

On average about 1500-1600 people die due to asthma every year in United Kingdom. According to the 1996 estimate and the children below 18 years are having the highest risk of asthma at 78.6 per 1000 populations. The present general pervasiveness in youngsters is assessed at 6.5 – 7.5 %, with a total of more than five million kids influenced.
Asthma is the 4\textsuperscript{th} leading reason for kid’s disability and most general reason for not attaining the school. The predominance in grown-ups is roughly 7%. Asthma predominance among African-Americans is extensively higher as compare to Caucasians or Hispanics, with Black kids having a 35% more prominent frequency examination with White youngsters in 1995-1996. (Bethesda 1997)

\begin{figure}[h]
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\caption{Figure No. 1.3 Airway constriction}
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1.1.1 The Characteristics of Asthma:
It is currently recognized that the asthma may be chronic or acute. The signs and symptoms and characteristic features of most cases of asthma are as follows:

\begin{itemize}
  \item Airway obstruction (reversible narrowing of the airways).
\end{itemize}
Airway inflammation with bronchial hyper activity. (Graig et al., 2004; Busse and Lemanske, 2001)

1.1.2 Types of Asthma

1) Extrinsic
   a) Atopic
   b) Non-atopic
   c) Occupational

2) Intrinsic (Idiosyncratic)
   a) Drug Induced
   b) Exercise induced asthma

3) Mixed.

1) Extrinsic asthma:
Type I hypersensitivity reactions Initiates the extrinsic asthma mainly due to the exposure to different allergic conditions or natural factor from environment like chemicals, fumes and dust etc.

a) Atopic asthma:
It is triggered by environmental natural antigens like pollens, foods, dusts and animal dander. In atopic asthmatic condition the number of T lymphocytes, eosinophils and mast cells get increased and it is mainly characterized by the airway inflammation. (Robbin’s and Cotran, 2004)

b) Nonatopic asthma:
This is activated by respiratory disease infections (e.g., parainfluenenza or rhinovirus, infection) rather than microbes are most regular reason. In non-atopic asthmatic condition increase in numbers of neutrophils and mast cells takes place. (Aletta et al., 2002)
c) Occupational asthma:
This is triggered by fumes, organic and chemical dusts, gases and other chemicals. In genetically susceptible individuals, allergens interact with dendritic cells. Resulting in stimulation B-lymphocytes to differentiate into plasma cell, which produce IgE antibodies, attach to mast cell & eosinophils, resulting in release of different inflammatory mediators. (Rang et al., 2003)

2) Intrinsic asthma:
Intrinsic asthma is mainly occurred by assorted, non-immune mechanism which mainly includes respiratory infection, intake of aspirin, inhalation of irritants, stress, cold environmental conditions as well as exercise.

a) Drug induced asthma:
Aspirin trigger asthma in aspirin sensitive asthma patient. Aspirin inhibits the cyclo-oxygenase pathway of arachidonic acid metabolism but it does not affect the lipo-oxygenase route, and so tipping the balance toward elaboration of the bronchoconstrictor leukotrienes.

b) Asthma Induced by Exercise:
It is a temporary airway narrowing or bronchospasm, which is induced by strenuous exercise.

Numerous patients don’t unmistakably fit into both of the two classifications and have blended highlights of both. These patients who create asthma in early life have solid sensitivity segment while

3) Mixed type asthma:
Some patients don’t fit in the above two categories and have combined features of both types. These patients who forms asthma in early life have strong sensitivity while those individuals who build up the illness late have a tendency to be non hypersensitive.
1.1.3 Pathogenesis Of Asthma:

The major factors are hereditary inclination to type1 hypersensitivity, acute airway inflammation, chronic airway inflammation and bronchial hyper responsiveness. The respiratory inflammation involves numerous cell types and different inflammatory mediators, like Type 2 helper T-cells and CD4+ helper T-cells, are major components of the bronchial irritation. Th2 cells releases the interleukin which promote inflammation due to hypersensitivity and thus β-cells get stimulated and produced IgE and different other antibodies.

Type 1-helper (T-1 cells), the other different CD4+ T-cells, releases interleukin-2 and interferon, and starts destroying of infectious microbes, viruses and different intracellular microbes with activation of cytotoxic T cells & macrophages.

Cytokines and other immunogenic stimulations released subgroup of T helper cells. They form a loop which forms inhibition of cytokines from T1 to T2 cells and in opposite direction. The disturbance in this opposite arrangement might be the cause of asthma. (Vinay et al., 1999)

Few Acute Inflammation And Associated Symptoms:

Sudden attack of asthma mainly causes due exposure to different allergens, viruses as well as outdoor and indoor pollutants. (Bousquet et al., 2000) When a person inhales allergen molecule, in allergic condition that leads to an initial phase of allergic symptoms that is followed by later phase symptoms. Activation of body cells which contains allergen-specific IgE gives start to the early phase reaction which further leads to sensitization of respiratory track macrophages and mast cells.
The sensitized cells release the pro-inflammatory mediators like eicosanoids, histamine and Reactive Oxygen moieties causing contraction of smooth muscles of respiratory system airway, vasodilatation and mucus secretion. (Jarjour et al., 1997) Inflammatory mediator activates the micro-vascular cells for secretion of plasma in the respiratory airway. (Bouquet et al., 2000) Plasma protein secretion induces thicker, edematous and engorged airway wall and reduction in the diameter of the airway lumen. Plasma release leads to reduced mucus clearance. (Wanners et al., 1996)

The late-phase inflammatory action occurs within 6-9 hours after the release of allergen and also involves formation as well as the activation of CD4+ T cells, macrophages, neutrophils, eosinophils and basophils (Bousquet et al., 2000). After allergen challenge activation of the T cells causes release of cytokines (T-helper cells) that is an important mechanism of late-phase responses (Kay, 1992). The release of cytokines by mast cells initially triggers the early recruitment of cells. This broncho constrictive reaction which is associated with the acute inflammation of cells results in minute symptoms which mainly include wheezing and dyspnea and shortness of that last for a day or less.
Figure No.1.4 Interaction between T-cells and β-cells
Chronic Inflammation Site and Cell Survival:
Eosinophilic apoptosis limits inflammatory tissue injury and promotes resolution rather than progression of inflammation. Reduction in apoptosis leads to self-perpetuating & chronic inflammatory survival. Chronic inflammation may be due to cell apoptosis reduction which causes self-perpetuating and chronic inflammatory survival and deposition of cells. There is increased expression of adhesion molecule on epithelial cells. Cytokines and chemokines that are over expressed in asthmatic airway may promote cell survival; these include granulocyte-macrophages-colony stimulating factor, IL-3, IL-5 and IL-8 (Busse and Lemanske, 2001). Antiasthmatic agents may resolve inflammation by causing apoptosis.

Development of asthma, mainly allergic type of asthma mainly occurs in two phases.
A) Immediate phase and 
B) Late phase.

I) The immediate phase of extrinsic asthma:
IgE –these are sensitized mast cells formed on the mucosal surface. These IgE’s initiates the initial phase and the resultant mediator release opens the intracellular tight junctions of the mucosal membrane and ultimately improves the entrance of antigen to the various submucosal mast cells.

In addition, direct incitement of sub epithelial parasympathetic receptor incites bronchoconstriction by the central as well as local reflexes. This happens immediately after stimulation and so called as acute or immediate response, which contains bronchoconstriction, edema (causing enhanced vascular permeability), mucus release and hypertension.

Mast cells also give the release of cytokines which causes influx of other leucocytes including lymphocytes, basophile, eosinophils, neutrophils and
monocytes. These inflammatory cells gives a reaction which begins 4-8 hrs and might remains for about 12 Hrs or more also.(Vinay et al., 1999)

II) The late phase of extrinsic asthma:-

It is late reaction which happens at a variable time after exposure to stimulus and may be nocturnal. It is a continuous progressive inflammatory response, start of which happens at the first phase, the influx of T-2 helper, lymphocytes having specific significance. It is caused by excessive mobilization of blood leukocytes that include basophils besides eosinophils and neutrophils. These result in further release of mediators, which accentuate the above-mentioned effects. Late phase leads to epithelial loss and the C fibers are most exposed to the irritant stimuli, which are believed to be the basis of hyper-sensitivity. Epithelial cells produce a large variety of cytokines when exposed to drugs, gases, and infectious agents along with to inflammatory mediators. (Sur et al., 1993)
Figure No. 1.5 Symptoms of asthma

- **Inflammation**: Asthma triggers irritate the lining of the bronchial tubes, causing them to become inflamed and swollen. Excess mucus makes breathing more difficult.

- **Bronchoconstriction**: During an asthma attack, bands of muscles surrounding the bronchial tubes contract, causing the airway to narrow.
Inflammatory Cells Involved In the Pathogenesis of Asthma:-

Chronic inflammatory disorder of respiratory system ia called as asthma. It is mainly contains various symptoms like respiratory track hyper sensitivity. Airway inflammation in asthma is characterized by an inflammation of eosinophils identified by an increase in number of activated eosinophils in broncho-alveolar lavage fluid (BAL), induced sputum and the bronchial biopsies, as well as increase in number of CD4⁺ T-helper type-2(Th2) lymphocytes. These lymphocytes are thought to arrange the degranulation of mast cells which are associated with the intense bronchospasm and eosinophilic inflammation which is a characteristic of the asthmatic airway.
Eosinophils:
Eosinophils release pro-inflammatory mediators like cytokines and cytotoxic mediators. Eosinophils moves through the airways by interaction with the selectin and at the same time it adheres to endothelium by binding to plasma protein integrins. Eosinophil survival is prolonged by GM-CSF and IL-5. Eosinophil activation releases mediators of inflammation like granule proteins, Leukotrienes to affect airway tissue. (Bousquet et al., 2000; Busse and Lemanske 2001)

Role of Eosinophils in Allergic Inflammatory condition:
Activation of mast cells and T2 cells takes place when an antigen gets inhaled in respiratory track. It induces formation of different inflammatory mediators like cytokines, leukotrienes and histamine which also includes interleukin-4 & 5.

This Interleukin-5 reaches to bone marrow & causes differentiation of eosinophils at terminal levels. Further during circulation eosinophils goes in allergic inflammation area; then starts migration towards lungs by interaction with protein selectin, attaching with the respiratory track endothelium of by binding with integrins to members adhesion proteins; immunoglobulin, intercellular ICAM-1, vascular-cell-1 (VCAM-1) adhesion molecule. Then eosinophiles pass through framework of airway with influence of different chemo-kines, granulocytes–macrophage colony-stimulation factor (GM-CSF), interleukin-5 and cytokines increases their survival.

On activation, the Eosinophils releases different inflammatory mediators like granule proteins and leukotrienes to injure respiratory track tissues. Along with this, eosinophils can also create GM-CSF to prolong as well as potentiates their contribution and survival for continuous inflammation of airway. (Busse and Lemanske, 2001)
**Lymphocytes**
Two types of T-helper cells are present. IL-2 & IFN-\(\gamma\) get produced by Th-1. Both are essentials for cellular defense mechanism. IL-4, 5, 6, 9 and IL-13 get produced by Th-2, that mediates inflammatory allergies. During the specimen evaluation of mucosal-biopsy of asthmatic patients activation of surface markers observed which mainly forms due to inhalation of allergenic lymphocytes. Two types of CD4 \(^{+}\)T helper cells are present in mice.
1. Helper T cell (Th1) which produced interferon-\(\gamma\) & interleukin-2. These are important for defense mechanism of cells.
2. Helper T cells (Th2) which forms different cytokines like interleukin -4, 5, 6, 9, 13 which transfers the allergic inflammation.

**Neutrophils:**
High quantities of neutrophils have been found in the respiratory track of patients who died due to sudden-onset of high risk asthma or fatal asthma. This recommends that neutrophils may have a important role in the onset of diseased condition. Neutrophils are major source for a different large variety of mediators’ like LTs, PGs, PAF, and TXs, which causes the bronchial hyper responsiveness and respiratory tract inflammation. (Sur et al., 1993)

**Mast Cells:**
These are formed by a specific precursor present in bone marrow & get matured under micro environmental factor of local tissue. These are essential in formation of hypersensitivity reaction development, by cross liking of the surface receptor for IgE which causing degranulation of mast cells and the release of vaso-active, nociceptive, and pro-inflammatory mediators which includes cytokines, histamine, and photolytic enzymes. The released mediators from mast cells each have more than one potent effect on airway inflammation. (Judith, 2002)
Figure No. 1.7 Interaction between T-Cells & β-Cells in IgE- Synthesis.
Phytochemical and Pharmacological Study of Traditional Indian Plants for Antiasthmatic Activity.

- **Inflammatory Mediators Of Asthma:-**

Asthma is chronic inflammatory disorder of resp. system which includes enactment of different structural and provocative cells, which releases the mediators of inflammation which results in the typical patho-physiological changes of asthma. (Peter *et al.*, 2003)

There are a few lines of identification that might shows presence of a mediator in the asthma. It might be mirror highlights of clinical asthma. The mediator might be formed in asthmatic patients.

Thus, mediators or their metabolites might found in the form of histamine in plasma, in the form of LTE4 in urine, more probably, in the respiratory track which can found in biopsies, broncho-alveolar lavage fluid, induced sputum, or in breathing air).

- **Amine Mediators:**

**Histamine:**
In asthmatic attacks Histamine plays the major role. Histamine is important for the immediate bronchoconstriction response. Histamine is a preformed mediator resent in the mast cells. The bridging between the adjacent IgE receptors present on mast cell surface gives Antigen-induced histamine secretion release. This causes a rise of cytosol Ca++. Calmodulin (CaM) seems to be the intracellular Ca++ receptor and Ca++-CaM complex through protein kinase causes protein phosphorylation and the enzyme activation. (Takei, 1992)

In the smooth muscle of bronchi, the stimulation of \( H_1 \) receptors leads to IP\(_3\) (Inositol 1, 4, 6 triphosphate) mediated release of intracellular Ca\(^{++}\) which leads to activation of Ca\(^{++}\)/calmodulin dependent myosin light chain kinase. In mammalian tissue Histamine show different response depending upon the receptors present on that tissue. (Kulkarni, 1976) As follows:
**Figure No.1.8** Inflammatory mediator release from mast cell.

<table>
<thead>
<tr>
<th>SN</th>
<th>Cell</th>
<th>Mediators</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eosinophiles</td>
<td>Major basic proteins like IL1, ECP, GM-CSF, ENDT, superoxide, LTC4, and IL-6.</td>
<td>Broncho-constriction, epithelial shedding, improvement of inflammation</td>
</tr>
<tr>
<td>2</td>
<td>T-lymphocytes</td>
<td>Different Cytokines</td>
<td>Promotion of aggravation</td>
</tr>
<tr>
<td>3</td>
<td>Basophiles</td>
<td>Histamine, IL-4 and LTC4,</td>
<td>Broncho-constriction</td>
</tr>
<tr>
<td>4</td>
<td>Macrophages</td>
<td>TNF-α, PGD₂, superoxide, protease,</td>
<td>Tissuadamage, chemo taxis, Mucus secretion, Broncho-constriction</td>
</tr>
</tbody>
</table>

**Table No.1.1** Cells involved during asthmatic inflammation

ECP- Eosinophil cationic protein, ENDT- Eosinophil derived neurotoxin, LT-leukotrienes, IL- interleukin, GM-CSF granulocyte macrophage colony-stimulating factor, TNF- tumour necrosis factor, PG- prostaglandin.
Adenosine:
During hypoxia the lung tissues released adenosine after allergen induced bronchoconstriction, mast cells also release adenosine. In normal health condition human bronchi doesn’t cause any contraction, but strongly constricts the asthmatic airway in vitro. Adenosine gives broncho-constrictor effect indirectly by activation of degranulation of mast cell. Adenosine releases histamine from mast cells. Adenosine percentage is more in the bronchoalveolar lavage fluid of asthmatic patient as compare to the normal human being. (Peter et al., 2003)

Lipid-derived mediators:

a) Leukotriens:
Arachidonic acid metabolism produces Leukotriens; a potent lipid mediator in cell or nuclear membrane. Different types of airway cells produces Leukotriens due to different types of stimuli, like eosinophils, mast cells, neutrophils, macrophages and epithelial cells. In pathogenesis of asthma Leukotrien acts as major mediator of inflammation. Leukotrien-A₄ hydrolyase synthesizes Leukotrien-B₄ in neutrophiles, which is extremely potent activator of neutrophils, which causes accumulation of degranulation and chemotaxis. (Peter et al, 2003).

Pharmacotherapy of Bronchial asthma:
The agents used in treatment of asthma can be categorised into two groups

1. Drugs acting on smooth muscle contraction. These drugs are also called as quick relief medications. E.g. β-adrenergic agonists, anticholinergics and methyl xanthenes.

2. Drugs preventing and acting against inflammation. These are also called as long term therapeutic agents. E.g. mast cell stabilizing agents, glucocorticoids, receptor antagonists and leukotriene inhibitors.
Drug Used In The Treatment Of Asthma:

1. Bronchodilator
2. Mast cell stabilizers
3. Anti-inflammatory
4. Antihistaminics

1) Bronchodilator:

Bronchodilator includes:

i) Sympathomimetic: e.g. Adrenaline, Ephedrine, Isoprenaline, Salbutamol, Salmeterol, Terbutaline.

They causes relaxation of smooth muscles of by stimulation of $\beta_2$-receptor, by a mechanism, which activates adenyl cyclase, in turn increasing level of intracellular 5-adenosine monophosphate (cAMP), which causes bronchodilation.

$\beta_2$ agonists are mainly used in relieving the bronchospasm which mainly occurs due to exercise. Terbutaline as non selective and epinephrine, isoproteorenol and ephedrine as selective $\beta_2$ agonists. Generally selective $\beta_2$ agonists are used as it has longer duration of action but minute side effects on cardiovascular system. When given by inhalation route it shows rapid onset of action within few minutes, peak effect within 30 min. And lasts till 3 -8 hours. $\beta_2$ agonists never given by I. V. Route.

In P2 adrenergic receptors, Isoetharine and Metaproterenoi are less selective and shorter acting, while in p1 and p2 adrenergic receptors isoproterenol activates both receptors equally and but it has shorter duration of action.

ii) Methylxanthines:

e.g. Theophylline, Aminophylline, theophylline, Hydroxyl ethyl-theophylline. They act as bronchodilation by inhibiting phosphodiesterase enzyme, there by
conversing c-AMP in bronchial smooth muscle cell. Thus, the sympathomimetic cause bronchodilation by promoting the formation of c-AMP, and the Methylxanthines act by inhibiting the destruction of c-AMP. Theophylline was used as important drug initially in treatment of asthma as bronchodilator. But due to its adverse effects and interactions with the other medications now a day’s not used commonly.

Theophylline also acts as an anti-inflammatory agent.

Xanthine acts by different mechanism of actions which gives pharmacological and physiologic effect it includes
1) Action on calcium concentration at intracellular,
2) Intracellular calcium uncoupling increases on contractile muscle moieties,
3) Cell membrane hyper polarisation by indirect effects on calcium concentration at intracellular,
4) phosphor-diesterases inhibition,
5) Adenosine receptors antagonism.

Methyl xanthenes are available orally. E.g. Caffeine, oxtiphylline and theophylline. Aminophylline is given by I. V. Route, it might causes cardiac arrhythmias. Methyl xanthenes never administered by inhalation. Theophylline causes fatal intoxication due to repeated use, but caffeine is safe as compare to Theophylline. It also causes palpitation, hypotension, nausea, dizziness, headache and precordial pain. (Barar, 2003)

iii) Anticholinergics

E.g. Atropine, Ipratropium bromide, Tiotropium bromide. Atropine and atropine like drug exert their bronchodilator effects by cholinergic muscarinic receptor competitive antagonism, which causes smooth muscle relaxation as well as causes bronchial muscle relaxation. Anticholinergic antiasthmatic drugs always given by inhalation route only. Ipratropium is one of the quaternary atropine derivatives it causes inhibition of bronchoconstriction. Ipratropium
causes bronchodilation with slow rate and it is less intense as compare to adrenergic agonists.

2) Mast cell stabilizers:
E.g. Sodium cromoglycate, Nedocromil sodium, Katotifen. They inhibit degranulation of mast cell by trigger stimuli. It inhibits the release of mediators like LTs, interleukin, PAF, and from different inflammatory cells as well as from mast cells. Used for maintenance therapy not in acute asthmatic attacks.

![Mechanisms of Action of Bronchodilator in Treatment of Asthma](image)

**Fig No.1.9** Mechanisms of Action of Bronchodilator in Treatment of Asthma

3) Anti-inflammatory drugs:

i) Corticosteroids:
Systemic: e.g. Hydrocortisone, Prednisolone
Inhalation: e.g. Beclomethasone, dipropionate, Flunisolide, Budesonide, Triamcinolone acetonide, Fluticasone, propionate. They reduce hyper reactivity of bronchi, suppressing inflammatory response to Antigen: Antibody reaction, reduction in mucosal edema as well as different other stimuli.
These are effective and most potent anti-inflammatory agents in asthma management. These can be given in form of inhalation but does not give quick relief. Triamcinolone, dexamethasone, budesonide, fluticasone, beclomethasone and flunisolide.

Examples of oral corticosteroids are prednisolone, prednisone, dexamethasone and methylprednisolone.

Methylprednisolone can administered by intramuscular route while methylprednisolone, dexamethasone and hydrocortisone will be administered by intravenously. These are helpful in treatment of all types of asthma in all patients. For long term use inhalational agents are always preferred as compare to oral steroids as inhalational asthmatics have fewer side effects as compare to oral. The dose of asthmatic drugs given by inhalation route should depend upon the condition of patient and severity of the disease.

It is generally twice a day. It causes irritation of throat, dysphonia, cough and oropharyngeal candidiasis. Higher dosages cause systemic adverse effects which mainly includes growth delay in children, adrenal suppression and osteoporosis.

Systemic corticosteroid which given for short term duration gives initial control on symptoms of asthma as well as on moderate to severe asthma symptoms. (Bethesda, 1998)

**ii) Leukotriene antagonists:**

e.g. Zafirlukast, Montelukast. They increase the recruitment of eosinophils and vascular permeability by acting as antagonist against cyst LT1 receptor mediated broncho-constriction.

These are potent anti inflammatory drugs in treatment of asthma and causes eosinophil infiltration, bronchoconstriction and increased mucus production.
These are potent in nature. It acts via lipoxygenase pathway via mast cells, eosinophils and alveolar macrophages. Eg. Zileuton and Zafirlukast

These are administered in the form of inhalation only and not by oral dosage form. E. g. nedocromil sodium & cromolyn sodium. These drugs are not useful in treatment of acute attack or on symptoms of attack but used only for prophylaxis of asthma.

Cromolyn releases histamine and forms inhibition of mast cell degranulation at pulmonary vessels. As well as it also causes leukotriene formation by interaction in between cell bound IgE and specific antigen. Cromolyn at less conc. Suppress the activation of chemo peptides on eosinophils, human neutrophils or monocytes.

Nedocromil gives similar effects but only with less conc. Nedocromil and Cromolyn both are safe with moderate to mild anti-inflammatory action, and act on exercise and allergen caused asthmatic condition.

iii) 5-Lipoxygenase inhibitor:
e.g. Zileuton. It mainly acts by inhibiting synthesis of leukotrienes from arachidonic acid by inhibiting release of 5- lipoxygenase, which mainly requires for the synthesis of leukotrienes.

4) Antihistaminic:
Terfenadine and Astemizole are few of newer potent H₁ –antagonist used in treatment of asthma. Terfenadine when administered orally it blocks the histamine-induced bronchospasm very effectively. It gives the maximum broncho-dilation and it also provides the limited protection from exercise-induced asthma. (Nagarajan et al., 1995)
Role of Herbs in management of asthma:
Synthetic medications give immediate cure in symptoms of asthma, but also causes large no of adverse effects like acidosis, drowsiness, ulcerative colitis, and anorexia. In addition, their effectiveness goes on diminishing with their persistent utilization. Natural medications like Ephedra, Boswelila, Tylophora, Coleus and Ginkgo etc., gives long term effects along with very less adverse reactions. In addition, dietary changes like natural vegetarian diet schedule and minimum use of beverages like coffee, salt, sugar, and chlorinated tap water; and intake of vitamin C might help the patient. Alternative therapy like- acupuncture may also be valuable for asthmatic patient along with naturals. About 30-35 % of today’s drugs contain plant derived constituents or molecules. (Jawla et al, 2010)

Herbal drugs against asthma are well known to India at the time of Atraya and Dhanvantari. In writings of Charaka and Shushruta, we find many herbs claimed to possess anti-asthmatic activity which is being used by practitioners of Indian medicine even today. Some of the important traditional herbs used as anti-asthmatics are – Atropa belladonna, Ephedra sinica, Albizzia lebbeck, Allium sativum. Clerodendron serratum, Piccrorrhiza kurroa, Tylophora asthmatica, Solanum xanthocarpum, Ficus racemosa etc.

Ephedra- It is the green stems of various Ephedra species –E.sinica, E.gerardiana (Ephedraceae), Ephedrine mainly contains alkaloids- Ephedrine, Pseudoephedrine, and Nor-ephedrine. Ephedrine has β1 and β2 agonist activity, Mostly β2-agonist activity accounts for ephedra’s effectiveness in treating bronchial asthma. When β2 agonist binds to the receptor on the cell membrane, a conformational change occurs in the receptor. This change causes transduction of a signal transduction which involves the activation of adenylate cyclase of G-protein. This leads to increase in cyclic AMP level in intracellular and cytoplasmic calcium concentration reduced which resulting in relaxation of smooth muscles. (Tylers et al 2000)
1.4 INTRODUCTION TO EXTRACTION OF CRUDE DRUGS

Extraction is the process of separation of required constituents from the crude drugs by using different processes or techniques. Generally solid – solid and solid- liquid extraction takes place on care of herbals.

Some process should be adopted before actual start of the extraction process which ensures the proper and maximum extraction of crude drugs.

1. **Authentication of the crude drug** - Proper identification and confirmation of the crude drug is required. Botanist can help for the identification of the crude drug and confirmation of its identity.

2. **Drying and Size reduction** - Crude drug should be dried immediately after collection in natural environmental conditions to avoid the growth of fungi and microbes as well as to avoid the deterioration of the active constituents. Crude drug can not used as it is in natural form due to its large particle size. Crude drug is size reduced for the proper and complete extraction. In case of large particle size solvent cant penetrate into the particles which affect the rate of extraction and if the particle size is very less then it will affect the process of extraction by blocking the extraction assembly as well as loss of solvent at the time of separation of extract, so proper particle size is required for extraction of crude drugs. Ideally it is about 60 -80 mesh size.

3. **Extraction Process:**

There are different types of extraction process:
1. Infusion
2. Decoction
3. maceration
4. percolation
1. **Infusion**: In this method of extraction boiling solvent is added in the powdered crude drug and it is kept for particular period of time in the mixed form of solvent and powder. The liquid then filtered and strained from the marc. And the extract I used as it is immediately after filtration. Or can be used for a week if it is stored in the refrigerator.

2. **Decoction**: in the preparation of decoction process powdered crude drugs are boiled in the suitable solvent for a sufficient period of time so that separation of soluble constituents will come in the solvent. The extract is filtered then and marc is separated.

3. **Maceration**: Maceration process is the process in which suitable solvent is mixed with powdered crude drug for about one day to seven days with intermediate shaking of the container. In the container suitable preservative is added to avoid the fungal growth or microbial contamination. After specific period of time the extract is filtered, marc is pressed and separated.

4. **Percolation**:  
Percolation is of two types- cold percolation and hot percolation.

**Cold Percolation**: In cold percolation process the powdered crude drug is kept in the percolator in a uniform form and required solvent is added from the top of the percolator slowly without disturbing the powdered crude drug. The extract is collected at the bottom of percolator.

**Hot Percolation**: In hot percolation process the powdered crude drug and solvent is mixed in sufficient quantity and heated for sufficient period of time.

**Soxhlet** extraction is the example of the continuous hot percolation method. This extraction is done with the help of soxhlet apparatus, which consist of three parts- RBF, Body and condenser of the apparatus.
Round bottom flask is attached at the bottom of the assembly which consists of sufficient quantity of solvent. Body of the soxhlet apparatus consist of extractor and side arm and syphon of the body. Extractor is filled with the powdered crude drug and side arms are useful for the flow of the solvent for extraction from RBF to condenser. Syphon of the body is useful for transfer of extract from body to the RBF. Condenser is attached above the body of soxhlet assembly. It is useful for the condensation of the vaporized solvent into the liquid form.

**Process of extraction by using Soxhlet apparatus**

Soxhlet assembly is arranged in the place with sufficient water supply and proper setup. Powdered crude drug is filled in the extractor of the assembly in the cloth bag. The solvent is filled in the round bottom flask. Condenser is assembled at the top of the body of soxhlet apparatus. Water supply is given to the condenser with adequate quantity.

Solvent required for the extraction is heated in the round bottom flask. Solvent get vaporized and transferred to the condenser through side arm of the body of soxhlet. In the condenser vapors get cooled and solvent get converted into liquid form. The condensed solvent fall down onto the powdered crude drug filled in the extractor and passes through it.

While solvent passes through the extractor, extraction of soluble constituents takes place and it passes through syphon tube into the round bottom flask. This process is continuing till extraction of the crude drug present in extractor takes place completely and the extract getting collected in the round bottom flask become more and more concentrated. The extract is separated, powdered is dried and again placed for extraction with another solvent.
Soxhlet extraction is mainly important for continuous extraction with different solvent useful for different types of crude drugs. Different types of solvent can be used with increasing polarity.

**Evaporation of the solvent**

Evaporation of solvent from the extract is done to concentrate the extract and to minimize the presence of solvent in the extract. Evaporation of the solvent is done carefully to avoid the loss of constituents in the extract like vaporization of volatile constituents. If the solvent for extraction is non-volatile like water then it is done without recovery from the evaporation. And if the solvent is volatile or inflammable and expensive then the recovery of the solvent from the extract is done by evaporation of the solvent in closed condition with help of distillation apparatus.
Fig. No. 1.10 Soxhlet Assembly
1.5. Major difficulties in Standardization of Herbals:

1.5.1. Uncertainty about activity and quality of constituents:
Herbal drugs are chemically complex in nature and it contains different no of constituents. Standardization of these large no of active constituents of the drugs does not gives any possible pharmacological activity, as the biological activity is not completely depending upon only one active constituents but it is mainly due to the overall chemical composition of the plants. Different other constituents are also present in plants which do not affect directly the pharmacological effect, but their presence might influence the bioavailability & excretion of the active constituents.

Sometimes the rate of side effects may be reduced and the stability of the active components might be increased due to presence of these inert constituents. If active constituents are more in crude drug then it might have
synergistic or additive action. As the biological activity depends on several components and it is not possible to optimize all the constituents during quality control.

1.5.2. Uneven composition of Chemical Constituents:

The chemical composition of the medicinal is depending on following factors which may change the chemical constituents of the raw materials.

1. Geographical variation,
2. Natural climatic condition,
3. Association with other plants,
4. Harvesting time,
5. Handling after harvesting of plants,
6. Storage of crude drugs,
7. Size reduction, separation, drying and extraction process,
8. Manufacturing of formulations,
9. Instruments used,
10. Storage of finished formulations.

To minimise some of above causes one can collect the crude drugs from cultivated source rather than wild source. Other complications include the presence of adulterations and substituents, toxic plant constituents, contamination due to the insecticides and pesticides, micro organisms, fumigating agents and heavy metals.

So during the quality control of medicinal plants, complicated composition of constituents and its complex nature are also considered. We should remember that herbal medicines are not similar to that of synthetic medicines and we cannot control it like allopathic medications. So for quality control of natural drugs we should have different approach than the synthetic one.
1.5.3. Current approach in standardization of herbal medicines:

Now a day’s different modern analytical technique like chromatographic techniques like HPTLC, TLC and HPLC & GC etc can utilised in evaluation of crude and processed herbals. Herbal medicines are evaluated by chemo profiling of the plant. Chemo profiling of the plant & its relation with the biological activity are by the following way-

- If the active constituents are unknown then the constituents which are present in large concentrations can be used as marker compounds for standardisation. For example In Aegle marmelos, aeglin is used as marker, even though aeglin is not linked with the recommended therapeutic activity of Bilva.

- If the active constituents present in the herbs, have the prominent pharmacological activity then they can be considered as marker compounds. And they can be used for the standardization of herbals. For example in Withania somnifera concentration of active constituents withanolides are used as marker for standardization of ashwagandha extract.

- In the process of preparative TLC of herbal extract, TLC of extract was done for number of times and the 4-5 compounds predominantly present in the TLC were taken as a marker for standardisation of extracts. The compounds were isolated, purified, chemically characterised and then correlated with the biological activity of that particular crude drug. In practical standardisation TLC of marker compound is compared with the TLC of the extract.

- More attention is given on the selection and manufacturing process of herbals should be done in presence of well qualified person.
• Utilisation of latest techniques for observation of process of manufacture,

• Formulation process should be done with latest facilities and processes to form best quality medicines,

• In all over India only one centre is available for standardisation of formulation and monitoring the quality of herbal drugs and preparations. It is done by ICMR (Indian Council of Medical Research).

**Development of standardization parameters for assessment of crude drugs according to WHO guidelines**

WHO has given small documentation which contains some general methods for testing the identity (macroscopic, microscopic, chemical, TLC finger printing) of the crude drug. It includes different quality parameters such as extractive value, ash value, moisture content, volatile oils determination & TLC for confirmation of quality and purity. World Health Organisation has given general limit for pesticidal residue, heavy metal content, microbial contamination and radioactive compounds.

In the process of testing and identification of crude drugs following steps are generally implemented:

1. **Sampling**

Before a drug can be evaluated, sample must be taken for analysis. The sample collected must be the representative of the material which is being undergoing tests. If the sample size is more than 100 kg then around 500gm is taken.
2. Authentication

The authenticity of a crude drug is established by comparing the plant material collected from the appropriate region of the country, at an appropriate stage of its growth with the description of is given in the pharmacopeia or the other official publications of that country.

3. Organoleptic Evaluation or Macroscopical Evaluation

Organoleptic evaluation is done by means of organs of sense, which is sufficient to evaluate the drug to be identified. This gives the idea about the quality of drugs. This evaluation includes general appearance, taste and odour of the drugs. For Example-

- In cases of barks, roots, rhizomes, etc shape and size.
- In cases of leaves types of shape, margin, bars, apex.
- In cases of flowers like pyrethrum, saffron, types of fluorescence.
- In case of fruits like cardamom, beal, their size, shape, surface characteristics are the identification features..
- Taste in case of *Phyllanthus amarus*, which are bitter in taste whereas *P. fraternus* is not bitter and *P. madresp enfens* is slight bitter in taste.

4. Microscopical Evaluation

This evaluation is not only helps in the study of the presence of adulterants but also helps in the correct identification of the medicinal plants. For this purpose the drug is soaked in water if it is not fresh then fine transverse section is taken and stained for the study of the arrangement of the cells important staining liquids used are Phloro-glucinol and HCL for lignified tissues, Ruthenium red for Gums and Mucilage containing cells, chlor-zinc iodide for cellulose tissues, Picric acid for Aleorane grains, Sudan red for Volatile oil content. The slides of this test drug are compared with slides of the
authentic crude drug description given in the references book. This helps in the study of substances like lignified content, volatile oils, starch grains, fixed oil, aleuronic grains, calcium oxalate, mucilage etc.

For Example: *P. amarus* shows wavy walled epidermal parenchyma whereas *P. Madraspatensis* shows straight walled epidermis parenchyma. The study of the surface constants is the important phenomenon of histological evaluation which includes palisade ratio, stomatal index, stomatal number, vein islet number etc. and using camera Lucida. These features are used for the authentication of leaf drugs and to detect adulterants and also help to differentiate closely related species. For Example- Anisocytic stomata in *P. madrespatensis* whereas both paracytic and anisocytic stomata in *P. amarus*. Microscopical evaluation is also essential in the powdered drugs. Such diagnostic microscopical feature helps in plants drugs Standardization.

5. Foreign Matter:

Other part of the plant or organic matter present in the crude drug which is not complying with authentic drug is to be considered as foreign matter. Other plants or parts of same plant might be present due to improper harvesting and garbling. During storage insects, moulds, animal excreta will add to the crude drug. The Medicinal plant material can be entirely free from them, which is difficult. So, Pharmacopoeias provide the limit for the presence of other parts of the plants.

6. Moisture contents:

The deterioration of the compound is occurred by moisture content. The moisture content in the compound activates the enzyme and promotes the growth of microbes or enhances the degradation of constituents of crude drugs which are responsible for the deterioration. Then these drugs have no
economical importance. There are a number of methods which are used to identify the moisture content; these are as follows-

- Karl Fischer method
- Loss on drying
- Azeotropic distillation method.
- Loss on drying:

The LOD of crude drug sample is calculated by heating the crude sample in an oven at 105 °C for 30 min. The process is repeated till it gives constant weight. LOD is done for the purpose of removal of water and moisture from crude drug and to determine the weight loss of drug after drying the sample. Water and moisture content in the sample mainly increases weight.

7. Extractive Values:

The pharmacological action of herbal mainly occurs when pharmacologically active constituents are present in particular drug. The solubility of these constituents is mainly depending upon the polarity of solvents used for extraction. If active constituents are completely soluble in a specific solvent, this is mainly called as extractive values. Thus extractive value gives the ideas about pharmacological activity of a crude drug. We can determine the polarity of compound.

8. Ash value:

After complete incineration of crude drug, the crude drug removes the inorganic salts which are considered as ash. The determination of ash values indicates the purity and quality of the crude drugs. There are various types of Ash value which are determined as
i) Total Ash:
The total ash is the ash in which carbon and organic matter of the drug are present. For the preparation of ash from drug is required the temperature 450 °C or above till it attains a constant weight. It mainly contains phosphates, carbonates, silica and silicates. Total ash can be used further to determine the following

ii) Water-soluble ash:
In this method water insoluble material is removed to find the water soluble ash content. It is obtained by separation of water-soluble content from the total ash, which is then dried to get water-soluble ash.

iii) Acid insoluble ash:
This is determined by treatment with dilute HCl. It removes different inorganic salts to yield the insoluble ash.

iv) Sulphated ash:
The crude drug is incinerated at a temperature of about 600° C with dil H₂SO₄ before ignition. This process converts all oxides & carbonates to sulphate salt.

NEED AND OBJECTIVE FOR THE STUDY:

Nature has an important part of mankind. It is required not to nourish us with nutrients but also gives effect in the cure and prevention of various types of disorders. This can be completed by use of natural sources of crude drugs like the medicinal plants. Generally the plants are most beneficial in cure of disorders or different ailments from different parts of countries in various geographical locations.

The effectiveness of the crude drugs along with their safety factors has made it to use it widely by traditional practitioners and physicians. In rural areas the herbals are used most commonly for treatment of different disorders as they
have not money to purchase the allopathic medications. Then, they utilised the crude drug which are easily available naturally in concern part of country or locality. Now a day the allopathic formulations are entering in the market, but these formulations are not suitable for different diseases like CVS disorder, liver disorder, asthma and GIT disorders etc.

Different numbers of allopathic medicaments are available in market for treatment of asthma at present situation but none of them is effective completely. As asthma takes place due to different number of mechanisms and the allopathic drugs are effective against any one or two mechanisms associated with the asthma, but not on all mechanisms of asthma. At the same time these allopathic medicines shows prominent side effects and adverse effects on human respiratory system. So it was required to search a new crude drug which will be effective against different mechanisms of asthma.

As per the review of literature the selected plants have different pharmacological actions therefore i selected these four plants for evaluation of antiasthmatic activity of them.
OBJECTIVE OF STUDY

- Review of literature of *Ficus racemosa*, *Cuscuta reflexa*, *Leucas linifolia* and *Sapindus trifoliatus* related to phytochemical profile, pharmacological profile and formulation.

- Collection and authentication of *Ficus racemosa*, *Cuscuta reflexa*, *Sapindus trifoliatus*, *Leucas linifolia*.

- Drying and size reduction of plant material of stem bark of *Ficus racemosa*, Stems of *Cuscuta reflexa*, Fruit pericarp of *Sapindus trifoliatus*, Leaves of *Leucas linifolia*.

- Extraction of powdered Fruit pericarp of *Sapindus trifoliatus*, stem bark of *Ficus racemosa*, leaves of *Leucas linifolia and stems of Cuscuta reflexa* by using 95% alcohol as solvent.

- Preliminary phytochemical evaluation of all the extracts.

- Pharmacological evaluation of all the extracts for anti-asthmatic activity using following screening models.
  1. Mast cell Degranulation,
  2. Clonidine Induced Catalepsy,
  3. Milk Induced eosinophilia
  4. Haloperidol Induced Catalepsy
  5. Milk Induced leukocytosis
  6. Histamine induced paw edema.

- Phytochemical Study of active extracts.