HPTLC Fingerprinting and Anti-asthmatic Activity of Roots of Two Different Sources of Bharangi

Sagar Soundalgekar¹, Atish Naik¹, Kirankumar Hullatti²,* , Sunil Jalalpure¹, Sneha Patil¹, Vishakha Parab Gaonkar¹

¹Department of Pharmacognosy, KLE College of Pharmacy, Belagavi, KLE Academy of Higher Education and Research, Belagavi, Karnataka, INDIA.
²Department of Pharmacognosy, KLE College of Pharmacy, Bengaluru, Karnataka, INDIA.

ABSTRACT
The present research work aims at developing HPTLC fingerprints of two sources of Bharangi Clerodendrum serratum (Linn.) and Clerodendrum indicum (Linn.), along with in vivo anti-asthmatic evaluation in OVA-induced Wistar rat model. Air dried roots of both plants were subjected for extraction by maceration followed by soxhlet using ethanol (80%). Further a HPTLC fingerprints was developed for the quantification of Oleanolic acid and Stigma sterol to distinguish both the plants. The anti-asthmatic activity of Clerodendrum serratum and Clerodendrum indicum was evaluated in Ovalbumin induced Wistar rat model and inflammatory parameters like absolute eosinophil count in BALF; total leukocyte count in BALF, absolute eosinophil count in the Blood, IgE antibodies in serum along with the histopathological changes of lungs were studied. HPTLC fingerprinting showed the presence of Oleanolic acid in Clerodendrum serratum and it was found to be absent in Clerodendrum indicum whereas Stigmasterol was found to be present in Clerodendrum indicum and absent in Clerodendrum serratum. In in vivo anti-asthmatic activity, test drugs have shown significant decrease in inflammatory parameters such as Absolute eosinophil count in Blood, Absolute eosinophil count in BALF; Total leukocyte count in the BALF and Concentration of IgE antibodies. Among Extract treated groups CSE1 and CSE2 showed good results with p<0.0001 when compared with asthmatic group. All the studied parameters clearly conclude that both these plants with controversial botanical identity can be distinguished based on their physiochemical and HPTLC fingerprint profiles. The results suggest that the hydroalcoholic extracts of both the plants significantly possess anti-asthmatic activity.

Key words: Bharangi, Clerodendrum serratum, Clerodendrum indicum, HPTLC, Anti-asthmatic activity.

INTRODUCTION
Traditional herbal drugs with various phytoconstituents and properties have been used as medicines for the treatment of a wide range of diseases from ancient times. These medicines have been considered to be intrinsically safe, due to their natural occurrence, efficacy and less side effects.¹² The special status for botanical medicines is due to their complex composition and the resulting challenges for analytical methodologies and activity test. Now a days a shift has been taking place from classical herbal drugs to phyto-pharmaceuticals, which defined as labeled or standardized extracts.²¹ A number of Indian medicinal plants are using for treating various disease conditions. They may be tonics, antimalarial, antipyretics, aphrodisiacs, expectorants, hepatoprotectives, antirheumatics, diuretics etc. However, proper methodologies for the research and development are the need of the day for tapping the full therapeutic potentials of plants.¹³

HPTLC is a powerful modern analytical method for qualitative, quantitative standardization and simultaneous assay of several components in a multi-component formulation.²² It has been investigated for Authentication of various species of plant along with evaluation of stability and consistency.²³ In this regard, this project was selected to develop the HPTLC fingerprint and quality control parameters for Clerodendrum serratum and Clerodendrum indicum belonging to Verbenaceaefamily, which are commonly known as Bharangi. It is one important herb used in many formulations in Ayurveda for various ailments. These plants are used clinically in treatment of bronchitis, asthma, fevers, blood disease, tumors, inflammations, burning sensation, epilepsy, malaria, ulcer and wounds. Leaves are used in fever and hiccough. Root bark contains mainly sapogenins, while leaves contain flavonoids and phenolic acids.⁶

Asthma is a chronic inflammatory condition associated with repeated wheezing, breathlessness, chest tightness and coughing.³¹ At present, it is managed by using beta-2 agonist, anticholinergics, methylxanthines, mast cell stabilizers, leukotrine antagonist, glucocorticoids, Anti-IgE antibody like omalizumab and need to be used for prolonged time. Since from ancient days people have found the relief from the disease by using natural products, the natural drugs used in the treatments of asthma are enumerated as Ephedra, Vásaka, Licorice, Coleus forsholii, Tylophoraindica, Ginkobilobo, Shinpi and Nyctanthesarboritristis Linn.⁶ Clerodendrum serratum and Clerodendrum indicum are used as antiasthmatic agents in traditional system of medicine under the name Bharangi. The proposed research work is adopted to provide beneficial information regarding the standardization and in vivo anti asthmatic activity of the two species of Bharangi.

Correspondence: Dr. Kiran Kumar Hullatti, Professor, Department of Pharmacognosy, KLE College of Pharmacy, Bengaluru, Karnataka, INDIA.
Phone No: +91-9448800184; E-mail: kkhullatti@gmail.com
MATERIALS AND METHODS

Chemicals and Reagents
The solvents used for HPTLC analysis such as chloroform, formic acid, toluene, methanol were purchased from Merck, Mumbai. Stigmasterol was procured from Sigma–Aldrich, Bangalore. All other reagents used were of laboratory grade.

Collection of Plant material
The plants of Clerodendrum serratum was collected from the local areas of kanburgi, Belagavi and its botanical identification was confirmed from RMRC-ICMR, Belagavi, (Karnataka), India. The herbarium specimens of the plants have been deposited at RMRC-ICMR with accession number RMRC-1286. Clerodendrum indicum was procured and authenticated by Dr. Madhava Chetty, Professor at Sri Venkateshwara University, Tirupati, Andhra Pradesh. The voucher specimens of the species have been deposited in RMRC and preserved with accession numbers RMRC-992 and SVU-1169.

Processing and Extraction of plant material
The roots of the collected plants were washed, shade dried and powdered then subjected to maceration with ethanol (80%) for 24 hr. The marc was then subjected to successive hot continuous extraction (soxhlet) with ethanol. The extract was filtered and concentrated in Rota evaporator at 50°C.

Pharmacognostic Evaluation[9]
Dry powdered roots were subjected for various quality control parameters like Extractive value, ash value, swelling index and foaming index by following standard procedures. Hydro alcoholic extracts of both the species were subjected to phytochemical screening for analyzing the presence of secondary metabolites.

HPTLC Fingerprinting analysis[10,11]
Preparation of standard and sample solutions
The stock solutions of standard Stigmasterol and Oleanolic acid were prepared by dissolving 5mg of each in 5ml of methanol (1.0mg/ml) separately. The above solution 1ml was taken and further diluted with 10ml of methanol (0.1mg/ml) this stock solution was used to make calibration curves of Stigmasterol and Oleanolic acid. Sample Solutions of the extracts of Clerodendrum serratum and Clerodendrum indicum were prepared by dissolving 500mg of each of the extracts in 5 ml of methanol.

Chromatographic conditions
Precoated TLC silica gel Aluminium plates 60 F 254 (20X10cm, 250µm thickness, Merck, Darmstadt, Germany) were used for chromatographic analysis. The sample solutions were applied on the plates in the form of bands of 8mm width with the help of Hamilton syringe(100µl) using CAMAG Linomat V (Camag, Muttenz, Switzerland) sample applicator and were controlled by WinCATS software 1.4.4. Plates were developed in 20 x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). A TLC scanner IV was used for scanning the HPTLC plates. Two different aliquots of solution of the extracts (10.0, 20.0µl) and that of standard (2.0, 5.0µl) were applied on 10 x 10 cm HPTLC plates for the generation of fingerprint profile.

For analysis with Stigmasterol, the mobile phase consisted of toluene: ethyl acetate: formic acid in the ratio of 14.5: 3.5: 0.1 and for the analysis with Oleanolic acid the mobile phase consisting of toluene: ethyl acetate: formic acid in the ratio of 14.5: 3.5: 0.1 was used per plate. The optimized chamber saturation time for mobile phase was 10 min at room temperature (25 ± 2°C) at relative humidity of 60 % ± 5 RH. The plates were developed and scanned within 10 min using densitometric scanner IV in the remission mode at 254, 366 and 540nm. The quantitative analysis was carried out by comparing the retention time and peak area of the standards and that of the sample extracts.

Pharmacological investigation
Animal selection
Albino Wistar rats weighing 100-120 gm were used in the experiment and female albino wistar rats weighing 100-120gm used for acute toxicity. The rats were kept on ad libitum feed and water. After fifteen days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC) Resolution No. KLECOP/IAEC/Res.17-31/08/2013.

Acute oral toxicity study and dose selection
The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD), revised draft guidelines 423. According to Acute Oral Toxicity study, hydro-alcoholic Clerodendrum serratum and Clerodendrum indicum extract did not show any significant toxic effect till 2000mg/kg dose. Thus, in the present study, the anti-asthmatic effect of Clerodendrum serratum and Clerodendrum indicum extracts was evaluated against Ovalbumin induced asthmatic rats with the dose of 400mg/kg (1/5 of LD50) and 200mg/kg(1/10 of LD50) for each extract.

Induction of Asthma[12]
Asthma was induced in rats by i.p administration of alum precipitated OVA and Ovalbumin exposure in histamine chamber. Sensitization: In this stage animals are sensitized with the alum precipitated Ovalbumin allergen through the intra peritoneal route of administration.

Challenge: To the sensitized animals the Ovalbumin allergen is administered through the aerosol form at 1% concentration in PBS solution from 14th day to 35th day. Then the dose is increased and aerosol of ovalbumin was administered from the 36th day to 42nd day at 2% concentration.

Animal groupings and treatment
Animals were randomly divided into six groups (6 rats/group). Asthma is induced by ovalbumin (OVA). Group 1 received only Phosphate Buffer saline which served as a normal control (untreated), group 2 OVA-aerosols, groups 3 and 4 received different doses of Clerodendrum serratum extracts (200 and 400 mg/kg p.o.), Group 5 and 6 received different doses of Clerodendrum indicum extracts (200 and 400 mg/kg p.o.). (Table 1)

Table 1: Animal groupings for in vivo anti-asthmatic study.

| Group type | Group | Description |
|------------|-------|-------------|
| Normal     | Group 1 | PBS (1 ml P.O) for 14 – 42nd day |
| Asthma     | Group 2 | Only OVA Aerosol |
| Asthma+CSE1| Group 3 | CSE (200mg/kg P.O) + OVA Aerosol |
| Asthma +CSE2| Group 4 | CSE (400mg/kg P.O) + OVA Aerosol |
| Asthma +CIE1| Group 5 | CIE (200mg/kg P.O) + OVA Aerosol |
| Asthma +CIE2| Group 5 | CIE (400mg/kg P.O) + OVA Aerosol |
Evaluation parameters
On the 43rd day animals were anaesthetized, blood was collected through retro-orbital puncture and subjected for biochemical studies, serum is separated for IgE estimation, lungs are separated for the Histopathology studies and BALF is collected from trachea by using catheter.

Eosinophil count in blood:
Blood was allowed to coagulate for 20 min and then centrifuged at 2000 rpm for 15 min. The serum was separated and used for estimations of eosinophils.

Estimation of eosinophil’s and WBC in BALF
Bronchiolar lavaging was done with the normal saline 7ml normal saline was flushed in to bronchi by using feeding needle. Cells in the Lavage treated with ACK buffer which kills RBC and WBC cells was collected by centrifuging at 5000rpm for 10 min they were stored in 20%foetal bovine serum in RPMI solution. WBC cells was counted by using WBC diluting fluid. By using total leukocyte count and differential leukocyte count, absolute eosinophil count was done by using indirect method.

IgE Estimation
The concentration of IgE in serum was determined using sandwich ELISA kits according to the manufacturer’s instruction. The absorbance was measured at 450 nm using a micro plate reader.

Histo-pathological evaluation of lungs
To obtain information about the histological changes in lungs was observed by performing Histopathology of Lungs in normal, diseased, CSE1, CSE2, CIE2, CIE2 group’s animals. Lungs are embedded in paraffin, sectioned and stained with hematoxylin and eosin. Pathological changes in lungs was observed and reported.

Statistical analysis
Results were expressed as Mean ± S.D., where n= 6. Differences among data were determined using one way ANOVA followed by Tukey’s multiple comparison test (Graph Pad Prism software, version 5.01). p<0.05 was considered statistically significant.

RESULTS AND DISCUSSION
Pharmacognostic evaluation
The dried powdered roots of both the plants species were evaluated for pharmacognostic evaluation and the results for each parameter is summarized in Table 2. The preliminary phytochemical studies revealed the presence of major chemical constituents in ethanol extracts of both the plants such as alkaloids, flavonoids, terpenoids and tannins.

Anthraquinone glycosides were found to be absent in both the plants. Whereas cardiac glycosides were found to be present in Clerodendrum serratum and absent in Clerodendrum indicum. Saponins were found to be present in Clerodendrum serratum and absent in Clerodendrum indicum.

HPTLC Fingerprinting
HPTLC fingerprint patterns have been evolved for extracts of Clerodendrum serratum and Clerodendrum indicum as shown in Figure 1 and Figure 2 respectively. The Rf value of Oleanolic acid matched with the Rf value of Clerodendrum serratum extract which was about 0.29. Oleanolic acid was found to be absent in extracts of Clerodendrum indicum. Whereas, the Rf value of stigmasterol matched with the Rf value of extract of Clerodendrum indicum which was about 0.34. Stigmasterol was found to be absent in extracts of Clerodendrum serratum.

Quantification of Oleanolic acid and Stigmasterol
The optimized chromatographic conditions were applied for the quantification of markers in the herbal extracts. The HPTLC analysis revealed the presence of 0.24% w/w of Oleanolic acid in Clerodendrum serratum extract. Whereas, the quantity of Stigmasterol in Clerodendrum indicum was found to be 1% w/w.

Table 2: Pharmacognostic evaluation parameters.

| Parameters                | Clerodendrum serratum (%w/w) | Clerodendrum indicum (%w/w) |
|--------------------------|------------------------------|-----------------------------|
| Alcohol soluble extractive value | 11.35±0.46                 | 8.12±0.58                   |
| Water soluble extractive value | 6.06±0.38                  | 1.08±0.45                   |
| Total ash value          | 13.65±0.12                  | 15.2±0.15                   |
| Water soluble ash value  | 0.87±0.28                   | 0.88±0.28                   |
| Acid insoluble ash value | 7.9±0.08                    | 8.9±0.14                    |
| Loss on drying           | 12.68±0.21                  | 10.11±0.45                  |
| Foaming index            | 125.35±9.0                  | <100                        |
| Swelling index           | 5.20±0.53                   | 5.62±0.57                   |

Figure 1: HPTLC fingerprint of Clerodendrum serratum (I, II) and Clerodendrum indicum (V, VI) with Oleanolic acid (III, IV).

Figure 2: HPTLC fingerprint of Clerodendrum serratum (I, II) and Clerodendrum indicum (V, VI) with Stigmasterol (III, IV).
**In vivo Anti-asthmatic activity**

**Evaluation of biochemical parameters**

**Eosinophil Count in BLOOD:**

Eosinophil count was significantly ($P<0.0001$) increased in Asthma group when compared to normal group. From Figure 3(A) it is evident that Eosinophil count was decreased significantly in the treatment groups (ASTHMA+CSE1, ASTHMA+CSE2, ASTHMA+CIE1 and ASTHMA+CIE2) when compared to the Asthma group, however there was a less significant result with ASTHMA+CIE2 group but there was marked decrease in the eosinophil count in comparison to Asthma group (Table 3).

**WBC count and Eosinophil count in BALF**

The WBC count and Eosinophil count was significantly ($P<0.0001$) increased in the Asthma group in comparison to the normal group. The treatment group (ASTHMA+CSE1, ASTHMA+CSE2, ASTHMA+CIE1 and ASTHMA+CIE2) showed significant decrease in the WBC count as well as in eosinophil count when compared to the Asthma group. However, a less significant result were seen with ASTHMA+CIE2 group.

*From Figure 3 (B-C) it can be seen that a ASTHMA+CSE2 group showed good significance among all other treatment groups (Table 4 and 5).*

**IgE antibody concentration in blood serum**

A significant increase of IgE concentration was observed in the Asthma group in comparison to the normal group. Consequently, a significant decrease in the IgE concentration was observed in the treatment groups (ASTHMA+CSE1, ASTHMA+CSE2, ASTHMA+CIE1 and ASTHMA+CIE2). However, there was a less significant result with ASTHMA+CSE2 group but there was marked decrease in the IgE concentration in comparison to Asthma group. The results are depicted in Figure 3(D) (Table 6).

**Histopathology of lungs**

The Histopathological changes of the normal, Asthma and treated group are shown in Figure 4 and 5. The Ovalbumin exposed group showed hyperplasia of the bronchial walls and also eosinophil infiltration in the lungs. The damage in the treatment (ASTHMA+CSE1, ASTHMA+CSE2, ASTHMA+CIE1 and ASTHMA+CIE2) groups was markedly attenuated in comparison to the Asthma group.

### Table 3: Effect of Clerodendrum serratum and Clerodendrum indicum root extract on AEC of blood in control and experimental rats.

| Animals | Normal | Asthma | Asthma+CSE1 (200mg/kg) | Asthma+CSE2 (400mg/kg) | Asthma+CIE1 (200mg/kg) | Asthma+CIE2 (400mg/kg) |
|---------|--------|--------|------------------------|------------------------|------------------------|------------------------|
| 1       | 50     | 375    | 50                     | 125                    | 212                    | 250                    |
| 2       | 50     | 350    | 175                    | 150                    | 175                    | 195                    |
| 3       | 50     | 360    | 125                    | 225                    | 215                    | 215                    |
| 4       | 75     | 325    | 75                     | 175                    | 186                    | 245                    |
| 5       | 60     | 320    | 125                    | 50                     | 150                    | 235                    |
| 6       | 59     | 350    | 150                    | 350                    | 195                    | 255                    |
| Mean±SEM| 57.33±4.01 | 346.7±8.53* | 116.7±19.0*** | 179.2±41.54*** | 188.8±9.94*** | 232.5±9.46*** |

*P<0.0001 when compared with normal, ***P<0.0001 when compared with Diseased, **P<0.001 when compared with Diseased

### Table 4: Effect of Clerodendrum serratum and Clerodendrum indicum root extract on Total Leukocyte Count in BALF in control and experimental rats.

| Animals | Normal | Asthma | Asthma+CSE1 (200mg/kg) | Asthma+CSE2 (400mg/kg) | Asthma+CIE1 (200mg/kg) | Asthma+CIE2 (400mg/kg) |
|---------|--------|--------|------------------------|------------------------|------------------------|------------------------|
| 1       | 1300   | 10000  | 3450                   | 2000                   | 3900                   | 5150                   |
| 2       | 1150   | 11300  | 4100                   | 2650                   | 3300                   | 4650                   |
| 3       | 2800   | 10150  | 4850                   | 2900                   | 3650                   | 4400                   |
| 4       | 1550   | 9900   | 3250                   | 2800                   | 4200                   | 3950                   |
| 5       | 1400   | 7650   | 3750                   | 3650                   | 3350                   | 4450                   |
| 6       | 1100   | 11800  | 3800                   | 3100                   | 3450                   | 5750                   |
| Mean±SEM| 1550±634.0 | 10133±1441* | 3867±230.5*** | 2850±221.4*** | 3642±143.4*** | 4725±259.4*** |

*P<0.0001 when compared with normal, ***P<0.0001 when compared with Diseased, **P<0.001 when compared with Diseased
Table 5: Effect of *Clerodendrum serratum* and *Clerodendrum indicum* root extract on Absolute Eosinophil Count in BALF in control and experimental rats.

| Animals | Normal | Asthma | Asthma+CSE1 (200mg/kg) | Asthma+CSE2 (400mg/kg) | Asthma+CIE1 (200mg/kg) | Asthma+CIE2 (400mg/kg) |
|---------|--------|-------|------------------------|------------------------|------------------------|------------------------|
| 1       | 52     | 400   | 138                    | 80                     | 156                    | 206                    |
| 2       | 46     | 452   | 164                    | 106                    | 132                    | 186                    |
| 3       | 112    | 406   | 194                    | 116                    | 146                    | 176                    |
| 4       | 62     | 396   | 130                    | 112                    | 168                    | 158                    |
| 5       | 56     | 306   | 150                    | 146                    | 134                    | 178                    |
| 6       | 44     | 472   | 152                    | 124                    | 138                    | 230                    |

Mean±SEM 62.00±25.36 405.3±57.63# 154.7±9.12*** 114.0±8.85*** 145.7±5.73*** 189.0±10.38***

#P<0.0001 when compared with normal, ***P<0.0001 when compared with Diseased, **P<0.001 when compared with Diseased

Table 6: Effect of *Clerodendrum serratum* and *Clerodendrum indicum* root extract on Absolute Eosinophil Count in BALF in control and experimental rats.

| Animals | Normal | Asthma | Asthma+CSE1 (200mg/kg) | Asthma+CSE2 (400mg/kg) | Asthma+CIE1 (200mg/kg) | Asthma+CIE2 (400mg/kg) |
|---------|--------|-------|------------------------|------------------------|------------------------|------------------------|
| 1       | 56.34  | 96.52 | 23.47                  | 46.54                  | 36.19                  | 33.68                  |
| 2       | 55.72  | 93.46 | 22.01                  | 50.37                  | 39.94                  | 35.28                  |
| 3       | 53.01  | 101.94| 24.02                  | 53.15                  | 37.23                  | 38.97                  |
| 4       | 54.95  | 90.89 | 44.74                  | 51.89                  | 39.04                  | 37.99                  |
| 5       | 59.33  | 108.96| 47.45                  | 54.26                  | 36.67                  | 40.36                  |
| 6       | 52.03  | 120.50| 27.36                  | 55.44                  | 35.98                  | 36.26                  |

Mean±SEM 55.24±2.59 102.0±11.11# 31.51±4.68*** 51.95±1.3*** 37.51±0.66*** 37.09±1.01***

#P<0.0001 when compared with normal, ***P<0.0001 when compared with Diseased, **P<0.001 when compared with Diseased

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**Figure 3:** Graphical representation of A) Effect of CSE and CIE on Absolute Eosinophil count in blood, B) Effect of CSE and CIE on Leucocyte count in BALF, C) Effect of CSE and CIE on Absolute Eosinophil count in BALF and D) Effect of CSE and CIE on IgE Concentration in Blood Serum.

**Figure 4:** Histopathological changes in Bronchiole in a) Normal group, b) Asthmatic group, c) ASTHMA+CSE1 Group, d) ASTHMA+CSE2 Group, e) ASTHMA+CIE1 Group, f) ASTHMA+CIE1 Group.
CONCLUSION

In the present research work the correct identification and authentication of two sources of Bharangi has been reported. The HPTLC fingerprinting analysis revealed that marker compound Oleanolic acid was found to be present in Clerodendrum serratum and absent in Clerodendrum indicum. Stigmastanol was found to be present in Clerodendrum indicum and absent in Clerodendrum serratum. Both these plants with controversial botanical identity can be clearly distinguished based on their physiochemical and HPTLC fingerprint profiles. An attempt was made in the present study to evaluate the effects of Clerodendrum serratum and Clerodendrum indicum on airway inflammation and remodelling were investigated. Clerodendrum serratum and Clerodendrum indicum reduces infiltration leukocytes and eosinophils in BALF. Also influx of eosinophils and plasma cells in lung tissue were decreased, hyperplasia of bronchioles were markedly reduced compared to asthma group. Anti-OVA IgE antibody levels were reduced in serum of OVA sensitised and challenged rats treated with Clerodendrum serratum and Clerodendrum indicum root extract. The results of present study suggest that the hydroalcoholic extracts of Clerodendrum serratum and Clerodendrum indicum significantly attenuated the airway inflammation induced by ovalbumin hence the plants possess anti-asthmatic activity.

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CONFLICT OF INTEREST

The authors declare no conflict of Interest.

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Figure 5: Histopathological changes in Alveolar cells in a) Normal group, b) Asthmatic group, c) ASTHMA+CSE1 Group, d) ASTHMA+CSE2 Group, e) ASTHMA+CIE1 Group, f) ASTHMA+CIE1 Group.
In the present research work HPTLC fingerprints of two sources of Bharangi *Clerodendrum serratum* (Linn.) and *Clerodendrum indicum* (Linn.), along with *in vivo* anti-asthmatic evaluation in OVA-induced Wistar rat model has been carried out. Further a HPTLC fingerprints was developed for the quantification of Oleanolic acid and Stigmasterol to distinguish both the plants. The anti-asthmatic activity of *Clerodendrum serratum* and *Clerodendrum indicum* was evaluated in Ova albumin induced Wistar rat model and inflammatory parameters like absolute eosinophil count in Blood, total leukocyte count in BALF, absolute eosinophil count in BALF, and Concentration of IgE antibodies in serum along with the histopathological changes of lungs were studied. HPTLC fingerprinting showed the presence of Oleanolic acid in *Clerodendrum serratum* and it was found to be absent in *Clerodendrum indicum* whereas Stigmasterol was found to be present in *Clerodendrum indicum* and absent in *Clerodendrum serratum*. In *in-vivo* anti-asthmatic activity, test drugs have shown significant decrease in inflammatory parameters such as Absolute eosinophil count in Blood, Absolute eosinophil count in BALF Total leukocyte count in the BALF and Concentration of IgE antibodies. Among Extract treated groups CSE1 and CSE2 showed good results with p<0.0001 when compared with asthmatic group. The results suggest that the hydroalcoholic extracts of both the plants significantly possess anti-asthmatic activity.