Asthma involves chronic inflammation of airway and is characterized by the hyper reactivity which causes limited airflow by producing bronchoconstriction, mucus plug and increased inflammation. There is increasing demand for the use of medicines obtained from natural origin in the treatment of asthma. In the same way, there are increasing demands for the use of traditional medicines in the therapy of asthma, because of few side effects compared to those of synthetic drugs.

**Collection, identification and authentication of plant material**

Leaves of *Vitex negundo* were collected in the month of august, aerial part of *B. diffusa* was collected in the month of august and leaves of *E. neriifolia* were collected in December month from local region of Bhopal district Madhya Pradesh (India). Herbarium specimens were prepared, identification and authentication were done from Dr Vinayak Naik, Senior Research Scientist Piramal Life Sciences India Ltd. Goregaon (E) Mumbai. A Voucher specimen of the plant Nos. NPIL/PLS/08-689, NPIL/PLS/05-378 and NPIL/PLS/10-1038 of *V. negundo*, *B. diffusa* and *E. neriifolia* respectively have been deposited for future reference. The plant materials were shade dried and coarsely powdered by using mechanical grinder. The powders were passed through sieve no. 40 and stored in airtight container for the extraction.

**Ethanolic extraction and fractionation of *Vitex negundo* leaves, *Boerhavia diffusa* herb and *Euphorbia neriifolia* leaves**

The air dried powdered leaves (1000g) of *Vitex negundo* were defatted with petroleum ether (60-80°C) and remaining marc was extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanolic extract (220.0g). The ethanolic extract was dissolved in ethanol and water (1:2 v/v) and partitioned with chloroform and ethyl acetate in 50mL portion for several times till complete extraction takes place. Resulted in chloroform fraction (102.0g) and ethyl acetate fraction (106.0g) upon concentration under reduced pressure. The air dried whole herb of *Boerhavia diffusa* (1000g) were
defatted with petroleum ether (60-80°C) and remaining marc was extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanolic extract (115.4g). Ethanolic extract was dissolved in ethanol and water (1:2 v/v) and partitioned with chloroform and ethyl acetate in 50mL portion for several times till complete extraction takes place. Resulted in chloroform fraction (43.2g) and ethyl acetate fraction (58.1g) upon concentration under reduced pressure. The air dried powdered leaves of *Euphorbia neriifolia* (1000g) were defatted with petroleum ether (60-80°C) and remaining marc was extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanolic extract (365g). Ethanolic extract was dissolved in ethanol and water (1:2 v/v) and partitioned with chloroform and ethyl acetate in 50mL portion for several times till complete extraction takes place. Resulted in chloroform fraction (149.8g) and ethyl acetate fraction (182.0g) fraction upon concentration under reduced pressure.

**Toxicological studies**

Toxicological studies of various extracts of *Vitex negundo*, *Boerhavia diffusa* and *Euphorbia neriifolia* was performed and it was found that it was safe and can be used for further pharmacological screening.

**Experimental Animals**

The experiments were conducted on Swiss albino mice weighing 20-25g, albino rats weighing 110-150g and healthy guinea pigs (Dunkin hartley) weighing 350-500g. The animals were maintained separately for various experiments. Mice and rats were used for studying eosinophil measurement while guinea pigs were used for histamine induced bronchospasm, isolated ileum preparation and trachea chain preparation. The animals were housed to animal house prior to experimentation at temperature of 25±2°C and 50±5% relative humidity in polypropylene cages with a 12 hours light/dark cycle and allowed free access to diet and sufficient water. The experiments were performed by following rules and regulations of CPCSEA (Committee for the Purpose of Control and Supervision...
on Experimental Animals) approved by the IAEC (Institutional Animal Ethical Committee), RKDF University, Bhopal.

Pharmacological screening

In-vitro and in-vivo study using soft tissues and experimental animal models were performed to evaluate the ethanolic extracts and its chloroform and ethyl acetate fractions of *Vitex negundo*, *Boerhavia diffusa* and *Euphorbia neriifolia* were evaluated for anti-asthmatic potential.

**Contractions produced by histamine on isolated guinea pig ileum preparation and effects of ethanolic extract and different fractions of Vitex negundo, Boerhavia diffusa and Euphorbia neriifolia on it**

The effect of ethanolic extract and its chloroform and ethyl acetate fractions of *Vitex negundo*, *Boerhavia diffusa* and *Euphorbia neriifolia* on histamine induced contractions on isolated guinea pig ileum preparation were evaluated at the concentration of 10mg.

The ethanolic extract of *V. negundo* (23.10±2.34, 31.99±3.05, 43.33±2.67, 48.44±2.34, 61.33±2.00), *B. diffusa* (21.99±1.76, 34.66±2.00, 41.55±2.03, 50.66±2.00, 61.77±1.67) and *E. neriifolia* (10.21±2.69, 40.88±3.00, 43.55±2.34, 48.22±3.42, 58.22±4.33) significantly antagonized the effect of histamine induced contraction on isolated guinea pig ileum preparation at the concentration of 10mg when compared with the control group (vehicle) (42.44±1.01, 54.66±1.33, 68.67±1.33, 85.10±2.03, 95.11±2.34), respectively at the log concentration of histamine at 4.00, 3.699, 3.3979, 3.0969 and 2.7960. The activity was found to be dose dependent (Table 3.1-3.3 and figure 3.1-3.3).

The chloroform and ethyl acetate fraction of *V. negundo* did not show any significant activity.

Chloroform fraction of *B. diffusa* showed maximum reduction in histamine induced contractions on isolated guinea pig ileum preparation (15.33±1.33, 27.10±2.69, 31.77±3.41, 36.44±2.33, 40.22±2.03) followed by ethyl acetate fraction of *E. neriifolia* (22.44±3.35, 29.77±3.67, 35.77±3.67, 41.10±2.69,
50.99±3.17), respectively at the log concentration of histamine 4.00, 3.699, 3.3979, 3.0969 and 2.7960 at the concentration of 10mg.

The above results revealed that ethanolic extract of *V. negundo*, *B. diffusa* and, *E. neriifolia*, chloroform fraction of *B. diffusa* and ethyl acetate fraction of *E. neriifolia* significantly antagonized the effect of histamine induced contraction on isolated guinea pig ileum preparation at the concentration of 10mg at the log concentration of histamine at 4.00, 3.699, 3.3979, 3.0969 and 2.7960.

Contractions produced by histamine on isolated guinea pig tracheal chain preparation and effects of ethanolic extract and different fractions of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* on it

The effect of ethanolic extract and its various fractions of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* on isolated guinea pig tracheal chain preparation were evaluated at the concentration of 10mg. The ethanolic extracts of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* significantly antagonized the effect of histamine induced contraction on isolated guinea pig tracheal chain. The ethanolic extracts of *V. negundo* (23.88±2.17, 34.44±3.00, 40.44±1.68, 47.77±4.33, 61.77±2.34), *B. diffusa* (24.33±2.84, 35.32±3.05, 41.77±3.00, 47.55±4.68, 62.44±3.35) and *E. neriifolia* (24.33±2.84, 34.88±3.67, 41.33±3.71, 47.33±5.03, 62.66±3.70), respectively antagonized the effect of histamine induced contraction, when compared with vehicle (25.20±1.40, 34.40±1.80, 38.06±1.85, 46.60±2.25, 66.66±3.05) at the log concentration of histamine (4.00, 3.699, 3.3979, 3.0969, 2.7960), respectively (Table 3.4-3.6 and Figure 3.4-3.6).

Chloroform and Ethyl acetate fraction of *V. negundo* did not show any activity. Chloroform fraction of *B. diffusa* (17.55±3.14, 21.33±3.71, 27.55±4.68, 34.66±3.05, 43.99±4.16) showed significant inhibition in contraction. Ethanolic extract and ethyl acetate fraction of *B. diffusa* did not show any significant activity.
Ethyl acetate fraction of *E. neriifolia* (20.22±1.68, 16.88±2.14, 15.99±2.40, 13.77±1.01, 13.33±1.76) significantly inhibited the contraction induced by histamine.

In the presence of ethanolic extracts of *V. neundo, B. diffusa* and *E. neriifolia* and chloroform fraction of *B. diffusa* and ethyl acetate fraction of *E. neriifolia* by guinea pig ileum and trachea chain method exhibited right side shift of dose response curve of histamine, indicated anti-asthmatic activity. The present study revealed that ethanolic extract of *V. negundo*, ethanolic extract of *B. diffusa* and its chloroform fraction and *E. neriifolia* extract and its ethyl acetate fraction at the concentration of 10mg significantly inhibited the contraction of isolated guinea pig ileum and trachea chain preparation produced by histamine, showed anti-asthmatic properties of these plants because of H1 receptor antagonist effect.

**Bronchospasm produced by histamine on guinea pig and effects of ethanolic extract and different fractions of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* on it**

Histamine is inflammatory mediators involved in asthma which causes inflammation and hyperresponsiveness of bronchial airway. The anti-asthmatic study of ethanolic extracts of *V. negundo, B. diffusa* and *E. neriifolia* on histamine induced bronchospasm was carried out at three dose levels 150, 300, and 600mg/kg body weight in guinea pig. Results showed that ethanolic extracts of *V. negundo, B. diffusa* and *E. neriifolia* significantly extended the latent period of convulsion as compared to standard (Ketotifen fumarate) upon histamine exposure. The percentage protection offered by the ethanolic extracts of *V. negundo, B. diffusa* and *E. neriifolia* were found to be 65.15, 70.48 and 75.96 respectively at the dose of 600mg/kg body weight (Table 3.7 to 3.9 and figure 3.7-3.9).

The chloroform and ethyl acetate fractions of *V. negundo, B. diffusa* and *E. neriifolia* were also evaluated on histamine induced bronchospasm at the doses of 150, 300 and 600 mg/kg body weight. Results showed that ethyl acetate
fraction of *B. diffusa* showed the maximum spasmolytic activity (90.42%) followed by ethyl acetate fraction of *E. neriifolia* (89.36%) at the dose of 150mg/kg.

**Bronchospasm produced by acetylcholine on guinea pig and effects of ethanolic extract and different fractions of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* on it**

The ethanolic extract of *V. negundo, B. diffusa* and *E. neriifolia* on acetylcholine induced bronchospasm were evaluated at three different dose levels 150, 300 and 600mg/kg body weight in guinea pigs. Results showed that ethanolic extracts of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* significantly extended the latent period of convulsion as compared to standard (Ketotifen fumarate). The percentage protection offered by the ethanolic extracts of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* were found to be 69.44, 65.22 and 77.41, respectively at dose level of 600mg/kg body weight. (Table 3.10 to 3.12 and figure 3.10 to 3.12).

Chloroform fraction of *B. diffusa*, and Ethyl acetate fraction of *E. neriifolia* offered protection, 88.62% and 90.07% at dose level of 600mg/kg body weight respectively.

**Effects on eosinophil count produced by ethanolic extract and different fractions of *Vitex negundo, Boerhavia diffusa* and *Euphorbia neriifolia* in mice**

The percentage protection of eosinophil was measured in milk induced mice with the treatment of ethanolic extracts of *V. negundo, B. diffusa* and *E. neriifolia* and their chloroform and ethyl acetate fractions at the doses of 150, 300 and 600mg/kg p.o. the percentage protection of eosinophils measured in milk induced mice was found to be 38.83, 39.18, 52.31, 52.48, 55.11, 58.55, and 56.10, 37.00, 34.29, respectively when treated with ethanolic extracts of *V. negundo, B. diffusa* and *E. neriifolia* at the dose level of 600mg/kg body weight. The protection in the Ethyl acetate fraction of *E. neriifolia* (79.08%) showed maximum protection followed by Ethyl acetate fraction of *V. negundo* (77.09%). A blood eosinophilia
tells us both allergic and non-allergic asthma. The eosinophil count increases in body fluids and tissues, emphasis is placed on the number of eosinophils in blood. Eosinophilia is often associated with allergic respiratory disorder together with pulmonary infiltrates which can be detectable on chest films. A recurrent milk aspiration produces changes in airway mechanics, lung eosinophilia in animal model, the observations of the present study indicated that ethyl acetate, chloroform and ethyl acetate fractions of ethanolic extract of *E. neriifolia*, *B. diffusa* and *V. negundo* reduced milk induced eosinophilia. In India *V. negundo*, *B. diffusa* and *E. neriifolia* are being used traditionally in the treatment of asthma. This study was carried out to evaluate these herbs scientifically to justify their traditional claim as anti-asthmatic drugs. Results obtained from all the parameters tested revealed that *V. negundo*, *B. diffusa* and *E. neriifolia* have significant anti-asthmatic activity. The ethanolic extract of *V. negundo*, chloroform fraction and ethyl acetate fraction of *B. diffusa* and *E. neriifolia* were found to be the most effective in all the models. This anti-asthmatic activity of ethanolic extract of *V. negundo* may be due to the presence of alkaloids, glycosides, tannins, sterols, flavonoids, phenolic compounds. The chloroform fraction and ethyl acetate fraction of *B. diffusa* was found the most significant. The phytochemical study revealed the presence of alkaloids, glycosides, tannins, flavonoids, phenolic compounds in chloroform and ethyl acetate fractions. Therefore these constituents may be responsible for anti-asthmatic activity. The ethyl acetate fraction of *E. neriifolia* was found to be the most effective. This fraction contains glycosides, saponins, flavonoids, phenolic compounds and these may be responsible for anti-asthmatic activity.

In the comparative study the ethanolic extract of *V. negundo* was found to be the most effective followed by *B. diffusa* and *E. neriifolia*. On the basis of all above results, it is concluded that the traditional anti-asthmatic use of *V. negundo*, *B. diffusa* and *E. neriifolia* is justifying.

The results obtained from all the parameters revealed that the ethanolic extract of *V. negundo*, chloroform and ethyl acetate fractions of *B. diffusa* and ethyl acetate fraction of *E. neriifolia* were found to be the most potent for their anti-
asthmatic activity. Therefore these particular fractions were selected for further
detailed phytochemical studies.

The ethanolic extract of *V. negundo*, chloroform fraction and ethyl acetate
fraction of *B. diffusa* and *E. neriifolia* were found to be the most effective in all the
models. This anti-asthmatic activity of ethanolic extract of *V. negundo* may be
due to the presence of betulinic acid, ursolic acid, p-hydroxybenzoic acid,
protocatechuic acid, oleanolic acid, flavonoids angusid, casticin, nishindine,
gluco-nonitol.

The chloroform fraction and ethyl acetate fraction of *B. diffusa* was found the
most significant. The phytochemical study revealed the presence of alkaloids
(Punarnavine-1, Punarnavine-2). Therefore these constituents may be
responsible for anti-asthmatic activity.

The ethyl acetate fraction of *E. neriifolia* was found to be the most effective. This
fraction contains triterpenes such as 3,12-di-O-acetyl-8-O-tigloylingol, (24R)-
cycloartane-3β,24,25-triol, 5,4′-dihydroxy-3,7,3′,5′-tetramethoxyflavone,
pachypodol (5,4′-dihydroxy-3,7,3′-trimethoxyflavone), combretol (5-hydroxy-
3,7,3′,4′,5′-pentamethoxyflavone). These may be responsible for anti-asthmatic
activity.

In the comparative study the ethanolic extract of *V. negundo* was found to be the
most effective followed by *B. diffusa* and *E. neriifolia*. On the basis of all above
results, it is concluded that the traditional anti-asthmatic use of *V. negundo*,
*B. diffusa* and *E. neriifolia* is justifying.

**Determination of extractive values**

The yield of water soluble extractives of *V. negundo, B. diffusa* and *E. neriifolia*
were found to be 26.33%w/w, 13.20%w/w and 37.60%w/w respectively as
represented in table 4.1.

The total ash value of *V. negundo, B. diffusa* and *E. neriifolia* was found to be
5.80%w/w, 8.32%w/w and 7.42%w/w respectively. Acid insoluble ash of *V.
egundo, B. diffusa* and *E. neriifolia* was found to be 0.82%w/w, 1.24%w/w and
1.18%w/w respectively. Water soluble ash value of *V. negundo*, *B. diffusa* and *E. neriifolia* was found to be 4.98%w/w, 7.14 and 6.24%w/w.

The ethanolic extract of *V. negundo*, *B. diffusa* and *E. neriifolia* were then partitioned with chloroform and ethyl acetate to get chloroform and ethyl acetate respectively. The yield of chloroform and ethyl acetate fractions of *V. negundo* were found to be 46.36%w/w and 48.18%w/w, respectively. The yield of chloroform and ethyl acetate fractions of *B. diffusa* were found to be 49.68%w/w and 50.52%w/w respectively. The yield of chloroform and ethyl acetate fractions of *E. neriifolia* were found to be 41.15%w/w and 50.00%w/w, respectively.

The phytochemical screening of ethanolic extract of *V. negundo* revealed the presence of alkaloids, terpenoids, sterols, glycosides, phenolic compounds, tannins and flavonoids, carbohydrates and proteins and amino acids. Its chloroform fraction showed presence of sterols, phenolic compounds and flavonoids. The ethyl acetate fraction showed positive test for presence of glycosides, phenolic compounds and flavonoids.

The phytochemical screening of ethanolic extract of *B. diffusa* revealed the presence of alkaloids, terpenoids, sterols, glycosides, phenolic compounds, flavonoids, carbohydrates and proteins and amino acids. Its chloroform fraction showed presence of alkaloids, sterols, phenolic compounds and flavonoids. The ethyl acetate fraction showed positive test for presence of glycosides, phenolic compounds and flavonoids.

The phytochemical screening of ethanolic extract of *E. neriifolia* revealed the presence of sterols, glycosides, phenolic compounds, flavonoids, saponins, carbohydrates and proteins and amino acids. Its chloroform fraction showed presence of sterols, phenolic compounds and flavonoids. The ethyl acetate fraction showed positive test for presence of glycosides, phenolic compounds and flavonoids.

The thin layer chromatographic studies on ethanolic extract and its chloroform and ethyl acetate fractions of *V. negundo*, *B. diffusa* and *E. neriifolia* were performed with various solvent systems. The ethanolic extract of *V. negundo* showed the best resolution in solvent system chloroform: methanol (9:1v/v) by
separating maximum 08 spots. The chloroform fraction showed the best resolution of maximum constituents i.e. 06 in mobile phase chloroform:methanol (6:4v/v). The ethyl acetate fraction showed best resolution of various constituents in solvent system toluene: ethyl acetate (6:4v/v) as represented in table 4.5.

The ethanolic extract of *B. diffusa* showed the best resolution in solvent system chloroform: methanol (7:3v/v) by separating maximum 06 spots. The chloroform fraction showed the best resolution of maximum constituents i.e. 06 in mobile phase chloroform:methanol (7:3v/v). The ethyl acetate fraction showed best resolution of various constituents in solvent system toluene: ethyl acetate (6:4v/v) as represented in table 4.7.

The ethanolic extract of *E. neriifolia* showed the best resolution in solvent system chloroform: methanol (9:1v/v) by separating maximum 08 spots. The chloroform fraction showed the best resolution of maximum constituents i.e. 06 in mobile phase chloroform:methanol (7:3v/v). Ethyl acetate fraction showed best resolution of various constituents in solvent system toluene: ethyl acetate (6:4v/v) as shown in table 4.9.

Since bioactive compounds present in plants are in combinations and have to be isolated by various chromatoigraphic techniques such as column chromatography and preparative TLC. An attempt has been made to isolate the various compounds present in pharmacological active fraction of the *V negundo*, *B. diffusa* and *E. neriifolia* and have been characterized by IR, NMR and Mass spectroscopic techniques.

**Column chromatography**

**Isolation and Characterization of Compounds VN1 and VN2 from ethyl acetate fraction of *V. negundo***

The air dried powdered leaves (1000g) were defatted with petroleum ether and remaining marc was extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanol extract (220.0g). Ethanol extract was dissolved in ethanol and water (1:2 v/v) and partitioned with ethyl acetate and n butanol in 50mL portion for several times till complete extraction
takes place. Resulting ethyl acetate fraction was concentrated under reduced pressure (106g) and was chromatographed on silica gel column (70cmX15cm, 60-120mesh, 2kg) chromatography and preparative TLC.

Column was initially eluted with chloroform, then polarity was gradually increased with methanol in different concentrations (100:0, 95:5, 90:10, 85:15, 80:20, 70:30, 60:40v/v). 232 fractions each of 50mL were collected and TLC was performed of each fraction individually and eluates were monitored for the presence of various constituents as represented in Table 4.10. Fractions were pooled on the basis of their TLC profile, pooled fractions (36-40) and (55-75) were selected for the isolation of constituents. Preparative TLC of isolated constituents offered VN1 and VN2.

Isolated compound VN1 was white amorphous powder which was soluble in chloroform and insoluble in water. The IR spectrum exhibited an intensely broad band at 3443 cm\(^{-1}\) showed presence of OH stretching, 2923, 1730 (carbonyl stretch), 1230 (C-O stretch, ether).

Mass spectrum showed molecular ion peak [M+1] m/z at 515(M+1) which corresponds to molecular formula C\(_{30}\)H\(_{48}\)O\(_3\).

The Compound A gave a positive Liebermann-Burchardt test. It showed a molecular ion at m/z 456 corresponding to C\(_{30}\)H\(_{48}\)O\(_3\). The \(^1\)H NMR spectrum of compound A showed seven tertiary methyl groups at δ0.67, 0.76, 0.80, 0.83, 0.85, 0.91 and 1.09 on an oleanane skeleton. A doublet of one proton at δ3.69 and a doublet of one vinyl proton at δ5.34 were assigned to H-18 and H-12, respectively, suggesting an olea12-ene skeleton. In \(^{13}\)C-NMR spectrum, the signal corresponding to the carboxyl C-28 appeared at δ143.62. The spectral data were similar to the ones reported for oleanolic acid.

Isolated compound VN2 was yellowish amorphous powder which was soluble in chloroform. The IR spectrum showed intensely broad band at 3454cm\(^{-1}\), moderate intense band at 1194cm\(^{-1}\) observed for the OH bond vibration of hydroxyl group. Carbonyl stretch was observed at 1696cm\(^{-1}\). The corresponding C=\(C\) vibrations was shown at 1453cm \(^{-1}\) was weakly intense band. Mass
spectrum of isolated compound showed molecular ion m/z 271.3[M+1] corresponding to the molecular formula C\textsubscript{30}H\textsubscript{50}O.

$^1$H NMR spectrum, revealed the presence of seven tertiary methyl protons at δ0.83, δ0.88, δ0.93, δ0.94, δ1.00, δ1.06, and δ1.18. A sextet of one proton at δ2.21 corresponds to 19 β –H is characteristic of lupeol. H-3 proton appeared as multiplet at δ3.18 while two broad singlets at δ5.05 and δ5.11 due to two exomethylene protons attached at C29.

$^{13}$C NMR spectrum, showed seven methyl groups at δ28.75 (C-23), δ27.28 (C-28), δ26.62 (C-25), δ25.99 (C-26), δ23.69(C-24), δ23.37(C-27), and δ22.69 (C-30). The deshielded signals at δ79.05 were due to presence of hydroxyl group at C-3. The comparison of the spectral data with reported led us to propose the structure as lupeol.

**Isolation and Characterization of Compound BD1 from *B. diffusa***

The air dried powdered aerial part (1000g) was extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanol extract (115.4g). Ethanol extract was dissolved in ethanol and water (1:2 v/v) and partitioned with ethyl acetate in 50mL portion for several times till complete extraction takes place. Resulting ethyl acetate fraction was concentrated under reduced pressure (58.1g) and was chromatographed on silica gel column (70cmX15cm, 60-120mesh, 2kg) chromatography and preparative TLC. Column was initially eluted with chloroform, then polarity was gradually increased with methanol in different concentrations (100:0, 95:5, 90:10, 85: 15, 80:20, 70:30, 60:40v/v). 110 fractions each of 50mL were collected and TLC was performed of each fraction individually and eluates were monitored for the presence of various constituents as represented in Table 4.12. Fractions were pooled on the basis of their TLC profile; pooled fractions (13-19) were selected for the isolation of constituents. Preparative TLC of isolated constituents offered BD1.

**Compound 1: Physical and spectral properties of isolated compound BD-1**
Appearance: Yellow
Solubility: Chloroform
TLC (Rf value): 0.6
IR (KBr, in cm\(^{-1}\)) : 3454, 2925, 1635, 1463
\(^1\)H NMR (300 MHz, CDCl\(_3\), δ, TMS=0): δ6.907 (1H, S), 5.284 (1H, S), 3.653 (1H, S), 7.609 (1H, m), 1.060 (2H, S), 1.134 (2H, S), 1.257 (2H, S), 1.625 (1H, d, J=7.2 MHz), 1.874 (1H, S), 2.046 (1H, S), 2.309 (S), 2.437 (S), 2.621(S), 2.842 (d, J= 10.8 MHz).
\(^13\)C NMR (75Hz, CDCl\(_3\), δ, TMS=0): δ122.06 (C-1’), 121.40 (C-2’), 143.96 (C-3’), 148.90 (C-4’), 128.22 (C-5’), 124.16 (C-6’), 170.66 (C-2), 131.42 (C-3), 177.45 (C-4), 131.42 (C-6), 170.98 (C-7), 77.43 (C-7).
Mass spectral data: Mass spectrum of isolated compound showed molecular ion m/z 343 [M+1] corresponding to the molecular formula C\(_{18}\)H\(_{16}\)O\(_7\).

Isolation and Characterization of Compound EN1 from ethyl acetate fraction of E. neriifolia

The air dried powdered leaves (1000g) were extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanol extract (365.0g). Ethanol extract was dissolved in ethanol and water (1:2 v/v) and partitioned with ethyl acetate and n butanol in 50mL portion for several times till complete extraction takes place. Resulting ethyl acetate fraction was concentrated under reduced pressure (182.0g) and was chromatographed on silica gel column (70cmX15cm, 60-120mesh, 2kg) chromatography and preparative TLC.

Column was initially eluted with hexane, then polarity was gradually increased with ethyl acetate in different concentrations (100:0, 95:5, 90:10, 85:15, 80:20 v/v). 117 fractions each of 50mL were collected and TLC was performed of each fraction individually and eluates were monitored for the presence of various constituents as represented in Table 4.15. Fractions were pooled on the basis of their TLC profile, pooled fractions (26-34) were selected
for the isolation of constituents. Further purification was performed by preparative TLC of isolated constituents to offered EN1.

**Compound 1: Physical and spectral properties of isolated compound EN-1**

**Appearance:** Yellow amorphous powder  
**Solubility:** Chloroform  
**TLC (Rf value):** 0.5  
**IR (KBr, in cm⁻¹):** 3437, 3018, 2925, 1736  
**¹H NMR (400 MHz, DMSO, δ, TMS=0):** δ4.57 (1H, t, H-24), 3.12 (1H, m, H-3), 1.58 (3H, S, H-27), 1.51 (3H, S, H-26), 1.07 (1H, S, H-29), 0.93 (1H, S, H-28), 0.92 (1H, S, H-30).  
**¹³C NMR (150 Hz, DMSO, δ, TMS=0):** δ32.54 (C-1), 30.17 (C-2), 58.93 (C-3), 40.73 (C-4), 47.51 (C-5), 20.77 (C-6), 28.24 (C-7), 48.67 (C-8), 20.77 (C-9), 25.97 (C-10), 25.47 (C-11), 25.97 (C-12), 46.57 (C-13), 48.67 (C-14), 32.54 (C-15), 27.01 (C-16), 55.17 (C-17), 18.31 (C-18), 29.79 (C-19), 36.86 (C-20), 19.20 (C-21), 37.06 (C-22), 24.92 (C-23), 121.93 (C-24), 128.17 (C-25), 19.13 (C-26), 25.16 (C-27), 33.37 (C-28), 14.22 (C-29), 25.16 (C-30).  
**Mass spectral data:** Mass spectrum of isolated compound showed molecular ion m/z 540.9[M+1] corresponding to the molecular formula C₃₀H₅₁O. Therefore it has been revealed that by comparison of their spectroscopic and physicochemical data with those of reported spectral data in the literature, the compounds VN1, VN2 were identified as oleonolic acid and lupeol respectively. The compounds EN1 and BD1 were identified as cycloartenol and eupalitin, respectively. The pharmacological studies revealed that ethanolic extract of *V. negundo* showed anti-asthmatic activity in all the models. The phytochemical studies revealed that ethanolic extract contains betulinic acid, ursolic acid, p-hydroxybenzoic acid, protocatechuic acid, oleanolic acid, flavonoids angusid, casticin, nishindine, gluco-nonitol. Therefore the antiasthmatic activity may be due to the presence of these constituents. The chloroform fraction of *B. diffusa* showed significant anti-asthmatic activity in all the models. The chloroform
fraction contains alkaloids (punarnavine1 and punarnavine2). Therefore presence of these constituents may be responsible for anti-asthmatic potential. Ethyl acetate fraction of *E. neriifolia* showed most significant anti-asthmatic potential in all the models. Ethyl acetate fraction contains 3,12-di-O-acetyl-8-O-tigloylingol, (24R)-cycloartane-3β,24,25-triol, 5,4′-dihydroxy-3,7,3′,5′-tetramethoxyflavone, pachypodol (5,4′-dihydroxy-3,7,3′-trimethoxyflavone), combretol (5-hydroxy-3,7,3′,4′,5′-pentamethoxyflavone). Therefore presence of these constituents may be responsible for anti-asthmatic activity. On the basis of above results it has been concluded that the traditional anti-asthmatic use of *V. negundo*, *B. diffusa* and *E. neriifolia* was justifying.