Isolation and characterization of oleonolic acid and lupeol from *Vitex negundo* leaves

JA Sawale, JR Patel and ML Kori

Abstract

*Vitex negundo* is commonly known as nirgundi belonging to family Verbenaceae and is found throughout India. It has been traditionally reported for the treatment of depression, malaria, venereal diseases, asthma, wounds, skin diseases, anti-inflammatory, analgesic, ulcers and snake bite. The secondary metabolites in a pure form such as flavonoids, iridoids, sesquiterpene, diterpenes, lignans and plant steroids have been isolated and identified previously. In the present research work a triterpenoids i.e. oleonolic acid and lupeol isolated and characterized for the first time from *Vitex negundo* leaves.

Keywords: Triterpene, lupeol, nirgundi, Vitex

Introduction

*Vitex negundo* is herb which is found throughout India, locally known as nirgundi and belongs to family Verbenaceae. In Sanskrit language, the word “nirgundi” is used for plant or any substance that protects the body from the disease(s). The usefulness of the nirgundi has been written in De Materia Medica as well as in the fundamental texts of Ayurveda and Charaka Samhita [1, 2]. It has been reported the use of *Vitex negundo* in traditional medicine for the treatment of depression, venereal diseases, malaria, asthma, allergy, wounds, skin diseases, anti-inflammatory, analgesic, ulcers and snake bite [3]. Various pure compounds such as flavonoids [4], iridoids [5], sesquiterpene [6], diterpenes [7], lignans [8] and plant steroids [9] have been isolated and identified.

Upon phytochemical investigations of about 30 species from the genus Vitex have revealed that the major secondary metabolites of these plants are diterpenoids, ecdysteroids, flavonoids and iridoid glycosides. Other components, such as lignans, phenylpropanoids, sesquiterpenoids and triterpenoids have been also found in some Vitex plants, but with less frequency [10]. But these studies are not enough to identify and characterize the phytochemicals present in the plant. Hence the present study initiated to isolate and characterize the constituents from *Vitex negundo* leaves.

Materials and Methods

**Collection and Authentication**

Leaves of *Vitex negundo* belonging to family verbenaceae were collected in the month of august from local region of Bhopal district, Madhya Pradesh (India) and were authenticated from Dr Vinayak Naik, Senior Research Scientist, Piramal Life Sciences India Ltd. Goregaon (E), Mumbai. A voucher specimen of the plant (No. NPIL/PLS/08-689) has been deposited for future reference.

**Extraction and isolation**

All solvents used for extraction were of technical grade, which were distilled and dried before use. Solvents used for Column Chromatography and preparative TLC were of Analytical Reagent grade. Adsorbent for column chromatography was silica gel G 60-120 (Merck).

**Procedure for Extraction and Isolation**

The air dried powdered leaves (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 (55g). Ethanolic 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 (22g) and was chromatographed on silica gel column (70cmX15cm, 60-120mesh, 2kg) chromatography and preparative TLC [11].
Column was first eluted with chloroform, then polarity of mobile phase was gradually increased by adding methanol in different concentrations (100:0, 95:5, 90:10, 85:15, 80:20, 70:30, 60:40 v/v). Total 232 fractions each of 50 mL were collected and TLC was performed of each fraction individually. Fractions were pooled on the basis of their TLC profile, pooled fractions (36-40) and (55-75) were selected for the isolation of constituents. Further purification was performed by preparative TLC of isolated constituents.

Detection of Phytosterols

Salkowski test

A few crystals of compound A and B were dissolved in chloroform, to this solution few drops of concentrated sulphuric acid was added. Reddish colour produces shows the presence of phytosterols [12].

Libermann-Burchard test

A few crystals of compound A and B were dissolved in chloroform, to this solution few drops of concentrated sulphuric acid followed by few drops of diluted acetic acid, 3mL of acetic anhydride. A bluish green colour formation indicates the presence of phytosterols [13].

Acetylation

The isolated pure compounds were acetylated by refluxing with acetic anhydride and previously distilled pyridine under dry conditions. The reaction mixture was allowed to stay overnight at room temperature and the mixture was concentrated to dryness under reduced pressure [14].

Analytical methods

TLC was performed on silica gel GF 254 precoated (Merck) plates. IR spectra was recorded with FTIR (Shimadzu), 1H and 13C spectra recorded on Bruker (300MHz and 75.4MHz) in CDC13 used TMS as internal standard. ESIMS were measured using a Q-TOF micro mass spectrometer (Waters, USA).

Results and Discussion

Ethanolic extract obtained was 5.5% w/w. ethyl acetate extract obtained after partitioned of ethanolic extract was 22g upon column chromatography of ethyl acetate fraction (36-40) yielded (12mg) and fraction (55-75) yielded (10mg) of pure compounds by preparative TLC. Upon qualitative test performed on pure compounds indicated, it is of triterpenoidal nature.

Structural elucidation of compound A

It was white amorphous powder. m.p: 172-173°C. Mass spectrum showed molecular ion peak m/z at 515(M+1) corresponding to the molecular formula C30H48O3. The 1HNMR spectrum of compound A showed seven tertiary methyl groups at δ 0.67, 0.76, 0.80, 0.83, 0.85, 0.91, 0.93, 0.98, 1.00, 1.00, 1.06, 1.18. A sextet of one proton at δ 3.18 while two broad singlets at δ 5.05 and δ 5.11 due to two exomethylene protons attached at C29. 13C NMR spectrum, revealed the presence of seven tertiary methyl protons at δ 0.83, δ 0.88, δ 0.93, δ 0.94, δ 1.00, δ 1.06, and δ 1.18. A sextet of one proton at δ 2.21 corresponds to 19 β –H is characteristic of lupeol. H-3 proton appeared as multiplet at δ 3.18 while two broad singlets at δ 5.05 and δ 5.11 due to two exomethylene protons attached at C29.

IR Spectrum: Intensely broad band at 3454 cm -1, moderate intense band at 1194 cm -1 observed for the OH bond vibration of hydroxyl group. Carbonyl stretch was observed at 1696 cm -1. The corresponding C=O vibrations was shown at 1453 cm -1 was weakly intense band.

1H NMR (300 MHz, CDCl3, δ, TMS=0): 0.83(3H, S), 0.88(3H, S), 0.93(3H, S), 0.94(3H, S), 1.00(3H, S), 1.06(3H, S), 1.18(3H, S), 2.21(1H, S), 3.18(1H, m), 5.05(2H, S), 5.11(2H, S), 5.12(2H, S).

13C NMR (75.4Hz, CDCl3, δ, TMS=0): 79.05, 77.43, 64.39, 59.07, 55.19, 47.72, 47.24, 46.83, 42.08, 41.72, 40.02, 39.80, 38.78, 37.15, 36.90, 34.74, 33.75, 32.94, 31.93, 29.70, 28.75, 27.28, 26.94, 26.62, 25.99, 23.69, 23.53, 23.37, 23.27 and 22.69.

Characterization of compound B

It was yellowish amorphous powder. m.p: 217-219°C. Mass spectrum of isolated compound showed molecular ion m/z 271.3[M+1] corresponding to the molecular formula C30H48O3.

IR Spectrum: Intensely broad band at 3454 cm -1, moderate intense band at 1194 cm -1 observed for the OH bond vibration of hydroxyl group. Carbonyl stretch was observed at 1696 cm -1. The corresponding C=O vibrations was shown at 1453 cm -1 was weakly intense band.

1H NMR (300 MHz, CDCl3, δ, TMS=0): 0.67(3H, S), 0.76(3H, S), 0.80(3H, S), 0.83(3H, S), 0.85(3H, S), 0.91(3H, S), 1.09(3H, S), 2.04(9H, S), 2.82(2H, d, J=10.8Hz), 3.69(1H, d, J=11.7Hz), 3.87(1H, d, J=11.7Hz), 4.79(1H, m), 5.34(1H, d, J=15.3Hz)

13C NMR (75.4Hz, CDCl3, δ, TMS=0): 143.62, 80.94, 77.43, 76.59, 74.54, 46.52, 45.85, 41.05, 40.52, 39.30, 38.11, 37.70, 36.79, 33.05, 32.29, 31.92, 31.23, 30.67, 30.20, 29.69, 28.04, 27.62, 25.88, 23.57, 22.46, and 22.69.

The Compound A gave a positive Liebermann-Burckhardt test. It showed a molecular ion at m/z 515 corresponding to C30H48O3. The 1HNMR spectrum of compound A showed seven tertiary methyl groups at δ 0.67, 0.76, 0.80, 0.83, 0.85, 0.91 and 1.09 on an oleane skeleton. A doublet of one proton at δ 3.69 and a doublet of one vinyl proton at δ 5.34 were assigned to H-18 and H-12, respectively, suggesting an olea12-ene skeleton. In 13C-NMR spectrum, the signal corresponding to the carboxyl C-28 appeared at δ 143.62. The spectral data were similar to the ones reported for oleanolic acid.
Fig 2: Chemical structure of Lupeol

Conclusion
The physical, chemical and spectral evidence of compound A and compound B confirms that the given constituents are oleonolic acid and lupeol respectively.

References
Research Article

Evaluation of Anti-asthmatic property of *Euphorbia neriifolia*

J A Sawale¹, J R Patel¹ and M L Kori*¹

¹ RKDF University, Airport Bypass Road, Gandhi Nagar, Bhopal (M.P.) 462 033

Received: 18 Oct 2017 Revised: 22 Oct 2017 Accepted: 24 Oct 2017

Abstract:

**Objective:** *Euphorbia neriifolia* belongs to family euphorbiaceae, is being used in traditional medicine for the treatment of severe bronchitis and asthma. So the aim of study was to evaluate anti-asthmatic activity of ethanol extract and its various fractions such as chloroform and ethyl acetate.

**Materials and Methods:** In the present study ethanol extract and its chloroform and ethyl acetate fractions of *Euphorbia neriifolia* leaves were evaluated for acute toxicity studies, preliminary phytochemical screening, and anti-asthmatic activity using histamine induced contraction on isolated guinea pig ileum and isolated guinea pig tracheal chain at doses (10mg/mL), histamine and acetylcholine induced bronchospasm in guinea pig and milk induced eusinophilia in mice, at doses (150–600mg/kg p.o.).

**Results and Conclusion:** Present investigation showed that ethanolic extract has showed no toxicity at dose 2000mg/kg p.o. Phytochemical studies indicated the presence of steroids, phenolic compounds saponin, flavonoids and glycosides. Results showed that ethyl acetate fraction of *E. neriifolia* revealed most significant anti-asthmatic potential in all the models. The present study concludes that the anti-asthmatic activity of ethyl acetate fraction may be due to the presence of flavonoids or saponins.

**Keywords:** Saponins, anti-asthmatic, saponins, glycoside, euphorbiaceae

Introduction

Asthma is a chronic inflammatory disorder of the airways. The chronic inflammation of airway is characterized by the hyper reactivity which causes limited airflow by producing broncho constriction, mucus plug and increased inflammation (Amanda, 2014).

The overall Burden of asthma in India is estimated at more than 15 Million patients. However, India is a vast country having immense geographical, economical and religious diversity. In a survey of more than 2000 individuals, asthma prevalence was found 2.0% in women, 3.65% in men and 11.9% in children. Boys had a significantly higher prevalence of asthma as compared with girls (12.8% and 10.7%, respectively). Allergies in Childhood (ISAAC) have provided data on asthma prevalence in 6-7 and 13-14 year old Indian children. The above findings represent that the burden of bronchial asthma in Indian children is higher (Bosquet and khaltaev, 2007).
There is increasing demand for the use of traditional medicines in the management of asthma, and *Euphorbia neriifolia*, Euphorbiaceae is such plant with acclaimed potency in asthma (Akaha, 2003). In the same way, there are increasing demands for the use of traditional medicines in the therapy of asthma, because of few side effect compared to those of synthetic drugs to prevent and treat such chronic disease (Shang, 2010).

*E. neriifolia* is large fleshy, glabrous, branched shrub or a small tree, 1.8-4.5 m high Green and with sharp stipular spines. Milk juice exuded from freshly injured stems is used by Vaidyas as drastic cathartic and to relieve earache. Dr M C Koman tried it and found it is very beneficial in asthma (Longman, 2005; Nadkarni, 1998).

In Ayurvedic treatment the plant has been used for its beneficial effects such as bronchitis, tumours, laxative, carminative, improves appetite, useful in abdominal troubles, loss of consciousness, leucoderma, piles, inflammations, anaemia, ulcers (Kirtikar and Basu, 2008). The chemical constituents reported are Euphorbon, resin, gum, caoutchouc, malate calcium, the triterpenoids, euphol, 24-methylene cycloartenol, euphorbol, haxacosonate, glut-5 (10)-en-1-one, glut-5-en-3 beta-yet-acetate, taraxerol, friedelan-3-alpha-ol and 3 beta-ol (Khare, 2007).

In consideration with traditional claim of *E. neriifolia* under study, we investigated the leaves of the plant for its anti-asthmatic potential.

**Materials and Methods**

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

*E. neriifolia* leaves were collected from local region of Bhopal district Madhya Pradesh (India). Herbarium specimen of the plant from which juice was collected 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 No. NPIL/PLS/10-1038 of *E. neriifolia* has been deposited for future reference. The collected leaves were shade dried and kept in air tight container for further use.

**Extraction and fractionation**

The air dried powdered leaves of *Euphorbia neriifolia* (1000g) were extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanolic extract (364.8g). 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 (7.38g) and ethyl acetate fraction (9.42g) upon concentration under reduced pressure (Mukherjee, 2010; Harborne, 1998).

**Phytochemical screening**

The ethanolic extract of *E. neriifolia* and its different fractions were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, carbohydrates, tannins, flavonoids, saponins, sugars and proteins using conventional method.

**Determination of acute toxicity**

The acute toxicity study of ethanolic extract of *Euphorbia neriifolia* was carried out on female albino rats. The rats were administered orally dose of 300, 600, 1000, 1500 and 2000mg/Kg body weight of ethanolic extract and the control group received standard laboratory diet and water ad libitum following OECD guidelines.

**Studies on smooth muscles**

**Guinea pig tracheal chain**

Guinea pigs of either sex (250–500 g), starved overnight but allowed free access to water, were used. The animals were sacrificed by a blow on the head and exsanguinated. The trachea was
dissected out and cut along its length on the dorsal surface. Incomplete transverse cuts were made along the segments of the cartilage to produce a zig-zag strip. The tracheal preparation was mounted at a resting tension of 1.0g in Krebs-Henseleit solution with small cell pins. The buffer was maintained at 37°C and continuously saturated with bubbled air. The tissue was left to equilibrate for 60 minutes, during which the bathing solution was changed at every 10 minutes. At the end of equilibrium period, histamine induced contractions as well as the effect of the ethanolic extracts and its various fractions (10mg/mL) of Euphorbia neriifolia on the contractions produced by histamine were recorded at the dose of 100, 200, 400, 800 and 1000µg. The tissues were bathed in the test substances for 5 minutes before the addition of histamine. A drug tissue contact time of 90 seconds was observed (Akaha, 2003).

**Guinea pig ileum preparation**

Overnight fasted guinea pigs of either sex weighing between 350-500g were sacrificed using cervical dislocation method. Ileum was quickly dissected out and mounted in a student organ bath (Dolphin) and maintained at 37±0.5°C containing 20mL of Tyrode’s solution under basal tension of 500mg. The solution was continuously bubbled with air. The tissue was allowed to equilibrate for 30 minutes, during which the bathing solution was changed at every 10 minutes. The contractile responses of ileum against histamine were recorded in presence and absence of ethanolic extracts of Euphorbia neriifolia and their chloroform and ethyl acetate fractions. Contact time for 30 seconds and 5 minutes time cycle was followed for recording dose response curve (DRC) of histamine at the dose of 100, 200,400, 800 and1600µg. after recording DRC of histamine; ethanolic extracts of Euphorbia neriifolia and their chloroform and ethyl acetate fractions (10mg/mL) were added to reservoir and same doses of histamine were repeated. Graph of maximum percentage of contractile response on ordinate and log concentration of histamine was plotted to record DRC of histamine.

**Histamine and acetylcholine induced broncho spasm in guinea pigs**

Guinea pigs of either sex weighing between 350-500g were selected and randomly divided into seventeen groups each containing six animals. The ethanolic extracts and its different fractions were administered orally in 2% Tween 80. Animals were exposed to an aerosol of 1.0% histamine under constant pressure (1 kg/cm²) prior and after ethanolic extracts, different fractions and standard drug (Ketotifen) treatment. The preconvulsion time (PCT) was noted for the time of exposure to the onset of dyspnea in minutes in each animal as described by Sheth et al., 1972; Gokhale and saraf, 1996. As soon as the convulsions started, the animals were removed from the aerosol chamber and placed in fresh air. Animals of Group I were treated with 2% v/v Tween 80 (Control) and animals of Group II were treated with Ketotifen fumarate (1mg/kg, standard). Animals of Group III to XI were treated with ethanolic extracts of E. neriifolia and its chloroform and ethyl acetate fractions at doses of 150, 300 and 600mg/kg, b.w, p.o., daily for 7 days. On day 7, 2 h after the administration of last dose of test extracts, the onset of convulsions recorded as for day 0. After next 15 days intervals of washout period, the same animals were given the above treatment and preconvulsion time (PCT) was noted for 0.5% acetylcholine bromide aerosol spray. The percentage protections offered by ethanolic extracts and their different fractions of E. neriifolia and standard drug treated animals were calculated by the following formula (Adusumalli, 2013).

Percent protection$=\{1-T_1/T_2\} \times 100$

Where $T_1$= Time for onset of symptoms before treatment

$T_2$= Time for onset of symptoms after treatment
Milk induced eosinophil measurement in mice

Adult albino mice (20-25g) were divided into seventeen groups, six animals in each. Animals of Group I received distilled water (10mL/kg, p.o.) and Group II only injection of boiled and cooled milk in doses of 4mL/kg, s.c. Animals of Group III to XI were received injections of boiled and cooled milk in doses of 4mL/kg, s.c. and ethanolic extract of *E. neriifolia* and their chloroform and ethyl acetate fractions at the doses of 150, 300 and 600mg/kg, p.o. respectively, 1 hour before milk injection. Blood was sucked in WBC pipette up to mark and further diluted with eosin solution. The eosin solution facilitated destruction of all corpuscles except eosinophil. Pipette was shaken for few seconds and kept aside for 5 minutes. Neuber’s chamber was charged with above fluid and eosinophil count was done (Kumar et al., 2010). Difference in eosinophil count before and after drug administration was calculated.

**Statistical analysis**

The results were analysed using One way Anova and expressed as mean ±standard deviation. Differences between means of treated groups and control were regarded as significant at P<0.05.

**Results and discussion**

Toxicological studies of ethanolic extract of *E. neriifolia* was found safe at 2000mg/kg body weight. The extractive yield of ethanolic extract, and its chloroform and ethyl acetate fractions were found to be 36.48 %w/w, 0.73%w/w and 0.94%w/w respectively. The ethanolic extract of *E. neriifolia* and its different fractions were subjected to preliminary phytochemical screening for the detection of various phytoconstituents and is represented in table 1.

**Effects on smooth muscles**

The results of the isolated tissue experiments revealed that the extracts neither contracted nor relaxed any of the isolated tissue preparation. However the extracts inhibited the contractions induced by the spasmogens to varying degrees (Fig 1 to Fig 3). The ethyl acetate fraction was found most potent in inhibiting the contraction produced by histamine in isolated guinea pig ileum (Fig.1) and trachea chain preparation (Fig. 2). The chloroform fraction was found least potent in above models.

On the histamine induced bronchospasm in guinea pigs ethyl acetate fraction shows dose dependent maximum % protection against bronchospasm followed by ethanolic extract and chloroform fraction was found least active (Fig. 3).

### Table 1. Data showing the preliminary phytochemical screening of the various extracts of *E. neriifolia*

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Constituents</th>
<th>Ethanolic extract</th>
<th>Chloroform fraction</th>
<th>Ethyl acetate fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Glycosides</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Fixed oils and fats</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Phenolic compounds</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Protein and amino acids</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Effect of ethanolic extract and its various fractions of *Euphorbia neriifolia* on isolated guinea pig ileum and isolated guinea tracheal chain preparation

Ethyl acetate fraction of *E. neriifolia* significantly inhibited contractions produced by histamine on isolated guinea pig ileum and isolated guinea tracheal chain preparation represented in Fig 1.1 and Fig. 1.2, respectively. Ethanolic extract and its Chloroform fraction did not show any significant activity.

*p*<0.03 when compared with vehicle

**Figure 1.1** Effect of ethanolic extract and different fractions of *E. neriifolia* on histamine induced contractions on isolated guinea pig ileum preparation
*p<0.0003 when compared with vehicle

**Figure 1.2** Effect of ethanolic extract and different fractions of *E. neriifolia* on histamine induced contractions on isolated guinea pig tracheal chain preparation

**Effect of ethanolic extract and different fractions of E. neriifolia on histamine and acetylcholine induced contractions on isolated guinea pig tracheal chain preparation**

Histamine is one of the major inflammatory mediators in the immediate phase of asthma causing airway hyper responsiveness and bronchial airway inflammation. The asthmatic study of ethanolic extracts of *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 ethyl acetate fraction of *E. neriifolia* significantly prolonged the latent period of convulsion as compared to control (Ketotifen fumarate) following exposure to histamine aerosol. The percentage protection offered by ethyl acetate fraction of *E. neriifolia* 89.36% and 90.07% at dose level of 150mg/kg. and 600mg/kg body weight respectively showed the maximum spasmolytic activity as represented in figure 1.3 and 1.4 respectively.
*p<0.0003 when compared with vehicle

Fig 1.3. Effect of ethanolic extract and different fractions of *E. neriifolia* on histamine induced bronchospasm in guinea pigs
Fig 1.4. Effect of ethanolic extract and different fractions of *E. neriifolia* on acetylcholine induced bronchospasm in guinea pigs

Milk induced eosinophil measurement in mice

Effect of ethanolic extract and its various fractions of *Euphorbia neriifolia* on milk induced eosinophil measurement in mice

The percentage protection of eosinophil was measured in milk induced mice with the treatment of ethanolic extracts of *E. neriifolia* and their chloroform and ethyl acetate fractions at the doses of 150, 300 and 600mg/kg p.o. The percentage protection of eosinophil measured in milk induced mice was found to be 79.08% when treated with ethyl acetate fraction of *E. neriifolia*as represented in figure 1.5.

*p*<0.0003 when compared with vehicle
*p<0.0003 when compared with vehicle

**Fig 1.5. Effect of ethanolic extract and different fractions of *E. neriifolia* on eosinophil in mice**

**Conclusion**

In India *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. Results obtained from all the parameters tested revealed that *E. neriifolia* has significant anti-asthmatic activity.

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, combretol. Therefore presence of these constituents may be responsible for anti-asthmatic activity.
References