SUMMARY AND CONCLUSION

Most common ailment of respiratory system is Asthma. Globally, the mortality and morbidity of asthma is increasing day by day. It is a heterogeneous disorder physiologically biochemically and immunologically. Etiology of asthma is multi factorial due to contraction of the smooth muscle narrowing of the bronchi takes place which is characteristic of Bronchial asthma. This causes mainly due to release of histamine and other chemical mediators.

In the present study the *Cuscuta reflexa* stem, pericarp of *Sapindus trifoliatus*, *Ficus racemosa* bark and leaves of *Leucas linifolia* were selected for antiasthmatic activity. In this research pharmacognostic, phytochemical and pharmacological activity of alcoholic extract of all the plants on various models of asthma like eosinophilia, mast cell degranulation, leucocytosis, catalepsy and allergy associated with inflammation were performed. The identification of constituents responsible for effectiveness was done using GCMS techniques.

The *Cuscuta reflexa* stem is a twinner known as akashvel having variety of traditional uses. It also contains different number of chemical constituents like cuscutine, cuscutalin, amarbelin, β-sitosterol, kaempferol, bergenin. The pericarp of *S. trifoliatus* mainly called as soap nut. It is the evergreen tree. The soapnut plants have various traditional uses like asthma, epilepsy, diarrhea, amenorrhoea, gout and in rheumatism.

*S. trifoliatus* contains alkaloids, saponin, cyanogenetic glycosides and different acids. *F. racemosa* known as umber found through out of India as a holy tree. The plant acts as astringent, carminative, protective used in treatment of diabetes, leprosy, menorrharia, urinary disorders. *F. racemosa* contains Tannins, Leucoanthocynins, Leucoanthocynidines, β-sitosterol and lupeol. *L. linifolia* mainly known as dudhani found as a weed. It is used in rheumatism, as expectorant, in anxiety, leprosy, itching, as hypoglycaemic and antiulcer. It contains alkaloids, glycosides and flavonoids.
Morphology and physical parameters of stem of *Cuscuta reflexa*, pericarp of *Sapindus trifoliatus*, bark of *Ficus racemosa* and leaves of *Leucas linifolia* were studied in pharmacognostic study.

*C. reflexa* stem have light green to dark green colour and it is about 10-15 cm long with internodes and characteristic odour. The pericarp of *S. trifoliatus* is fleshy with dark brown colour and 1.3 to 2 cm in diameter with bitter taste and characteristic odour.

The *F. racemosa* bark is soft from inner side and have uneven outer surface with fibrous fracture. It is brown to dull brown in colour, 0.5-1.8 cm thick, having characteristic odour and astringent taste. *L. linifolia* leaves are dark green coloured with tapering end and characteristic odour and taste.

The mature stems of *C. reflexa* bark of *F. racemosa*, pericarp of *S. trifoliatus*, and Leaves of *L. linifolia* were collected from Ahmednagar district of Maharashtra state, authenticated by “Botanical Survey of India, Koregaon road, Pune.” The samples were cleaned, dried in shade, powdered and evaluated for the physical standardization.

All the crude drugs are freed from foreign organic matters. *C. reflexa* stems have 4% w/w, *S. trifoliatus* pericarp contains 7.5% w/w, *F. racemosa* bark possess 6.9% w/w and 5% w/w of moisture was observed in leaves of *L. linifolia*. It was observed that the *S. trifoliatus* pericarp contains maximum percentage of moisture content as compare to others.

Ash values were determined for all the powdered samples of *C. reflexa* stem, *S. trifoliatus* pericarp, *F. racemosa* bark and *L. linifolia* leaves. *C. reflexa* stem contains 1.4 %w/w, 8.6 %w/w, 4.9 %w/w and 3.1 %w/w of water soluble ash value, total ash value, acid sulphated ash value and insoluble ash value respectively.
Pericarp of *S. trifoliatus* contains total ash of 4.9 %w/w, water soluble ash of 0.8 %w/w, acid insoluble ash 0.5 %w/w and sulphated ash 1.4 %w/w.

*F. racemosa* bark contains total ash value 10.7 %w/w, water soluble ash value 1.9 %w/w, acid insoluble ash value 1.2 %w/w and sulphated ash value were found 3.6 %w/w while *L. linifolia* leaves contain total ash value 14.3 %w/w, water soluble ash value 3.9 %w/w, acid insoluble ash value 2.8 %w/w and sulphated ash value 6.5 %w/w respectively. All extracts contain more water soluble ash value as compare to acid insoluble ash value. It shows that the crude drug contains less percentage of inorganic variables.

Water soluble extractive value were identified as 14.3%w/w, 23.6%w/w, 16.2%w/w and 9%w/w while soluble extractive of alcohol value were found to be 10.1%w/w, 18.2%w/w, 20%w/w and 13%w/w in stem of *C. reflexa*, *S. trifoliatus* pericarp, bark of *F. racemosa* and leaves of *L. linifolia*.

Alcohol soluble extractive value were found to be 10.1%w/w, 18.2%w/w, 20%w/w and 13%w/w while Water soluble extractive value were identified as 14.3%w/w, 23.6%w/w, 16.2%w/w and 9%w/w respectively in stem of *C. reflexa*, *S. trifoliatus* pericarp, bark of *F. racemosa* and Leaves of *L. linifolia*.

Water soluble extractive value were more as compare to alcohol soluble extractive values, it indicates that the crude drugs contains less non polar content as compare to polar constituents.

The alcohol soluble extractive value was found to be more in bark of *F. racemosa* and Leaves of *L. linifolia* which indicates that more constituents are present in both which can be extracted into alcohol.

The phytochemical evaluation of stem of *C. reflexa*, pericarp of *S. trifoliatus*, *F. racemosa* bark and Leaves of *L. linifolia* were done. The extraction was carried out by using soxhlet extraction process and yield in percentage was found to be 12.80 %w/w in *C. reflexa*, 16.50 %w/w in *S. trifoliatus* and 17.50 %w/w in *F. racemosa* while *L. linifolia* extract contains 11.40%w/w of extracts.
For evaluation of presence of constituents for preliminary phytochemical screening of the extracts were characterized. Presence of Flavonoids, saponins, tannins, steroids, alkaloids and carbohydrates in all the extracts were detected, while *L. linifolia* shows presence of cynogenitic gylcosides.

The acute toxicity study was carried out for all the extracts to determine LD50. Oral administration of alcoholic extracts of all the extracts does not shown any toxic symptoms or death till maximum dose of 3000 miligram per kg body weight. So the LD50 was considered as more than 3000 miligram per kg body weight. 200 mg per kg body weight was considered as a safe dose for evaluation of antiasthmatic activity of different extracts on different models.

The antiasthmatic activity of alcoholic extracts of *C. reflexa, S. trifoliatus, F. racemosa* and *L. linifolia* was evaluated for Clonidine Induced Catalepsy, Milk Induced eosinophilia, Mast cell Degranulation, Milk Induced leucocytosis, Haloperidol Induced Catalepsy and Histamine induced paw edema.

In evaluation of antiasthmatic activity using Clonidine Induced Catalepsy, Clonidine was given to induce catalepsy. All the extracts were given at dose of 200 mg per kg i. p. and standard Chlorpheniramine maleate at dose of 10 mg per kg, i.p. and duration of catalepsy was observed. Vehicle treated group shown catalepsy for about three hours, and maximum at 120 minutes. All the extracts produced significant inhibition of catalepsy as compare to standard.

Clonidine is a $\alpha_2$ adrenoreceptor agonist, which produces dose dependent catalepsy in mice, which is not inhibited by $H_2$ receptor but inhibited by histamine $H_1$ receptor antagonists. Different asthmatic conditions are caused due to releases of histamine when Clonidine acts on mast cells. By histamine (via $H_1$ receptors) Catalepsy is produced by Clonidine is mediated and released from the brain mast cells and not via $H_2$ receptors (Taur D. J., et. al., 2011).
In Milk Induced Eosinophilia vehicle treated group without administration of milk does not shown any eosinophilia while Milk treated control group shown prominent increase in eosinophil count. From all the extracts alcoholic extract of *S. trifoliatus* pericarp shown prominent effect on milk induced eosinophilia which was followed by bark of *F. racemosa*. The standard drug dexamethasone when 50 mg/kg, i. p .dose is administered has shown prominent difference in total eosinophil count.

Increase in eosinophil count is one of the significant symptoms of allergic asthma as it contains increase in eosinophil count in bronchial mucosa. After 24 hr of parental administration of milk, it was evaluated that it produces a marked increase in the eosinophils and leukocytes count.

In present investigation of Mast cell Degranulation, Sodium cromoglycate used as standard at 0.5 mg/kg i. p. dose, given maximum protection of mast cells which was followed by alcoholic extract of *S. trifoliatus*, *F. racemosa*, *C. reflexa* and *L. linifolia* respectively.

Mast cells are present at different location in body where entry of pathogens takes place easily. It reacts with pathogens produces different moieties which gives immune responses. Mast cells activate the antibody-antigen immune complexes.

The mast cells granules are biologically active mediator of histamine and protease. On exposure to asthmatic condition mast cells releases granules as a result of activation of mast cell.

Mast cell degranulation was caused by Compound 48/80 when administered by parenteral route in mice. Mast cell degranulation is induced by the injection of 48/80 compound which further releases histamine and other mediators such as proteases, histamine and numerous cytokines.
In milk-induced leukocytosis, the leukocyte count increased within 24 hours after the administration of milk by subcutaneous route. In the present study, all the extracts shown prominent decrease in total leukocytes count in mice but as compared to vehicle-treated group alcoholic extract of *S. trifoliatus* and *F. racemosa* shown maximum decrease.

For different allergic reactions, parenteral administration of milk is responsible as total leukocytes counts get increased drastically after 24 hours. In asthmatic condition leukocytes release different inflammatory mediators like histamine, cytokines as well as some basic proteins, which enhances the inflammation.

In surrounding tissue system, release of reactive oxygen species increases leukocyte count that potentiates the inflammatory process which results into different pathogenic symptoms of asthma with increased oxidative stress.

Chlorpheniramine maleate given as standard does not show any inhibition at any duration in Haloperidol-induced catalepsy. It shows cataleptic effect on all the groups of the mice. *S. trifoliatus* and *L. linifolia* extracts shown prominent inhibition of catalepsy as compare to standard. *L. linifolia* extract shows prominent inhibition of catalepsy as compare to other extracts.

Catalepsy induced by Haloperidol occurs mainly by inhibition of D2 dopaminergic receptors and inhibits secretion of dopamine. Adrenaline is physiological antagonist of histamine and dopamine is agonist for adrenaline. Hence increases histamine level takes place with decrease in dopamine levels physiologically. Due to blockade of dopamine receptors in the striatum Haloperidol catalepsy is primarily caused. Haloperidol-induced catalepsy is inhibited by the agents increasing dopamine transmission. Haloperidol induced catalepsy is not inhibited by antihistaminic drug.

The significant reduction in histamine induced rat paw oedema was observed in all groups of animals treated with different extracts upto 4 hours. Alcoholic
extract of *L. linifolia* leaf shown significant reduction in histamine induced oedema, at the end of first and second hour. So it can be identified that the main mediator is histamine, histamine liberation from mast cells is inhibited by the crude extract and its fractions that contain constituents which are capable of it. inflammatory and allergic effects are caused by the histamine which is a basic amine that shows increase in vascular permeability and vasodilatation effects.

GCMS technique gives idea about separation of different constituents along with its possible chemical constituents.

GC-MS analysis of alcoholic extract of *F. racemosa* shown presence of Silane; 2, 6, 11- trimethoxy dodecane; Eicosane and 3, 5- dimethyl octane.

*Cuscuta reflexa* extract contains different decane derivatives -3, 5- dimethyloctane; pentadecane; dodecane; trtradecane; hexadecane; Eicosane and heptadecane. Also some anhydrides and carboxylic acid derivatives palmitic anhydride; 3, 5, di-tert- butyl- 4- hydroxyl anisole are also found in the sample.

The alcoholic extract of *Leucas linifolia* contains decane and acid derivatives. It also contains (Z, Z, Z) Methyl 8, 11, 14-heptadecatrienoate; 9, 12, 15-octadecatrienoic acid and some ester derivatives are also found in the extract. Eicosane is present in extracts of *Ficus racemosa* and *Cuscuta reflexa*.

The alcoholic extract of *Sapindus trifoliatus* contains different constituents mainly, Cis and trans-13-Octadecenoic acid; cis-Vaccenic acid; 3-O-Methyl-d-glucose; Mannofuranoside; methyl and Inositol derivatives. Some acid and ester derivatives like l(+) Ascorbic acid 2, 6-dihexadecanoate; n-Hexadecanoic acid; Pentadecanoic acid are also present. Saturated carbon chain compounds such as Hexadecane; Pentadecane and Heptadecane are also present in it.
Variations in the percentage of various constituents of different extracts might take place due to different environmental factors like collection of crude drug, processing of crude drug, climatic conditions, seasonal factors, development stage and location. Against various plant, human and food-borne pathogenic pests and fungi generally, the secondary metabolites of the crude drug are considered potentially effective and non-phytotoxic.