RESULTS AND DISCUSSION

From ancient time, human beings are utilizing natural sources of medicines as major source for treatment of different ailments, for providing better well being, curing body troubles, and giving flavour in nourishment of human body. In China and India maximum treatment was depends upon plants from about 5000 yrs ago.

In Europe, plants were utilised as one of the standard drug of treatment from around 70 years before. India, Arabic nations and China have built up their individual and unique indigenous way of treatment of disorders. Ayurvedic medication of pharmaceutical field is counted as the most important system of medication in terms of study.

Before the improvement in allopathic medications natural medications was the most useful way of treatment for maximum illness. Because of immediate onset of action, allopathic medications are adopted quickly by the human beings. Allopathic system of medications is utilized to incline toward manufactured drugs over traditional drugs. After long term utilization of allopathic medications, now we know different adverse reactions of allopathic medications. Consequently it was identified that there is requirement for re-establishment of natural prescriptions.

As per World Health Organization (WHO) assessment out of 80 % of the medications enlisted as per pharmacopoeia about less than 25 % of medications are either manufactured from molecules derived from plants. In 1970 war natural medications got importance in treatment. WHO guided all nations to invent their own natural medications to save their national legacy with respect to ethano-pharmacology.

Natural prescriptions are the medications based on plant origin, mainly called as phytomedicine. To maintain the quality of natural medications and to build open trust and to convert normal thing into standard, experts and authorities
must apply careful consistent procedures, systems and clinical trials to maintain and ensure the quality and consistency of standard natural medications.

Generally natural medications prepared now a day does not characterized and now there are no any standardisation steps involved in manufacturing of natural medications. The quality of natural medications depends generally on quality control of initial materials and their assembling into final medications. The quality and consistency of heterogeneous natural items can be checked for maintaining the quality of finished product.

Since long time, human beings are mainly dependent upon natural source as medication for treatment, longitivity and search for good health and to get relief from discomfort and pain and to complete the requirements of flavours, fragrance and foods.

In earlier stage human being explored his immediate natural surrounding and used different natural sources like plant, animal and mineral sources to develop different number of therapeutic agent. In developing countries different medicinal plants yet play important role in the both preventive and curative effect instead of advancement in modern western medicine. It is an important source of food and living for people of many developing countries.

The medicinal plants are considered as very important national resource of developing countries. European herbal medicines have importance in Graeco-Roman era and remained important until six decades ago. Different countries like India, china, Arab, Greece and other different countries across globe has discovered different plant based traditional systems of medicine which are quite different from each other.

Besides all traditional systems of medicine Ayurveda is possibly prime traditional system of medicine. (Kalia AN, 2011) Ayurveda and Chinese system of medicines are having great importance and also plays the major
role in discovery of new medicines. Many researches on pharmacognosy, chemistry and pharmacology have been performed on ayurveda traditional plants. (Patwardhan B. et al., 2004)

Various molecules have been existed from Ayurveda system of medicine e.g. Curcumines as anti-inflammatory agents, Rauwolfia alkaloids for hypertension, Guggulsterons as antihyperlipidemic agent, Holarrhena alkaloids as hypolipidemic agents. Mucuna as anti-parkinsons agent, Psoralens for vertigo, Phyllanthis as antiviral agent, Picroside as hepatoprotective agent, baccosides for mental retention, Piperidines for enhancing bioavalability, steroidal lactones, their glycosides and withanolides as immunomodulators. (Patwardhan B., 2000)

In India Ayurveda is well known traditional system of medicine from ancient time. Ayurveda is considered as one of the holy systems of medicine of India. It provides information and guidance related to lifestyle and food for keeping people healthy. Peoples use different parts of medicinal plants as herbal medicines. People use Charak, Sushutra and Veda etc for preparation of drugs.

In the process of discovery of new medicines by researchers the traditional medicines plays an important role. Ethno-pharmacology and drug discovery are correlated because both involves use of the natural products and it remains as important. Different modern medications have been invented from the knowledge of ethno pharmacology. (Patwardhan B., et al., 2004)

Now a day different number of advanced techniques are available but instead of availability of different advanced techniques, drug discovery from natural sources faces large number of difficulties which affect majorly the growth of pharmaceutical industry. (Joseph Di Masi and Cherie Paquette, 2004)

The demand of natural medicines is increased. Crude drugs are not useful in treatment of acute disorders. Plant based preparations are having less side
effects. These are often considered as major source of medications for treatment of minor diseases, this is helpful for additional and supportive treatment for chronic disorders. Medicinal plants need continuous attention of researchers for screening of chemical and pharmacological properties in developed and developing countries. The researchers discovered the different bioactive compounds with good medicinal value through the ethno pharmacological surveys on traditional medicines. (Jadhav R., 2003)

Plants represent primary source of medicine. The 88% of world’s population depends fundamentally on traditional drugs for their essential medications. The plant origin drugs are generally used for basic health care purpose. About 25% drugs are of plant origin and are semi-synthetic or depending on prototype compounds isolated from natural origin. (Patwardhan B., et al., 2004)

Plants are major components of all indigenous system of medicines. There are different elements in homeopathy, naturopathy and Ayurveda. Demand for herbal drugs is increasing throughout world because of recognition of growing natural plant-based products, which are generally non-toxic, does not have adverse reactions, easily obtainable at reasonable prices and many times unique source of medication for poor peoples. The plant occupied significant role in socio-cultural, economic, spiritual values of tribal and rural lives of different developed and developing countries. Different rural human beings are using medicinal plants. (Kalia A. N., 2011)

Internationally, it has observed that instead of inventing and formulating synthetic drug medicines, human beings are mainly involved in evolving plants to meet their healthcare needs. Due to these trials human being causes appraisal of novel medicines. (Kalia A. N., 2011)

Natural medicines are the most established type of human services and these can be utilized by all the societies all through history. Now a day we lives in age of globalization and this opens the gates of nations has created new
challenges as well as new opportunities. Standardization of drug is the most complex and most difficult task. To maintain the efficiency and public acceptance, ayurvedic medications required the standardization at different stages. From the identification of source of plants till the formation of finished product, that also includes storage and shelf-life. (Nair V.R., 2008)

The plants are helpful because it contains essential and auxiliary metabolites. Auxiliary metabolites are called as synthetic elements or gathering of intensifies which create clear physiological activity in human body. Essential metabolites are required for physiological advancement and general development of plant like proteins, sugars and lipids. Secondary metabolites are biosynthetically obtained from essential metabolites. Optional metabolites are biosynthetically gotten from essential metabolites. They may serve as defensive, guarded or hostile chemicals e.g. alkaloids, glycosides, tannins, terpenes and tars. (Edeoga et al., 2005)

Natural drugs are classified depending upon the basis of their chemical constituents, as they are responsible for therapeutic significance and pharmacological activity of these drugs. Naturally occurring compounds are derived from living systems. These compounds are basically organic in nature and are of thee types. Some compounds responsible for reproduction of cells and metabolism which play vital role and found in all cells. These compounds are mainly known as primary metabolites and it consists of common sugars, nucleic acids and amino acids.

Second category includes polymeric materials having high molecular weight like cellulose, lignins and proteins which found in cellular structure. And last category consists of secondary metabolites which are characteristic of species of limited range. Maximum primary metabolites show their biological action within the organism or cell which causes production. Secondary metabolites have their own biological action on other organisms. Now a day more attention of researchers are turning to structure elucidation of actual biosynthetic pathways found in natural sources. These studies were done by
introduction of new techniques of isolation and analysis. Herbal medicines generally contain lots of therapeutically active materials and these are utilized for curing both acute and chronic disorders. (Trease and Evans, 2002)

Objectives of this study are the active principles of medicinal plants are to be identified, and in order to ensure their safety, effectiveness and of constant activity investigation of extracts to be carried out. Determination of structure and their isolation are more efficient. Phytochemical of these active principles examination of plant might include authentication and extraction of plant, pharmacological evaluation and isolation of compounds. (Mukharjee P. K., 2007; Trease and Evans, 2002)

As a branch of science, herbal prescription is known which deals with medicines based on plant. It is also called as phytomedicine or natural prescription. This term has been presented specifically as a phytotherapy, the most exact equivalent word for herbal medication or herbal medications. Phytopharmacoherapy term is used for utilization of natural medicines in treatment of different disorders. Advancement of nature based dietary supplements is one more accomplishment in field of herbal drugs.

Natural drugs are having multidisciplinary approach which joins with pharmacognosy and phytochemistry, organic chemistry, ethno pharmacology, toxicology. Phytomedicine is consists of different dynamic constituents present in different parts of plant having specific pharmacological activity on human beings. These phyto-medicines are mainly called as phyto-constituents, which are mainly used for long time or in different forms.

4.1 PLANT PROFILES

A heterogeneous disorder
- Immunologically
- Physiologically
- Biochemically
- Etiology is multi factorial of asthma.

I decided to work on Stem of *Cuscuta reflexa*, pericarp of *Sapindus trifoliatus*, bark of *Ficus racemosa* and leaves of *Leucas linifolia* to find out their usefulness to human being. The present study was planned to study Pharmacognostic, Phytochemical Investigation of different parts of the plants.

The pharmacological activity of extract of alcohol of plants on different aspects of asthma like mast cell degranulation, eosinophilia, leucocytosis, catalepsy and allergy associated with inflammation using various screening models were evaluated. The identification of constituents responsible for effectiveness was done using GCMS techniques.

*C. reflexa* (Convolvulaceae) is a herbaceous climbing, parasitic plant having scaly leaves mainly grows as a twinner and climber on shrubs grows well in late monsoon. It is mainly known as amarvel due to its immortal nature. The plant mainly used as antipyretic, expectorant, purgative, carminative, anthelmintic. It is applied externally in itching, other skin diseases and as blood purifier. It is mainly used in the form of decoctions, cold infusions, as powder and poultices. This is well known drug in ayurveda and unani system of medication. It is also used in flatulence, constipation, bilious affections and liver complaints treatment.

It consists of alkaloids, lactons, flavonoids, resins as well as waxes and semidrying oil. Different constituents were isolated from the *C. reflexa* like cuscutine, β-sitosterol, cuscutalin, kaempferol, amarbelin, bergenin and luteolin.

*S. trifoliatus* (Sapindaceae) mainly known as ritha or soap nut. *S. trifoliatus* is available all over the world, maximum found in forests and dry regions of tropical countries. It is a large to medium sized tree having height up to 18 M. in India it is mainly available in Andhra Pradesh and Karnataka. The plant has pubescent, pinnate leaves, with 2-3 pairs of leaflets having entire, oblong, and
retuse margins and the upper surface is glabrous. *S. trifoliatus* consists of 3 lobed fruits present in group of 1-4. Internally consist of black seed with reddish brown fleshy pericarp. The flowers are small and white in colour.

Different parts of *S. trifoliatus* are used as herbal drugs of treatment like whole plant, fruit pulp/ pericarp, seeds, stem, root, fruit and leaves. Seeds have acrid and narcotic effect. Fruit pericarp acts as tonic, expectorant, anthelmintic action. It is also used in treatment of asthma, hemicranias, hysteria, epilepsy, severe diarrhea, cholera, reptile bite. It also shows emetic effect at small close and at large dose it acts as purgative.

Root extract administered by nostrils is used in treatment of epilepsy, hemicranias and hysteria, which causes irritation to mucous membrane and increases the secretions and also at as expectorant. Seed kernels used in the form of pessaries as used in amenorrhoea and to stimulate the uterus in delivery. Leaf bath is given in treatment of gout, rheumatism and for painful joints and paralysis. The roots and bark are mainly used as demulcent and mild expectorant.

Fruit pericarp mainly used as detergents and also used as fish poison. Fruit saponins are used in shampoo and soaps preparation. Aqueous extract of pulp is used in treatment of as severe diarrhea and cholera as an anthelmintic and in snake bite.

*S. trifoliatus* fruits contain seeds and pericarp. It mainly contains alkaloids, saponin, cyanogenetic glycosides, oligoglycoside, cyclic sesquiterpene, trifolioside as well as pectin, glucose and white fat. Saponins contains aesculin, fraxin. Emarginatoside contains hederagenin as sapogenin and d-glucose, d-xylose and l-rhamnose as aglycones. Fruit kernels mainly consist of different percentages of non drying fixed oil mainly stearic, lignoceric, arachidic, linoleic, palmitic, behenic and oleic acids.
*Ficus racemosa* (Moraceae) mainly known as Gular is found through India mainly in outer and sub-himalayan tracks as well as in Western, Central and Southern India. *F. racemosa* is a holy plant of Indians.

*F. racemosa* is large lactiferous and deciduous tree with small aerial prominent roots. It consists of elliptic to ovate oblong, lanceolate, glabrous and stipules ovate dark green colored leaves and clusters of elliptic fruit receptacles (2-5 cm diameter) on main trunk and large branches. Fig fruits are very smooth having minute soft hairs; ripped fruits have pleasant odor and orange to dull reddish color. The bark is dull white or reddish brown colored, smooth and having remarkable translucent flakes of whitish tissue.

Barks, ripe and unripe fruits, roots, galls, leaves and milky juice are used as crude drug from *F. racemosa* plant. Bark and fruits are used as astringent in treatment of leprosy, diabetes. Leaves, unripe fruits and bark have carminative, vermicide, stomachic and astringent effect. Seed powder is used in diabetes, in thirst and in polyuria of diabetes.

Bark has protective action in pregnancy along with galactogogic effect and in menorrharia, as well as it is used in diabetes and dysentery. As mouth wash in sore throat and in spongy gum infusion of leaves, bark and fruits can be used. Fruit juice is used in diabetes and other urinary complaints. Fruits are carminative, astringent, stomachic, menorrhea and haemoptysis. Root sap is applied externally in mumps, gonorrhea and in glandular enlargements and internally in treatment of diabetes.

*F. racemosa* bark mainly contains β-sitosterol, Tannins, Stigmosterol, Leucoanthocynins, Leucopelargonidine, Leucoanthocynidines, Lupeol and amyrine.

*Leucas linifolia* (Labitae) known as dudhani found throughout India a a weed from Ceylon to Himalayan region. The *L. linifolia* is herbaceous, erect and slightly pubescent in nature.
Leaves are entire, oblong, serrated, linear and subequal in nature. Plant bears large no of flowers and linear hoary bracts having oblique lower and elongated calyx above with white flowers. Leaves, flowers and whole plant can be used as crude drugs. Plant is used in rheumatism. Flowers have diaphoretic, emmenagogue, expectorant and insecticidal action. Leaves juice is useful in headache, psorea and different chronic eruptions. By nasal route is useful in snake bite. Whole plant is used in stomach ache, anxiety, leprosy, fever, constipation, anthelmintic, joint pain, itching. It is also used as antiulcer, stimulant, hypoglycaemic, antimicrobial agent.

Flowers of *L. linifolia* contain alkaloids and small quantity of volatile oil. The plant contains glycosides, alkaloids and flavonoids.

**4. 2 PHARMACOGNOSTIC STUDY**

Different morphological and physical parameters were studied in Pharmacognostic study of *Cuscuta reflexa* Stem, *Sapindus trifoliatus* pericarp, *Ficus racemosa* bark as well as *Leucas linifolia* leaves.

**4.2.1. Macroscopy**

*C. reflexa* stems was observed that has light green to dark green in colour with characteristic odor and contains 10-15 cm long stems with internodes.

*S. trifoliatus* contains fleshy pericarp with fulvous hairs. It is dark brown to light brown in color with 1.3-2 cm diameter. It has characteristic odor with bitter taste.

*F. racemosa* bark have fibrous fracture with soft inner surface and uneven outer surface. It is 0.5-1.8 cm in thickness with grayish to dull brown color having astringent taste and characteristic odor.
Leaves of *L. linifolia* are petiolate with or without intervening stalk oval with tapering end. It is dark green to light green in color with characteristic odour and taste.

**4.2.2. Evaluation of physical parameter:**

The mature stems of *C. reflexa*, pericarp of *S. trifoliatus*, bark of *F. racemosa* and Leaves of *L. linifolia* were collected from Ahmednagar district of Maharashtra state. All the plants were authenticated by S. C. MAJUMADAR SCIENTIST FOR JOINT DIRECTOR, “Botanical Survey of India, Koregaon road; Pune.” Further all the samples were cleaned and dried in shade at room temperature, powdered and further evaluated for the standardization using different physical parameters.

All the samples were evaluated for the Foreign Organic Matter. It was observed that all the samples of crude drugs are freed from any foreign organic matters. Loss on drying was carried out for all the samples it was observed that *C. reflexa* stems possess 4% w/w, *S. trifoliatus* pericarp having 7.5% w/w, *F. racemosa* bark contains 6.9% w/w and Leaves of *L. linifolia* consists of 5% w/w of moisture. It was found that the pericarp of *S. trifoliatus* possess maximum percentage of moisture content as compare to other samples.

Ash values were determined for all the powdered samples of *C. reflexa* stem, *S. trifoliatus* pericarp, *F. racemosa* bark and *L. linifolia* leaves. In this sulphated ash value, acid insoluble ash value, water soluble ash value and total ash value were evaluated.

*C. reflexa* stem contains 8.6 %w/w of total ash value and 1.4 %w/w of water soluble ash value. It also possesses acid insoluble ash value of 3.1 %w/w and sulphated ash value of 4.9 %w/w was found. Pericarp of *S. trifoliatus* has 4.9 %w/w of water soluble ash value, total ash value 0.8 %w/w and 0.5 %w/w of acid insoluble ash value was found while sulphated ash value was 1.4 %w/w.
*F. racemosa* bark possess 10.7 %w/w of total ash value, 1.9 %w/w of water soluble ash value, 1.2 %w/w of acid insoluble ash value and sulphated ash value as 3.6 %w/w was found. *L. linifolia* Leaves possess 14.3 %w/w total ash value and 3.9 %w/w of water soluble ash value while acid insoluble ash value of 2.8 %w/w and sulphated ash value of 6.5 %w/w were identified.

All the samples possess more water soluble ash value than acid insoluble ash value, it indicates that samples possess minute quantity of inorganic variables, like silica, calcium oxalate or carbonate content.

Alcohol soluble extractive value and water soluble extractive value were found in *C. reflexa* stem as 10.1%w/w, 14.3%w/w, and *S. trifoliatu*s pericarp as 18.2%w/w, 23.6%w/w, *F. racemosa* bark as 20%w/w, 16.2%w/w and Leaves of *L. linifolia* as 13%w/w and 9%w/w respectively. The water soluble extractive value was found to be more than alcohol soluble extractive value in *C. reflexa* stem and *S. trifoliatu*s pericarp which indicates that more amount of polar compounds present in both the samples that can be extracted maximum into water than alcohol.

It was found that soluble extractive value for alcohol to be greater than soluble extractive value in water in *F. racemosa* bark and Leaves of *L. linifolia* this indicates that more quantity of constituents are present in both the samples which can be extracted maximum into alcohol than water.

### 4.3 Phytochemical Evaluation

The phytochemical evaluation of stem of *C. reflexa*, pericarp of *S. trifoliatu*s, *F. racemosa* bark and Leaves of *L. linifolia* reveals the following findings.

#### 4.3.1 Extraction

The percentage yield of *C. reflexa* extract was found to be 12.80 %w/w, and *S. trifoliatu*s extract 16.50 %w/w while *F. racemosa* 17.50 %w/w and L.
linifolia extract was found to be 11.40 % w/w respectively. When 500 grams of powdered crude drugs were extracted in Soxhlet apparatus.

### 4.3.2 Preliminary Phytochemical screening

Preliminary phytochemical tests were carried out for rough idea of constituents present in the extracts after extraction.

Alcoholic extract of *C. reflexa* and *F. racemosa* extracts showed presence of Saponins, steroids, alkaloids, flavonoids, tannins and carbohydrates. While presence of steroids, flavonoids, Saponins, alkaloids, tannins and carbohydrates is shown by the alcoholic extract of *S. trifoliatus*. It also gives positive reactions for gums.

Presence of steroids, carbohydrates, alkaloids, saponins, flavonoids and tannins is shown by the *L. linifolia* extracts. It also gives positive tests for cyanogenetic glycosides.

### 4.4. Pharmacological Evaluation

#### 4.4.1 Acute Toxicity Study

OECD guidelines was followed to determine LD50 as well as to evaluate acute toxicity study of all extracts.

All the extracts were given upto 3000 mg per kg body weight and observed for two weeks. It does not given any unwanted symptoms. The results showed that there were no such clinical signs and mortality of the animal therefore the LD$_{50}$ of the plant was assumed to be more than 3000 mg/kg body weight.
4.4.2. Antiasthmatic Activity

The pharmacological evaluation of all the extracts was tested for different pharmacological models like Milk Induced eosinophilia, Clonidine Induced Catalepsy, Haloperidol Induced Catalepsy, Mast cell Degranulation; Histamine induced paw edema and Milk Induced leucocytosis.

1. Clonidine Induced Catalepsy

Clonidine at dose of 1 mg/kg, by subcutaneous route produced catalepsy at all the groups of the mice. In group of animals treated with controlled group i.e. 1% Tween 80 solution at dose of 5 ml/kg, i. p. it shows maximum duration of catalepsy at 120 minutes (201.4±8.32) after the administration of Clonidine. Vehicle treated group shown catalepsy for about three hours. All the extracts give significant inhibition of catalepsy. *F. racemosa* and *L. linifolia* extracts at a dose of (200 milligram/kg, i.p.) shows prominent inhibition in catalepsy when compared to standard drug Chlorpheniramine maleate (10 miligram/kg, intraperitoneally) treated group.

After administration of Clonidine, all extracts at 2.5 hrs. has shown maximum duration of catalepsy. Clonidine induced catalepsy shows significant inhibition by all groups pretreated with alcoholic extracts of Leaves of *L. linifolia*, stem of *C. reflexa*, pericarp of *S. trifoliatius* and *F. racemosa* bark at 200 mili gram per kg dose o and the duration of catalepsy was found to be 96.39± 3.32, 134.7±3.12, 109 ± 3.97, 122.5 ± 2.3 seconds respectively, at 120 minutes.

Catalepsy is induced by inducing dopamine D2 receptor in the substantia nigra by neuroleptic agents. (Sanberg PR 1980) Anticholinergic activity of antidepressant was suggested in perphenazine-induce catalepsy by studying the relative role of acetylcholine and histamine by Chopra and Dandiya. (1975) this is probably because of their ability of acetylcholine release is inhibited or dopamine content in brain is enhanced. Direct correlation with
brain histamine content with various stages of catalepsy is also shown by them.

In mice dose dependent catalepsy is induced by $\alpha_2$ adrenoreceptor agonist i.e. Clonidine, which was not inhibited by H2 receptor but inhibited by histamine H1 receptor antagonists. Clonidine is responsible for different asthmatic conditions due to release of histamine from mast cells. Clonidine produced catalepsy is mediated by histamine (via H1 receptors) released from the brain mast cells and not via H2 receptors. (Taur D. J., et. al., 2011)

1. **Milk Induced Eosinophilia**

In the present evaluation, the vehicle treated group without administration of milk does not show any significant change in total eosinophil count (22± 5.75). Milk treated control group shows remarkable difference in eosinophil count (129± 20.35).

Groups pretreated with milk when administered with alcoholic extracts of different drugs shows prominent difference in eosinophil count. *L. linifolia* leaves, *C. reflexa* stem, *S. trifoliatus* pericarp and bark of *F. racemosa* at 200 mg/kg dose of and the difference in eosinophil count was found to be 61± 4.98, 65±9.8, 54±8.4 and 57±7.6 respectively. While the dexamethasone (50 mg/kg, i.p.) standard drug shown 63±40.20 difference in the eosinophil count.

The alcoholic extract of *S. trifoliatus* pericarp shown prominent effect on milk induced eosinophilia, which was followed by bark of *F. racemosa*.

Eosinophilia is one of the prominent symptoms of asthma. Symptoms of allergic asthma are significant in milk induced blood eosinophilia due to increase eosinophil count as it involves increase of eosinophil count into bronchial mucosa where allergic reactions and inflammation takes place. (Barry R. H., et. al., 1975)
Excessive secretion of mucus and congestion of bronchi are symptomatic manifestation of late asthmatic reaction. In pathology asthma involves different sorts of mediator. After 24 hr of parental administration of milk, marked increase in the eosinophils and leukocytes count is seen which was demonstrated by Bhargava K. P., 1981; Soman I., 2004; Brekhman et al., 1969.

2. Mast cell Degranulation

Mast cells play important role in immunity. These are mainly located in large number where entry of pathogens in the body is easily possible like Airways, GIT and skin. Thus, the immune cells are the mast cells which has ability to produce different moiety which gives immune responses. Mast cells act as an effector cells in adaptive immune reactions by their activation with antibody-antigen immune complexes. These are long lived prematopoietic cells and highly granulated. (Da Silva EZ et. al., 2014)

The presence of mast cells allows them to respond the external encounter with invading pathogens and environmental stimulus. The matured mast cell contains more no. of granules as compare to others. Along with it they have different morphological structure. (Wernersson S et. al., 2014)

The granules present on mast cells are biologically active mediator of protease and histamine. On exposure to the extracellular environmental condition the mast cells releases granules as a result of mast cell activation. IgE dependent activation is the most well known pathway for activation of mast cell during allergic responses.

From β lymphocytes, the formation of allergen specific IgE antibodies is stimulated by the allergens. In the sensitization of mast cells the allergen-specific IgE molecules bind to the high-affinity IgE receptor on the surface of mast cells (Galli SJ et al., 2008). On repeated exposure of allergens the IgE
antibodies binds to the allergens and induces cross linking and thus results into strong activation and further cause degranulation. (Rivera J., et. al., 2008)

Induction of mast cell degranulation in the skin of mice by compound 48/80 injection is a second approach. Mast cell degranulation produced by injection of 48/80 compound with the release of mediators like numerous cytokines histamine, proteases and histamine.

Their biological function is increased vascular permeability and recruitment of inflammatory leucocytes as part of both innate and acquired immunity. (Shimizu T. et. al., 2003, Oliver N., et. al., 2003) Sodium cromoglycate acts as stabilizer for mast cell degranulation. Thus taken as standard.

In present investigation Sodium cromoglycate when given at dose of 0.5 miligram/kg by intraperitoneal route showed maximum protection of about 82.43 %. The alcoholic extract of Sapindus trifoliatus at dose of 200 mg per kg, i.p. shows maximum inhibition 70.41 % followed by Ficus racemosa (64.60%), then extract of Cuscuta reflexa and Leucas linifolia (57.46 %) and 52.97% respectively.

All the extracts having prominent mast cell stabilizing effect as compared with control group treated with 10 ml per kg dose of distilled water by i. p.

**2. Milk Induced leukocytosis**

Ayurveda provides different number of sources of asthma treatment and natural formulations, which mainly contains some herbs to minimize stress. As it indirectly increases total leucocytes count or nervous disturbances which might causes severe symptoms of asthma (Yamaya M., 2012).

The anti-stress agent increases capacity of person to overcome various adverse effects of a biological, chemical and physical nature. Increase in total leucocytes count takes place after parental administration of milk, which
causes different allergic reactions. Significant increase in the leukocytes count occurs after subcutaneous administration of milk after 24 hours. A release of different inflammatory mediator occurs in leukocytosis situation of asthmatic inflammation like cytokines, histamine as well as some basic proteins, which enhances the inflammation. (Brekhman L. I. et. al., 1969)

The inflammatory process get potentiated with increase in leukocyte count due to reactive oxygen release from species into surrounding tissue system which results into increased oxidative stress (Nadeem A, 2003) and it is associated with different pathogenic symptoms of asthma. (MacNee W., Rahman I., 2001)

Increase in leukocyte count occurs after administration of 4 ml per kg of milk by subcutaneous route. In present study mice pretreated with alcoholic extracts of *L. linifolia* leaves, *C. reflexa* stem, pericarp of *S. trifoliatus* and *F. racemosa* bark at dose 200 miligram per kg, i.p. has shown significant decrease in total leucocytes count 2590± 129.80, 2714±113.90, 2375±90.87 and 2419±140.30 respectively as compared to (4569±230.90) control group.

In the present study it was observed that alcoholic extract of *S. trifoliatus* decreased leukocyte count in mice at 200 miligram/kg of body weight, when compared to group of animals treated with vehicle only, followed by alcoholic extract of *F. racemosa* bark.

5. **Haloperidol Induced Catalepsy**

Typical blockade of postsynaptic striatal dopamine D1 and D2 receptors was linked to neuroleptic-induced catalepsy. (Sanberg P. R., et. al., 1980, Farde L et. al., 1992)

Along with this other different types of neurotransmitters such as acetylcholine, GABA and serotonin have also causes dysfunction. Involvement of reactive oxygen species in haloperidol induced catalepsy

At 1 mg per kg dose when given by intraperitoneal route Haloperidol shows cataleptic effect on all the groups of the mice. The controlled group when treated with 1% Tween 80 solution showed maximum catalepsy (229±10.16) after 120 minutes of Haloperidol administration. Chlorpheniramine maleate at dose of 10 mg per kg, i. p., when given as Standard does not shows any inhibition at any duration.

_S. trifoliatus_ and _L. linifolia_ extracts at 200 miligram per kg, i.p. shows prominent inhibition of catalepsy as compare to standard Chlorpheniramine maleate (10 mg per kg, i.p.) treated group. The duration of catalepsy was found to be 153.6±6.9 and 145.8±4.70 seconds respectively, at 120 minutes. _L. linifolia_ extract shows prominent inhibition of catalepsy as compare to other extracts.

Catalepsy occurred by haloperidol mainly occurs due to inhibition of D2 receptors, dopamine and inhibits dopamine secretion. For adrenaline, Dopamine is agonist, and adrenaline is physiological antagonist of histamine. So decrease in dopamine levels increases physiological histamine. (S. K. Mahajan and R. Y. Chaudhary 2011)

haloperidol-induced catalepsy inhibits increase in dopamine transmission. So these antihistaminic drugs does not inhibits haloperidol induced catalepsy.

6. **Histamine Induced Rat paw oedema**

The significant reduction in rat paw oedema occurs due to histamine induced paw oedema. It was observed in all groups of animals treated with alcoholic extracts of leaves of _L. linifolia, C. reflexa_ stem, pericarp of _S. trifoliatus_ and _F. racemosa_ bark at dose of 200 mg per kg, orally. The oedema was observed upto 4 hours of evaluation at different time intervals 0 min., 30 min. and 1, 2, 3 hours.
Alcoholic extracts *F. racemosa, C. reflexa, L. linifolia* and *S. trifoliatus* at dose of 200 miligram per kg, and standard drug Cyproheptadine (10 mg per kg) significantly reduced the formation of oedema, observed at the 3rd hr (maximum oedema formation) in 1.16±0.04, 1.07±0.09, 1.12±0.09, 1.17±0.07 and 1.23±0.07 resp. it shows significant results when compared to the control 1.42±0.03. At 1st and 2nd hour significant reduction in histamine induced oedema occurs in different groups of animals administered by alcoholic extract of *L. linifolia* leaf was found 0.82±0.08 and 0.88±0.07 respectively.

Carrageenan and dextran/ histamine induced significant rat paw oedema by different techniques. Dextran is high molecular weight polysaccharide which induces anaphylactic reaction when administered by parentral route, which is identified by oedema formation and causes release of histamine and serotonin from mast cells. The histamine is basic amine which gives inflammatory and allergic reactions which further causes vascular permeability or vasodilatation. (Van Wauve J. P., and Goosens J. G., 1989, Rang H. P., *et al.*, 2001)

In the present analysis, depending on results obtained, it was observed that it shows an antihistaminic activity. However, alcoholic extract of *L. linifolia* leaf was effective in inhibition of rat paw oedema caused due to histamine. So it can be observed that main mediator is histamine, it can be suggested that all the extracts and its fractions contain different compounds that are responsible for inhibiting histamine liberation from mast cells, but not responsible from tissue release. (Oliveira F. A., *et al.*, 2004)
4.4. CHARACTERIZATION OF CONSTITUENTS BY GCMS TECHNIQUE

GCMS technique gives idea about separation of different constituents along with its possible composition, by using mass spectroscopy as one of the part of it.

GC-MS analysis alcoholic extract of *Ficus racemosa* showed that some major compounds identified with gas chromatography were Silane (29.32%), 2,6,11-trimethoxy dodecane (18.45%) and Eicosane (14.39%) also 3,5- dimethyl octane (22.31%). Previous studies have shown that different extracts of *Ficus racemosa* extracted from different parts as well as depending upon the storage duration of different regions are characterized by different contents of Silane, Eicosane, dodecane and Octadecane. Some derivatives such as tetradecane, hexadecane (4.39%) and 2, 6, 10- trimethyl pentadecane (5.39%) and some carboxylic acid derivatives (5.76%) are also detected.

In the extract of *Cuscuta reflexa* the major component identified with GCMS were decane derivatives 3,5- dimethly octane (11.63%), dodecane(16.59%), trtradecane, pentadecane, hexadecane (20.93%), Eciosane (10.42%), heptadecane(14.15%). Some anhydrides and carboxylic acid derivatives palmitic anhydride (11.20%), 3, 5, di-ter- butyl- 4- hydroxyl anisole (15.08%) were also found in the sample.

The alcoholic extract of *Leucas linifolia* shows presence of various constituents mainly belonging to decane and acid derivatives. Along with these all other major constituents were found to be 9, 12, 15- Octa decatrienoic acid, (Z, Z, Z) Methyl 8, 11, 14-heptadecatrienoate (38.63%). Some ester derivatives (15.72%) are also found in the extract. Decane derivatives were found with varying concentration from 7.19 to 11.12 %.

The alcoholic extract of *Sapindus trifoliatus* contains seven different constituents with varying $R_f$ values. 25.79 % constituent is 3-O-Methyl-d-glucose ád Mannofuranoside, methyl and Inositol derivatives. The cis-
Vaccenic acid, Cis, trans-13-Octadecenoic acid also present (21.28%). Some acid and ester derivatives like n-Hexadecanoic acid, I (+) Ascorbic acid 2,6-dihexadecanoate, Pentadecanoic acid (17.08%) are also present. Some saturated carbon chain compounds (15.29%) are also present in the given extract such as Hexadecane, Pentadecane and Heptadecane. Some other carbon chain compounds are also present in the range of 6.02 to 8.02%.

We may observe the variations in the percentage area of various constituents of the extract which is attributed to environmental conditions such as climate, location, seasonal factors, and development stage. Secondary metabolites of the crude drug are known as potentially effective against various food-borne, human, and plant pathogenic fungi and pests and nontoxic in nature.

Eicosane is present in extracts of *Ficus racemosa* and *Cuscuta reflexa*. 