



Preliminary report

Assessment of inhibitory potential of *Pothos scandens* L. on ovalbumin-induced airway hyperresponsiveness in balb/c miceSaurabh Gupta^{a,*}, Duraiswamy Basavan^a, Satish Kumar Muthureddy Nataraj^b, K. Rama Satyanarayana Raju^b, U.V. Babu^c, Sharath Kumar L.M.^c, Renu Gupta^d^a Department of Pharmacognosy, J.S.S. College of Pharmacy (Off Campus JSS University), Ootacamund 643 001, TN, India^b Department of Pharmacology, J.S.S. College of Pharmacy (Off Campus JSS University), Ootacamund 643 001, TN, India^c The Himalaya Drug Company, Makali, Bangalore-562 123, Karnataka, India^d Dr. Batra's Clinic, Nirala Bazaar, Aurangabad 431 001, Maharashtra, India

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ABSTRACT

Pothos scandens L. was used in Indian traditional medicine as an antiasthmatic drug. The ethanolic and aqueous extracts were prepared with aerial parts of *P. scandens* (PSE & PSA). ESI MS/MS of PSE ethanolic extract was carried out for the determination of chemical constituents. CP1 is isolated from the PSE, structurally confirmed with NMR and LCMS/MS. PSE, PSA and CP1 are evaluated against ovalbumin (OVA) induced airway hyperresponsiveness (AHR) in balb/c mice. The test drugs are administered *p.o.* prior to challenge with aerosolized 2.5% w/v OVA. Total and differential leucocyte count, nitrite (NO₂), nitrate (NO₃), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and interleukin-13 (IL-13) are estimated in bronchoalveolar lavage fluid (BALF). Similarly, myeloperoxidase (MPO), malonaldehyde (MDA) and total lung protein (TLP) are estimated in the lungs. The results reveal a significant increase in total and differential leucocyte count, NO₂, NO₃, TNF- α , IL-6, and IL-13 in OVA induced AHR. However, these parameters are significantly decreased in PSE and PSA tested doses (PSE 100 & 200 mg/kg). While, treatment with CP1 is less effective at 5 & 10 mg/kg doses. Similar observations obtain for MPO and MDA in lungs. However, the mean value indicated that the PSE at 200 mg/kg showed a significant restoration in all the parameters. Pro-inflammatory mediators are known to be responsible for AHR. Histopathology revealed justifies the effectiveness. The present investigations suggest PSE are interesting molecules for further research for asthma, with an approach through pro-inflammatory inhibitory pathway. *P. scandens* is a potential herbal medicine for allergy induced asthma.

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1. Introduction

"Air is life" is an old proverb but how many of us really think about it during our day-to-day life? The line focuses towards the direction of respiratory disorder. Asthma is now one of the world's most common long-term disease conditions. According to the global burden of asthma report, over 50 million people in Central and Southern Asia are asthmatic. The disease is estimated to affect as many as 300 million people worldwide, a number that could increase by a further 100 million by 2025 [1]. WHO also estimates that 235 million people currently suffer from asthma worldwide. This is the most common chronic disease condition among pediatric [2]. In India an estimated 57,000 deaths were reported for asthma in 2004 and it was considered as one of the leading causes of morbidity and mortality in rural India [3,4].

Asthma is characterized by airway inflammation, excess reversible constriction of airway smooth muscles, and AHR to a wide variety of

spasmogens [5]. Pathophysiological features of asthma show infiltration of inflammatory cells like eosinophils, [6] neutrophils, [7] lymphocytes, and monocytes [8]. In asthma, mast cells play a central role in inflammatory and immediate allergic reactions [9]. Many mediators derived from these inflammatory cells have been implicated in asthma pathophysiology viz. histamine, [10] cytokines, [11] leukotrienes, prostaglandins, thromboxanes, [12] free radicals like reactive oxidative species (ROS) [13] and reactive nitrogen species (RNS) [14] etc.

Several studies have demonstrated elevated levels of histamine in the plasma of asthma patients [15]; similar effects have been noted in the plasma [16] and lung tissues [17] of murine animals. Many cytokines have been detected in increased quantity in bronchial biopsies from asthmatic subjects [18]. The classical role play by eosinophils and mast cells activates the T cells through increased production of Th2 cytokines such as tumor necrosis factor TNF- α , interleukin-4 (IL-4), IL-5, IL-6 and IL-13 [19,20]. Airway allergen exposure produces a Th2-dominated response by recruiting and activating inflammatory cells including eosinophils, and increases the levels of IL-4, IL-5, IL-6 and IL-13 in asthmatic patient [21]. Furthermore, recent studies have shown the presence of these mediators in bronchoalveolar lavage fluid (BALF)

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and urine of asthmatic patients after allergen challenge [22]. Medicinal plants are alternatives to conventional therapies in many diseases.

Pothos scandens L. (genus—*Pothos*) belonging to the family Araceae is a climbing shrub. The leaves are used to treat skin disorders. An ethnobotanical survey carried out among the ethnic groups (Kanikaran) in Southern Western Ghats of India revealed the use of *P. scandens* leaves mixed with the fruits of *Capsicum annum* and rhizome of *Allium sativum* [23]. The mixture is ground into a paste with coconut oil and applied topically on affected places to heal wounds [24,25]. Sri Lankan tribal people use the leaf of *P. scandens* to reduce swelling speedily in trauma area [26]. In China the plants are used as blood coagulant for wounds, tumors and drinking for anti-cough [27]. In India, the infusion of the leaves of this plant as a bath is used for curing convulsions and epilepsy. Apart from that, the stem is also reportedly used to treat asthma, after being cut with camphor and smoked like tobacco [28]. The previous literature reported that *P. scandens* methanolic extract possesses antipyretic activity [29]. In continuation another author reported that *P. scandens* alcoholic extract formulated gel showed significant improvement in burn wound contraction [23]. The previous literature reported that the phytochemical investigation of *P. scandens* leaf extracts showed the presence of chemical compounds such as alkaloid, catechin, coumarin, tannin, saponin, flavonoid, phenol, sugar, glycoside and xanthoprotein [30]. The GC–MS analysis of ethanolic extract of *P. scandens* leaves detected nineteen compounds. The major compounds are dodecanoic acid, tetradecanoic acid, 3,7,11,15-tetramethyl-2-hexadecan-1-ol, n-hexadecanoic acid, phytol, 9,12-octadecadienoic acid (Z,Z), 9,12,15-octadecatrienoic acid (Z,Z,Z), 1,2-benzenedicarboxylic acid, and diisooctylester. 9,12-Octadecadienoic acid (Z,Z) – and 9, 12,15-octadecatrienoic acid (Z,Z,Z) – have the anti-inflammatory and anti-arthritis property. Among the identified phytochemicals, dodecanoic acid, tetradecanoic acid and n-hexadecanoic acid have the antioxidant property [31].

In our previous investigation in vitro mast cell stabilization potential of *P. scandens* extracts by C40/80 on rat mesentery. The finding provides evidence that the *P. scandens* inhibits mast cell derived immediate type-I allergic reactions and mast cell degranulation. As the mast cell plays a major role in Type I hypersensitivity mediated diseases like allergic asthma and rhinitis [32]. We hypothesized that *P. scandens* extract and isolated compound would have an antiasthmatic effect on AHR in murine model of allergic asthma.

2. Material and methods

2.1. Collection

P. scandens L. aerial parts were collected in the month of August, 2010, from Tirupati district, Andhra Pradesh, India. Dr. K. Madhava Chetty, Botanist, Department of Botany, Sri Venkateswara University, Tirupati authenticated the collected plant. Voucher specimen has been preserved in our laboratory (SVU/SC/09/25/10-11) for future reference.

2.2. Chemicals

Ova albumin, bovine serum albumin (BSA), O-dianisidine, flavin adenine dinucleotide (FAD), may-grunwald, hexa decyl trimethyl ammonium bromide (HTAB), thio barbituric acid, N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulphonic acid] (HEPES), griess reagent, 1,1,3,3-tetraethoxypropane, reduced nicotinamide adenine dinucleotide phosphate (NADPH), and nitrate reductase were purchased from sigma Pvt. Ltd. etc. Thiopentone sodium was procured from Abbott Laboratories, India. ELISA Kits IL-6 & TNF- α were procured from Koma Biotech, South Korea where as IL-13 from Ray Biotech, Inc., USA. All chemicals used were of analytical grade. Dexamethasone was obtained as a gift sample from Ranbaxy Pvt. Ltd.

2.3. Preparation of extraction

The dried *P. scandens* (1 kg) powdered was ground using a milling machine and extracted with cold maceration process using absolute ethanol (99.5%) by intermittent shaking for 10 days, filtered and the dried marcleft was macerated with aqueous for another 10 days with intermittent shaking. The solvent was dried by rotary flash evaporator (Rota vapor, R-210/215, Buchi, Switzerland) under reduced pressure at a temperature of maximally 55 °C. The concentrated semi solid material was kept in a desiccator for drying to give dark green ethanolic 2.98% and dark brown aqueous extract 5.39% w/w on the dry weight henceforth called PSE and PSA, respectively. The extract was subjected into qualitative phytochemical tests [33].

2.3.1. Qualitative estimation of crude extract by ESI-MS/MS spectrometric analysis

ESI-MS fingerprints of the extract by API 2000 (Applied biosystem/ MDS SCIEX, Canada) mass spectrometer coupled with ESI (Electro spray ionization) source and a chromatographic system. Batch acquisition and data processing were controlled by Analyst 1.5 version software. The optimized parameters like declustering potential (DP), ion source gas (O_2) (GS1) and (GS2), curtain gas (N_2) (CUR), focusing potential (FP) and source temperature (TEM) were optimized with respect to ionization intensity response. Acquisition was performed by setting the mass of the analysts with appropriate scan range (Table 1). The extracts were diluted in a solution containing 100% (v/v) HPLC grade methanol (Merck, India). The extracts were analyzed by direct infusion directly into the source by means of a syringe pump (Harvard Apparatus) at a flow rate 20 μ l/min continuously in mass spectrometer. Intensity of ionization response of extracts was analyzed by direct insertion into positive and negative ionization in both modes of ESI-MS/MS fingerprinting. This method provides a sensitive and selective method for the identification of polar organic compounds with acidic sites, such as the phenolic compounds found in *P. scandens*. Compounds of interest were then mass selected and their ESI-MS/MS was compared to those found in references, for the identification of these compounds.

2.3.2. Isolation of compound by column chromatography

The 20 g of PSE was charged over silica gel (60–120 mesh) column (100 cm $\times$ 5 cm) eluted gradually with solvents and the solvent mixtures of increasing polarities. Fractions were collected in 50 ml portions and monitored on TLC and the fractions showing similar spots were combined. Fractions 17 of PSE eluted in methanol mobile phase, showed one major spot when subjected for TLC using the mobile phase toluene: methanol: formic acid (3.4: 1.4: 0.2) solvent system and obtained the R_f value of 0.435 with minor impurities (yield: 200 mg). Re-crystallization of fraction 17 in acetone:methanol (1:9) yielded a light green crystalline powder designated as compound 1 (CP1) (yield: 165.5 mg). The structure of CP1 was elucidated by extensive spectroscopic methods including IR, ^1H and ^{13}C NMR experiments as well as ESI-MS/MS.

Table 1

Optimization values and condition of the ESI-MS/MS instrument.

Parameter	Optimization values
Ion Source	ESI (Turbo spray)
Declustering potential (DP)	40 V
Focusing potential (FP)	400 V
Entrance potential (EP)	10 V
Curtain gas (CUR)	20 psi
Ion spray voltage (IS)	5500 V
Temperature (TEM)	0 °C
Source gas (GS1)	30 psi
Source gas (GS1)	40 psi

3. Animals

Healthy female Swiss albino mice and male balb/c mice (20–28 g) were obtained from the animal house, J.S.S. College of Pharmacy, Ootacamund, India, and were maintained under standard environmental conditions (22–28 °C, 60–70% relative humidity, 12-h dark : 12-h light cycle). Animals had free access of standard laboratory feed (M/S Hindustan Lever Ltd., Bangalore, India) and water ad libitum. The experimental protocol was approved by the institutional animal ethics committee (IAEC) constituted in accordance with the rules and guidelines of the committee for the purpose of control and supervision on experimental animals (CPCSEA), India (Approval no. JSSCP/IAEC/Ph.D/P.Cog/02/2011-12).

3.1. Acute toxicity studies

Acute oral toxicity study in female Swiss albino of weight (20–28 g) was carried out as per OECD-423 guidelines. The dose level was selected from one of the four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The mice were observed for mortality, clinical signs and body weight changes daily for a period of 30 days and at the end of the study period, all the animals were subjected to gross necropsy.

3.2. Sensitization and airway challenge

Male balb/c mice (20–28 g) were injected i.p. with a mixture containing ovalbumin (OVA) (50 µg) and alum (1 mg) in 0.2 ml of normal saline except for the saline control group on days 0 and 7. At days 14 and 21, the mice were challenged with 2.5% (w/v) OVA aerosol through a nebulizer (Omron, UK, Model No. CX3) delivering particles of 0.5–5 µ size at a pressure range of 30–36 psi for 20 min. The saline control mice were exposed to saline aerosol for 20 min on days 14 and 21 [34]. Animal grouping consists of eight male balb/c mice. 1 h before each OVA sensitization and challenge on day 14 and 21st day after the initial sensitization. PSE and PSA (50, 100 and 200 mg/kg) and isolated CP1 (5 and 10 mg/kg) were administered orally once daily on days 14–21. Negative and positive control mice were treated orally with phosphate buffered saline (PBS) and dexamethasone (DEXA; 30 mg/kg) [35], respectively, once daily on days 14–21. Animals were sacrificed 48 h after the last challenge at day 22 to characterize the suppressive effects of tested drug. A schematic diagram of the treatment schedule is shown in (Fig. 1).

3.3. Collection of bronchoalveolar lavage fluid

The mice were sacrificed by administering thiopentone (80 mg/kg, i.p.) after 24 h of the last challenge. A tracheal cannula was inserted via mid cervical incision and lavaged twice with the 1 ml of ice-cold PBS, pH = 7.4. After collecting the bronchoalveolar lavage fluid (BALF), the lungs of the mice were removed and a part of it was stored at –20 °C for the estimation of MDA, MPO and total protein [36].

3.3.1. Total and differential cell count

The BALF was centrifuged at 170 g for 10 min at 4 °C and the supernatant was removed and stored at –80 °C for the estimation of nitrite and nitrates. The pellets obtained after the centrifugation were resuspended in 0.5 ml of the PBS and total leukocyte count was performed using neubauer chamber and WBC diluting fluid. A smear was prepared using the BALF. The air dried smeared slide was stained for 10 min with giemsa and washed with distilled water for 8 min. Counter staining was latter carried out with may-grunwald stain for 10 min. The differential cell count was carried out using a digital light microscope (Motic, Japan, Cat. No. B1 Series) at 100× magnification by oil immersion technique. At least 100 cells were differentiated on each slide [36].

3.3.2. Estimation of nitric oxide (NO) production

BALF supernatant (100 µl) was incubated for 30 min at 37 °C with N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulphonic acid] (HEPES) acid free buffer (50 mM; pH = 7.4), flavin adenine dinucleotide (FAD) disodium salt, (5 µM), nicotinamide adenine dinucleotide phosphate (NADPH) (0.1 mM), distilled water 290 µl and nitrate reductase (0.2 U/ml). Any unreacted NADPH in the solution was oxidized by incubating the solution with potassium ferricyanide (1 mM) at 25 °C for 10 min for the conversion of nitrate to nitrite. Later the sample solution was incubated with 1 ml of the griess reagent (NED: 0.2% (w/v), sulfanilamide: 2% (w/v), solubilized in double distilled water: 95% and phosphoric acid: 5% (v/v)), for 10 min and the absorbance was measured at 543 nm using a UV spectrophotometer (PerkinElmer, Model No. Lambda 25). For the estimation of nitrite in identical set of tubes, nitrate reductase was omitted. A standard curve plotted with absorbance (543 nm) vs. concentration (µM) standards and was used to determine the concentrations of nitric oxide metabolites in the BALF samples [37].

3.3.3. Enzyme-linked immunosorbent assay (ELISA)

Estimation of TNF-α (Cat#: K0331186), IL-6 (Cat#: K0331230) (Koma Biotech, South Korea) and IL-13 (Cat#: ELM-IL13-001) (Ray Biotech, Inc., USA) in the BALF was performed using ELISA kits according to the manufacturer's instructions.

3.3.4. Estimation of lung tissue malonyldialdehyde (MDA) production

The lung tissue (100 mg) was homogenated with 1 ml normal saline using Teflon coated glass high speed homogenizer (Remi, India; Cat. No. 4148). 1 ml of the tissue homogenate was mixed with the 2 ml mixture of thiobarbituric acid 0.375% (w/v), trichloroacetic acid 5% (v/v) and HCl (0.25 N). The mixture in the test tube was incubated in the boiling water for 15 min. Later it was cooled and centrifuged at 1500 rpm for 10 min. The pink colored solution was measured at 535 nm using a UV spectrophotometer. 1,1,3,3 tetraethoxypropane (in amounts of 2, 4, 6 and 8 nmol) served as an external standard and the standard plot was used for the estimation of MDA. MDA levels in the lungs were expressed as nM/mg of tissue protein [38].

3.3.5. Estimation of lung tissue myeloperoxidase (MPO) concentration

The frozen isolated lung was weighed and the tissue was minced and homogenized with 1 ml of the 50 mM phosphate buffer (pH = 6) using Teflon coated glass high speed homogenizer (Remi, India ; Cat. No. 4148). The homogenate was centrifuged at 40,000 g at 4 °C for 15 min. The pellets were resuspended in 1 ml 50 mM potassium phosphate buffer (pH = 6) containing 0.5% (w/v) hexadecyltrimethylammonium bromide (HTAB) to neutralize the pseudoperoxidase activity of hemoglobin and to solubilize membrane bound MPO. The suspension was freeze and thawed 3 times and sonicated (Bandelin Sonorex, Germany; Model No. RK100H) on ice for 10 s. 0.1 ml of the sample was added with 2.9 ml of potassium phosphate buffer (50 mM) containing O-dianisidine (0.19 mg/ml). The solution was transferred to the cuvette and hydrogen peroxide (0.0005% (v/v)) was added. Immediately the absorbance was measured at 460 nm and after 3 min using UV spectrophotometer. The MPO activity per gram wet lung was calculated as follows: MPO activity (µg/lung) = $\Delta A \times 4.05 / \text{lung weight (g)}$, where (ΔA = rate of change in absorbance at 460 nm between 1 and 3 min). The MPO units were defined by the quantity of enzyme catalyzing 1 µM of the substrate with 1 µM of hydrogen peroxide per min at 25 °C and were expressed in mU/mg protein [39,40].

3.3.6. Estimation of total lung protein concentration

A part of the lung homogenate from the MDA estimation procedure was mixed with the 5 ml of the Lowry reagent (A mixture 100 ml sodium carbonate (2% w/v), 1 ml of sodium potassium tartrate (2% w/v), 1 ml of 1% (w/v) of cupric sulfate) and 0.5 ml of the Folin-Ciocalteu's reagent. The mixture was mixed thoroughly and incubated for 30 min at 25 °C.

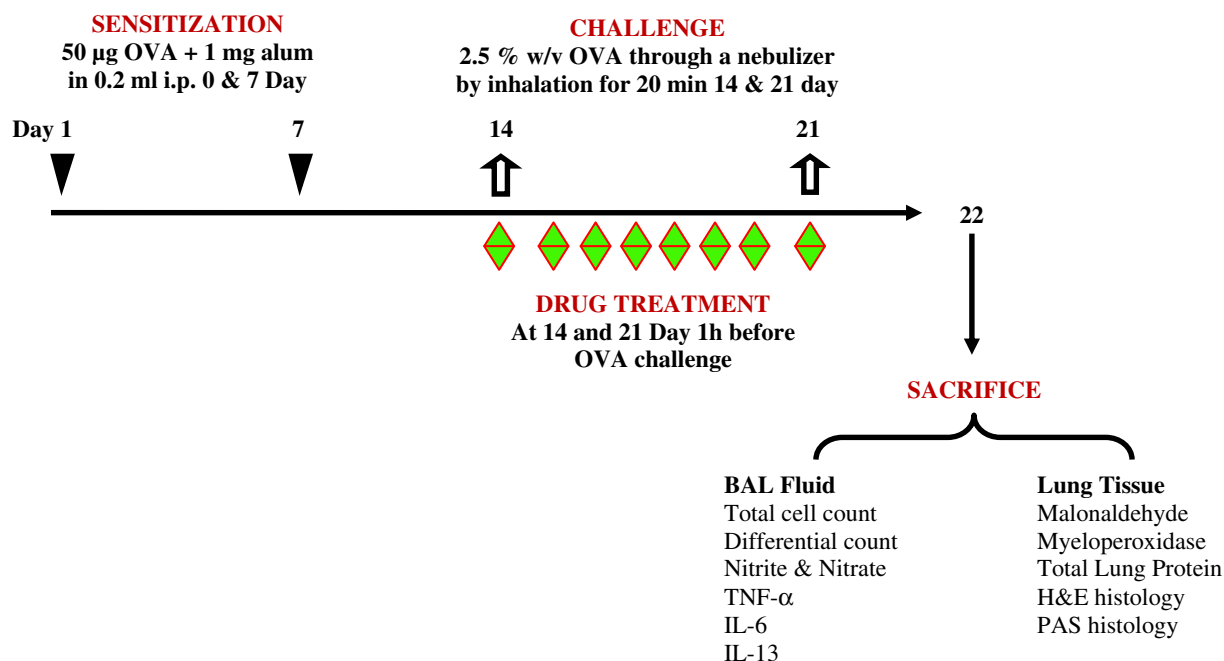


Fig. 1. Scheme of OVA sensitization and challenge protocol (INJ—injection; IN—inhalation; OVA—ovalbumin).

The absorbance of the blue colored solution was measured at 750 nm using a UV spectrophotometer. A standard plot was obtained using bovine serum albumin at concentrations of 10–100 µg/ml [41].

3.3.7. Histopathology of lung tissue

The lung tissue was stored in neutral buffer 10% (v/v) formalin. The paraffin embedded blocks were cut into 50 µm section using a microtome (Lecia, UK; Model No. RM2135) mounted and stained with hematoxylin and eosin (H&E) for routine histology and periodic acid–Schiff stain (PAS) for goblet cell study [42].

3.4. Statistical analysis

Statistical analysis was carried out by using One-Way Analysis of Variance (ANOVA) followed by Tukey's multiple comparison tests. P value < 0.05 was considered statistically significant. The analysis was carried out using GraphPad Prism software V.5.04.

4. Result

4.1. Qualitative phytochemical screening

The phytochemical screening on ethanolic and aqueous extract of *P. scandens* revealed the presence of primary metabolites such as, carbohydrates, fixed oil and proteins the secondary metabolites such as alkaloids, glycosides, flavonoids and phenolic compounds.

4.2. Qualitative studies of *P. scandens* ethanolic extract by ESI MS/MS

The intense response were observed in negative ion $[M-H]^-$, fingerprinting of ESI-MS/MS for PSE. The event was a full scan ESI-MS/MS FIA (flow injection analysis) studies to acquire data on ions in the range 140–500 m/z (Fig. 2). The eleven identified compounds were precursor ion at represented in (Table 2).

4.2.1. Characterization of phytoconstituents

Compound 1: Light green crystalline powder; m.p.: 270–280 °C. IR (KBr), $\max$ spectra show absorption bands at C–O–C (1056 cm^{-1}),

–CH=CH– (1616 cm^{-1}), RCR=O (1650 cm^{-1}), CH₃ (2853 cm^{-1}), 2923 cm^{-1} (–CH₂–) and –OH (3419 cm^{-1}). ¹H NMR (500.0 MHz, DMSO-*d*₆) δ 8.383 (*m*, 2H, CH=CH), 3.18–3.16 (*m*, 5H, CH₂, OH–CH and OH), 2.51–2.50 (*t*, 6H, O=C–CH₂), 2.17 (*m*, 6H, C=C–CH₂ and CH–OH–CH₂), 1.75–1.20 (*m*, 6H, –CH₂), 0.83 (*t*, 6H, –CH₃). ¹³C NMR (125.75 MHz, DMSO-*d*₆) δ 163.58 (HC=O, C-10), 158.52 (>C=O, C-5), 74.04 (>CH–OH, C-3), 130.18–78.16 (C=C, C-8, C-9), 24.78 (–CH₂, C-2, C-7, C-11–15), 9.90 (CH₃, C-1, C-16). ESI-MS/MS of compound 1 (negative mode) acquire data range of 100–300 m/z . The product mass ions, m/z of 284.46 [M – H]. The precursor ion at m/z 126.75 [M – H]; base peak ion at m/z 156.22 [M – H + K]. The IR, ¹H NMR, ¹³C NMR and ESI-MS/MS signals indicated that compound 1 proved its proposed structure are unsaturated long chain fatty acid or ester with molecular formula C₁₆H₃₀O₄ and compound name is 5-oxoundecyl-3-hydroxypentanoate (Fig. 3).

4.3. Acute toxicity study of *P. scandens* extracts

Acute toxicity study was carried out according to OECD guideline 423, none of the mice show observable signs of toxicity upon single administration of PSE and PSA (2000 mg/kg) on day one. Observations twice daily for 14 days also did not reveal any drug related observable changes. Based on the LD₅₀ value the test compounds were classified as GHS category V (LD₅₀ > 2000 mg/kg) and 1/10 (200 mg/kg), 1/20 (100 mg/kg), and 1/40th (50 mg/kg) of LD₅₀ dose was selected for pharmacological studies.

4.4. Effect of *P. scandens* extracts on total and differential cell count in the BALF

Mice were immunized with OVA and submitted to two OVA aerosol challenges show statistically significant ($p < 0.001$) increase in total cells, monocytes and neutrophils, eosinophils in the BALF collected at 24th h when compared to vehicle control mice. When compared to the OVA control, PSE and PSA showed statistically significant ($p < 0.001$) reduction in total cell count. However, the numbers of circulating eosinophils ($p < 0.001$) and neutrophils ($p < 0.001$) were significantly decreased by PSE 200 mg/kg treated animals (Table 3). While, the

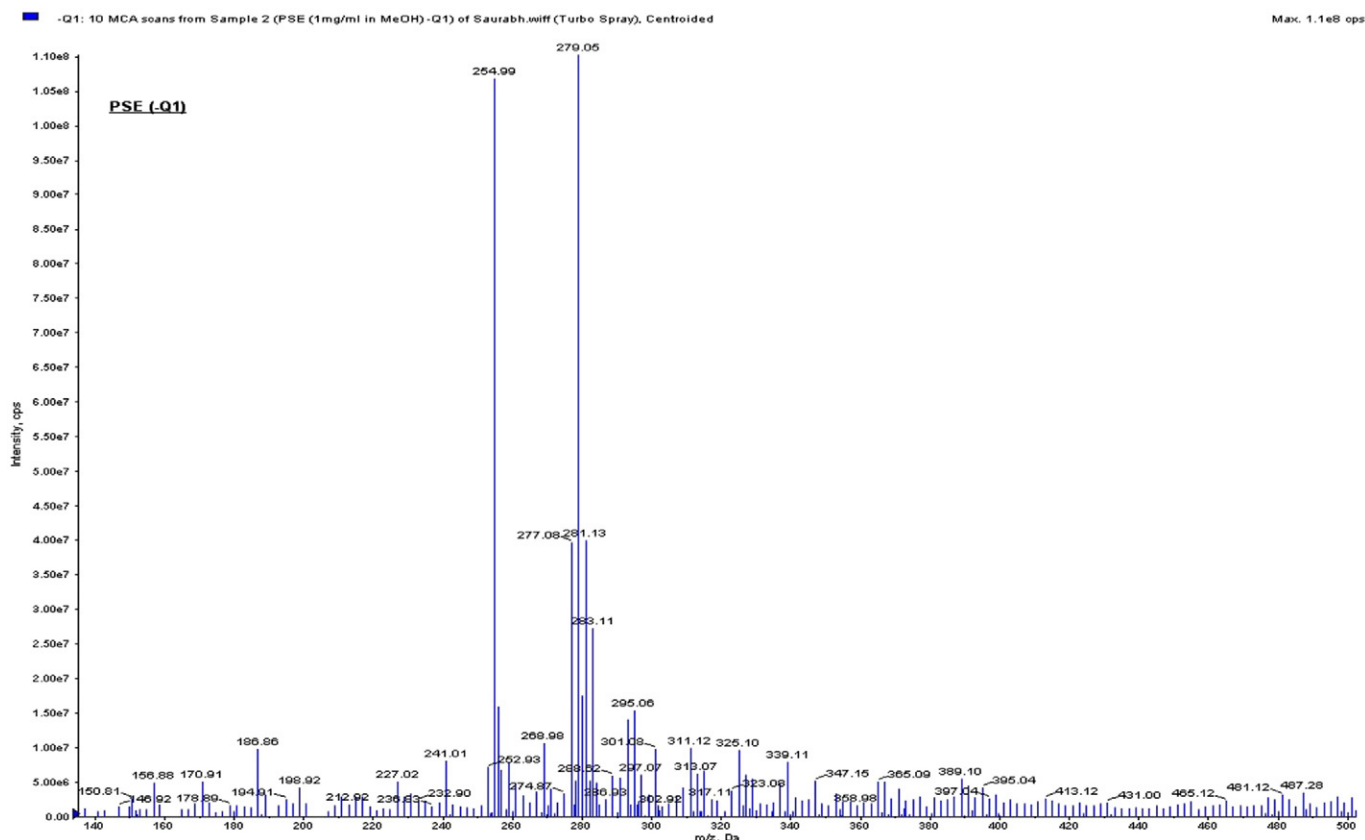


Fig. 2. ESI MS/MS full spectrum (140–500 m/z) of *P. scandens* ethanolic extract shows the presence of molecular ion m/z 179.99 and 241.01 etc. respectively.

mean values indicate that DEXA 30 mg/kg and PSE 200 mg/kg extract proved to be more potent in reducing the differential cell count in BALF when compared to all the other extracts and isolated compounds.

4.5. Effect of *P. scandens* extracts on nitrite (NO_2) and nitrate (NO_3) levels in the BALF

Increased levels of nitrite and nitrate in the lungs lead to formation of NO which causes oxidative stress. The combination of increased oxidative stress and NO may lead to the formation of the potent radical peroxynitrite that may result in nitrosylation of proteins in the airways. The nitrite and nitrate levels in BALF (μM) were significantly ($p < 0.001$) increased, when OVA control was compared to vehicle control. When compared to OVA control, elevated nitrite and nitrate levels were significantly ($p < 0.001$) decreased by PSE 200 mg/kg exhibited a

significant ($p < 0.001$) decrease in elevated nitrite and nitrate levels respectively, when compared to PSA at the same dose level (Fig. 4). However, the mean value indicates that CP1 is less effective in reducing the elevated level of nitrite and nitrate in BALF.

4.6. Effect of *P. scandens* extracts on cytokine production in the BALF

4.6.1. TNF- α level in the BALF

TNF- α is an amplifying mediator in asthma and is produced in increased amounts in the asthmatic airways. TNF- α activates the proinflammatory transcription factors, viz. nuclear factor- κB (NF- κB) and activator protein-1 (AP-1) which in turn activate on many inflammatory genes in the asthmatic airway. The TNF- α levels in BALF (pg/ml) were significantly ($p < 0.001$) increased, in OVA control when compared with vehicle control. When compared to OVA control, elevated TNF- α levels were significantly decreased ($p < 0.001$) by PSE and PSA at 100 and 200 mg/kg. Isolated CP1 at 10 mg/kg also showed significantly ($p < 0.001$) decreased elevated TNF- α levels. However, the mean value indicates that PSE 200 mg/kg showed ($p < 0.001$) significant decrease in the elevated TNF- α levels activity among the entire dose level (Fig. 5).

4.6.2. IL-6 level in the BALF

IL-6 is responsible for the activation of platelet derived growth factor (PDGF), erythroid differentiation factor (EDF), insulin-like growth factor 1 (IGF-1) and IL-11 which leads to smooth muscle hyperplasia and fibroblast activation. The IL-6 levels in BALF (pg/ml) were significantly ($p < 0.001$) increased in OVA control when compared to vehicle control mice. On comparing with OVA control, elevated IL-6 levels were significantly decreased ($p < 0.001$) by PSE and PSA at 200 mg/kg dose levels. Isolated CP1 at 10 mg/kg also showed significantly ($p < 0.001$) decreased elevated IL-6 levels. However, the mean value indicates that PSE at 200 mg/kg ($p < 0.001$) exhibited a significant decrease in the elevated IL-6 level (Fig. 6).

Table 2

ESI-MS/MS precursor ion m/z of identified compounds in the crude extracts of *P. scandens* ethanolic extract.

ESI-MS/MS ions (m/z)	
Compound name	Precursor ion [$\text{M}-\text{H}$] ⁺ m/z
D-Mannose	179.99
2-Hexadecanol	241.01
1-Hexadecanol, 2-methyl-	254.99
Dodecanoic acid	198.92
Tetradecanoic acid	227.02
Ethanol, 2-((-9-octadecenyl oxy)-(Z)-	311.12
Phytol	295.06
9,12-Octadecadienoic acid (Z,Z)-	279.05
9,12, 15-Octadecadienoic acid (Z,Z,Z)-	277.08
Octadecanoic acid	283.11
1,2-Benzenedicarboxylic acid, diisooctyl ester	389.10

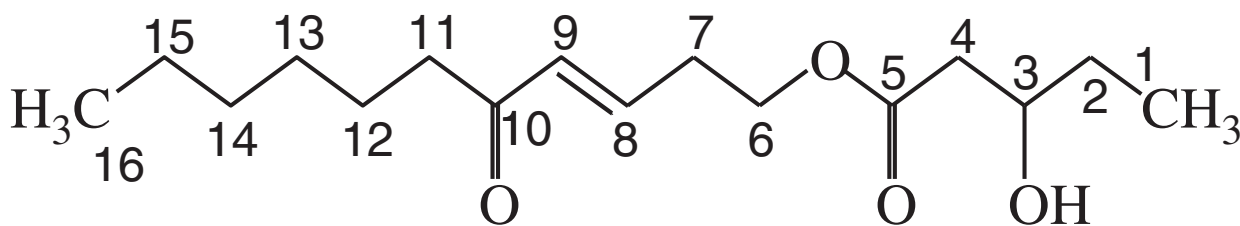


Fig. 3. Chemical structure of isolated CP1 (5-oxoundecyl-3-hydroxypentanoate).

4.6.3. IL-13 level in the BALF

IL-13 plays a key role in the allergic inflammatory response since they determine the isotype switching in B-cells that result in IgE formation. The IL-13 levels in BALF (pg/ml) were significantly ($p < 0.001$) increased in the OVA control when compared with vehicle control mice. When compared to OVA control, elevated IL-13 levels were significantly decreased by PSE and PSA at 200 mg/kg dose level ($p < 0.001$; $p < 0.01$), respectively. Whereas, treatment with CP1 at 10 mg/kg is less effective ($p < 0.05$) towards IL-13. However, the mean value indicates that overall PSE at 200 mg/kg ($p < 0.001$) showed significant decrease in elevated IL-13 levels (Fig. 7).

4.7. Effect of *P. scandens* extracts on MDA level

Estimation of this parameter helps in recognizing the degree of lipid peroxidation taking place in the lungs. The MDA level in lung tissue (nM of TBAR/mg protein) was significantly ($p < 0.001$) increased in OVA control when compared with vehicle control. When compared to the OVA control, the elevated MDA level was significantly ($p < 0.01$; $p < 0.001$) decreased by PSE and PSA at 100 and 200 mg/kg. Whereas, treatment with CP1 at 5 mg/kg is less effective when compared with 10 mg/kg ($p < 0.01$) treated animal. However, the overall mean value indicated that a dose of PSE at 200 mg/kg ($p < 0.001$) showed a significant decrease in elevated MDA levels (Fig. 8).

4.8. Effect of *P. scandens* extracts on MPO level

This parameter reveals the level of cell injury and degree of cell damage in the lungs. The MPO level in lung tissue (mU/mg) was significantly ($p < 0.001$) increased in OVA control when compared with vehicle control. When compared to the OVA control, the elevated MPO level was significantly ($p < 0.001$) decreased by PSE and PSA at 200 mg/kg. Whereas, isolated CP1 at 5 and 10 mg/kg are not effective in reducing the MPO levels. However, the mean value indicated that treatment with PSE at 200 mg/kg dose level showed most significant reduction ($p < 0.001$) in the elevated MPO level (Fig. 9).

4.9. Effect of *P. scandens* extracts on TLP level

This parameter shows the level of protein degeneration in the lungs. The TLP level in lung tissue ($\mu\text{g}/\text{mg}$) was significantly ($p < 0.001$) increased in the OVA control when compared to the vehicle control mice. On comparison with the OVA control, the elevated TLP level was decreased significantly ($p < 0.05$; $p < 0.001$) by PSE and PSA at 100 and 200 mg/kg. Whereas, treatment with CP1 at 5 mg/kg is less effective when compared with 10 mg/kg ($p < 0.01$) treated animal. Moreover, the mean value indicates that treatment with PSE 200 mg/kg displayed a significant ($p < 0.001$) decrease in elevated TLP level among all the tested dose level (Fig. 10).

4.10. Histopathology

4.10.1. Histology of bronchi with hematoxylin and eosin (H&E)

The histology of bronchi after staining with H&E revealed normal architecture of subepithelial membrane, submucosal mucus glands, but mild degree of peribronchial inflammation and active goblet cells in the mice group treated with vehicle control. Whereas, in OVA control, showed severe increase in peribronchial inflammation, epithelial fragility, wall thickening, abnormalities in elastin, cuffing and active metaplastic goblet cells. Among all the treated dose level maximum restoration of bronchi was observed in PSE (200 mg/kg) which showed occasional areas of focal inflammation with functional goblet cells in bronchi when compared with OVA control. However, similar observation was seen in mice treated with DEXA 30 mg/kg. The histological plates are shown below (Fig. 11A).

4.10.2. Histology bronchi goblet cell with periodic acid–Schiff (PAS)

Histology of bronchi under PAS stain revealed normal architecture of goblet cells with regular arrangement and also active condition in the group treated with vehicle control. Whereas, in OVA control showed lost architecture and metaplastic goblet cell arrangement. Among all the treated groups maximum restoration of bronchi and goblet cells was observed in PSE (200 mg/kg) which showed no metaplasticity

Table 3

Effect of *P. scandens* extract on WBC and DC distribution in BALF of balb/c induced with OVA allergen.

Groups (N = 6)	Dose (mg/kg; p.o.)	Total cell ($10^3/\text{ml}$)	Percentage of			
			Lymphocytes	Monocytes	Neutrophils	Eosinophils
Vehicle control	–	7.2 ± 1.2	69.7 ± 1.9	1.83 ± 1.0	27.8 ± 1.2	0.67 ± 0.2
OVA control	–	$25.5 \pm 1.9^{###}$	$30.2 \pm 2.9^{###}$	$10.1 \pm 1.1^{###}$	$39.5 \pm 1.4^{###}$	$20.2 \pm 1.5^{###}$
DEXA	30	$9.3 \pm 1.3^{***}$	$61.8 \pm 1.6^{***}$	$3.5 \pm 1.2^{**}$	$28.2 \pm 1.4^{***}$	$6.5 \pm 1.1^{***}$
PSE	50	23.2 ± 1.4	40.8 ± 2.3	8.5 ± 1.5	34.2 ± 1.7	16.5 ± 1.1
	100	$14.7 \pm 1.7^{***}$	$49.5 \pm 1.3^{***}$	6.5 ± 1.4	$30.8 \pm 1.2^{**}$	$13.2 \pm 1.6^{**}$
	200	$9.8 \pm 1.2^{***}$	$62.3 \pm 2.4^{***}$	$3.7 \pm 1.0^{**}$	$27.7 \pm 1.5^{***}$	$6.3 \pm 1.4^{***}$
PSA	50	24.7 ± 1.3	32.8 ± 3.1	10.2 ± 1.2	37.3 ± 1.2	19.7 ± 1.1
	100	$18.7 \pm 1.5^*$	$44.5 \pm 1.3^{**}$	8.5 ± 1.3	$32.2 \pm 1.6^{**}$	$14.8 \pm 1.1^*$
	200	$11.8 \pm 1.1^{***}$	$55.3 \pm 2.7^{***}$	$4.5 \pm 1.2^*$	$31.5 \pm 1.8^{**}$	$8.7 \pm 1.1^{***}$
CP1	5	21.7 ± 1.6	42.2 ± 3.4	8.7 ± 1.7	34.3 ± 1.9	14.8 ± 1.5
	10	$16.5 \pm 1.6^*$	$48.7 \pm 3.0^{***}$	5.2 ± 1.4	$32.5 \pm 1.5^*$	$13.6 \pm 1.9^{**}$

Values expressed as mean $\pm$ SEM of six independent experiments of male balb/c mice (N = 6). Statistical significance; $^{###}p < 0.001$ vs. vehicle control; $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. Ova control; One Way ANOVA followed by Tukey's multiple comparison test.

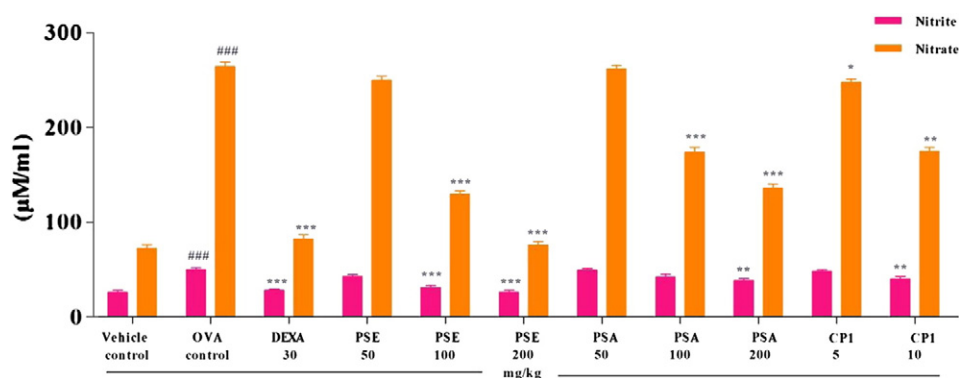


Fig. 4. Effect of *P. scandens* extract and isolated compound (PSE, PSA and CP1) at various tested doses (mg/kg) on the production of nitrite and nitrate ($\mu\text{M/ml}$) in 100 μl of BALF of OVA sensitized balb/c mice (except control). Each column and vertical bar represents the mean $\pm$ SEM for six mice. ### $p < 0.001$ vs. Control; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. OVA control; One Way ANOVA followed by Tukey's multiple comparison test.

reaching towards normal goblet cells when compared with OVA control. However, similar observation was seen in mice treated with DEXA 30 mg/kg. The histological plates are shown below (Fig. 11 B).

5. Discussion

One of the common diseases that affect humankind with diverse manifestations is allergic asthma, which is responsible for significant morbidity with severe economic impact [43]. Various epidemiological studies have identified the cause for an increase in the prevalence of upper and lower respiratory tract allergic disorders [44]. Some of the postulated reasons are increasing environmental pollution and increased predisposition of individuals producing excessive IgE through a major change in the gene pool and changing life style [45]. Allopathic drugs, which are available, develop drug resistance on continuous use of them. So there is a need for searching new potentially effective compounds from natural source against allergic asthma. One of the most promising targets in the search for new biologically active compounds is plants used in folk medicine, many of which have never been investigated for their chemical composition or pharmacological activity in a scientific manner. In this search for finding potential leads from medicinal plants that may be effective against different types of hypersensitivity reactions involving various phases of allergic asthma and also on the basis of ethnobotanical claims of those plants having antiasthmatic properties, the ethanolic and aqueous extracts of *P. scandens* extract and isolated compound were investigated in the present study for their in vivo antiasthmatic activity on OVA induced animal models. Efforts were also made to identify the phytoconstituents that are responsible for the antiasthmatic activity.

The phytochemical investigations on the extracts of *P. scandens* have revealed the presence of carbohydrates, fixed oils, proteins, alkaloids, glycosides, flavonoids and phenolic compounds. The phytochemical tests help in laying down the pharmacopoeial standards [46]. The qualitative analysis on the crude extracts has been carried out by ESI-MS/MS by FIA methods. It has been observed that the peak intensities are exactly matching with compounds, which are claimed to be present in this plant as per the literature. The result has revealed that in the *P. scandens* ethanolic extract at negative mode, eleven good intense response peaks were identified and are characterized as D-mannose at m/z 179.99, 2-hexadecanol at m/z 241.01, 1-hexadecanol 2-methyl- at m/z 254.99, dodecanoic acid at m/z 198.92, tetradecanoic acid at m/z 227.02, ethanol,2-(-9-octadecenyloxy)-(Z)- at m/z 311.12, phytol at m/z 295.06, 9,12-octadecadienoic acid (Z,Z)- at m/z 279.05, 9,12,15-octadecadienoic acid (Z,Z,Z) at m/z 277.08, octadecanoic acid at m/z 283.11 and 1,2-benzenedicarboxylic acid, diisooctyl ester at m/z 389.10. Lalitharani et al. (2009) have reported that the above mentioned compounds are present in the ethanolic extract of leaf of *P. scandens* by GC/MS analysis [31]. Among the identified phytochemicals, dodecanoic acid, tetradecanoic acid and n-hexadecanoic acid have the property of antioxidant activity. The compounds 9,12-octadecadienoic acid (Z,Z) and 9,12,15-octadecatrienoic acid (Z,Z,Z) have the anti-inflammatory property [29]. The compound isolated from the ethanolic extract of *P. scandens* namely compound 1 (CP1) indicating its fatty acid or ester nature, evidenced by IR, ^1H NMR, and ^{13}C NMR. The assignments are complemented by the corresponding ^{13}C NMR signals. The ^{13}C NMR signals indicate that the compound may be unsaturated long chain fatty acid or ester. The MS/MS spectral data of the isolated compound has indicated that the molecular formula is $\text{C}_{16}\text{H}_{30}\text{O}_4$ having the

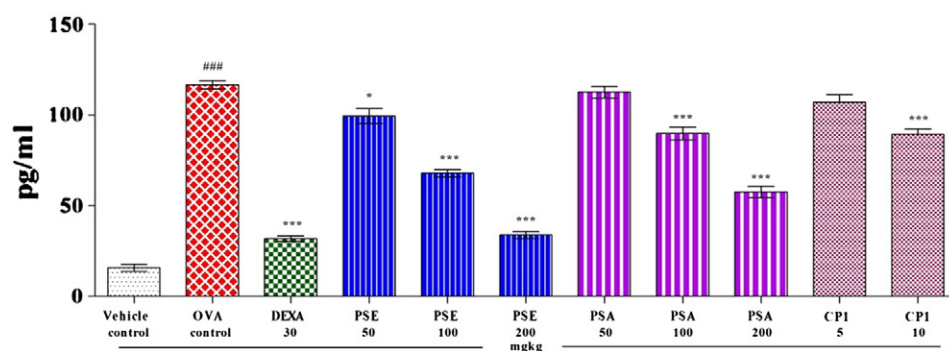


Fig. 5. Effect of *P. scandens* extract and isolated compound (PSE, PSA and CP1) at various tested doses (mg/kg) on the extent of suppression of inflammatory biomarker TNF- α (pg/ml) in BALF of OVA sensitized balb/c mice (except control). Each column and vertical bar represents the mean $\pm$ SEM for six mice. ### $p < 0.001$ vs. Control; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. OVA control; One Way ANOVA followed by Tukey's multiple comparison test.

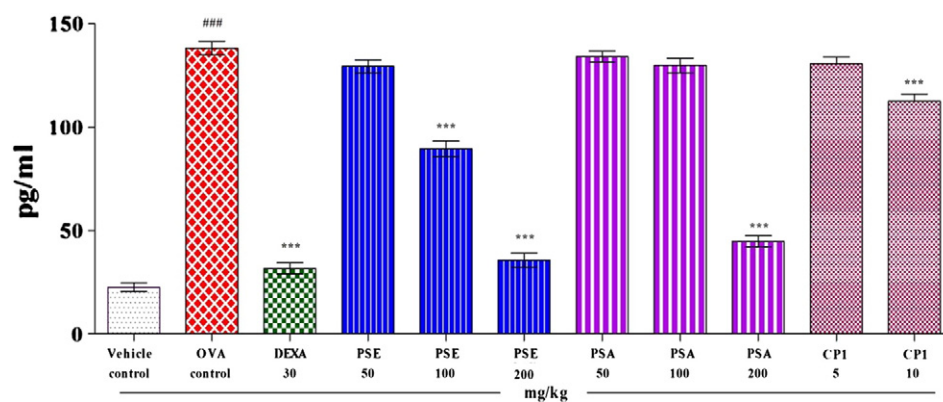


Fig. 6. Effect of *P. scandens* extract and isolated compound (PSE, PSA and CP1) at various tested doses (mg/kg) on the extent of suppression of inflammatory biomarker IL-6 (pg/ml) in BALF of OVA sensitized balb/c mice (except control). Each column and vertical bar represents the mean $\pm$ SEM for six mice. ###p < 0.001 vs. Control; *p < 0.05, **p < 0.01, ***p < 0.001 vs. OVA control; One Way ANOVA followed by Tukey's multiple comparison test.

molecular weight of 284.46. The IUPAC name for the compound is 5-oxoundecyl-3-hydroxypentanoate.

In the acute toxicity study, the extracts PSE and PSA at the dose of 2000 mg/kg have shown no abnormal clinical signs and no mortality. Based on the LD₅₀ value the test compounds have been selected for pharmacological studies. Park et al. (2010) have reported that the presence of various phytochemical constituents, such as phenolics, carotenoids, alkaloids, nitrogen and organosulfur compounds, and vitamins have been responsible for the antiasthmatic activity. The antioxidant and anti-inflammatory properties of some important natural bioactive compounds like *Curcumin*, resveratrol (3,4,5-trihydroxystilbene), vitamin A (retinol) and carotenoids etc. also exert favorable effects on bronchial asthma. These agents would be useful not only for reducing oxidative stress in asthma but also for the control of inflammation. Most of their actions have been related to their ability to direct enzymatic breakdown via endogenous anti-oxidative enzymes and synthesis of various anti-oxidants and quenchers, thereby inhibiting cytokine, chemokine or adhesion molecule synthesis [47,48]. From a therapeutic point of view, saponin containing plants are used in traditional medicine to treat asthma. *Gardenia latifolia*, Ginkgolides from *Ginkgo biloba* leaves have been used to treat asthma. The plants *Ziziphus polygala*, *Panax ginseng* and *Glycyrrhiza glabra* have been useful as antifatigue agents, antiphlogistics, expectorants for bronchitis and asthma, and hepatoprotective etc [49]. These herbs have shown interesting results in various target specific biological activities such as bronchodilation, mast cell stabilization, anti-anaphylactic, anti-inflammatory, antispasmodic, anti-allergic, immunomodulatory and inhibition of mediators viz., leukotrienes, lipoxigenase,

cyclooxygenase, platelet activating, phosphodiesterase and cytokine, in the treatment of asthma [49].

The use of ovalbumin challenge in murine mice, one of the "Classical" animal model of asthma, has significantly increased the understanding of the basic mechanisms of allergic inflammation and the underlying immunological response, allowing investigators to address specific questions that are difficult to answer in patients [50]. The ova model suggests that in atopic/allergic asthma, the early response to inhalation of allergen is a result of an IgE-dependent type I hypersensitivity reaction driven by mast cell-derived mediators, whereas the late response develops as a result of activation of antigen-specific T cells. The secretion of cytokines by activated T-lymphocytes, mast cells and injured airway epithelial cells contributes to the pathogenesis of airway hyperreactivity and other features of asthma [51]. The key regulatory role of CD4 + T-lymphocytes in asthmatic inflammation and the importance of Th2 cytokines have been highlighted by numerous studies in mice. In acute sensitization multiple systemic administration of the allergen protocol used in the presence of adjuvant such as aluminum hydroxide (Al(OH)₃) is known to promote Th2 phenotype by the immune system when its contact with an antigen [52]. Thus, allergic inflammations are investigated on their basis including the role of CD4 + Th2 cells. In the same way various cellular and molecular pathways are also involved to regulate the activity. However, there is a difference in the pattern of airway inflammation compared to human asthmatics [53]. In particular, studies on IL-6, IL-13 and TNF- α in such models have led to an understanding of the signaling pathways and the cellular feedback loops that are involved. The contribution of eosinophils to the

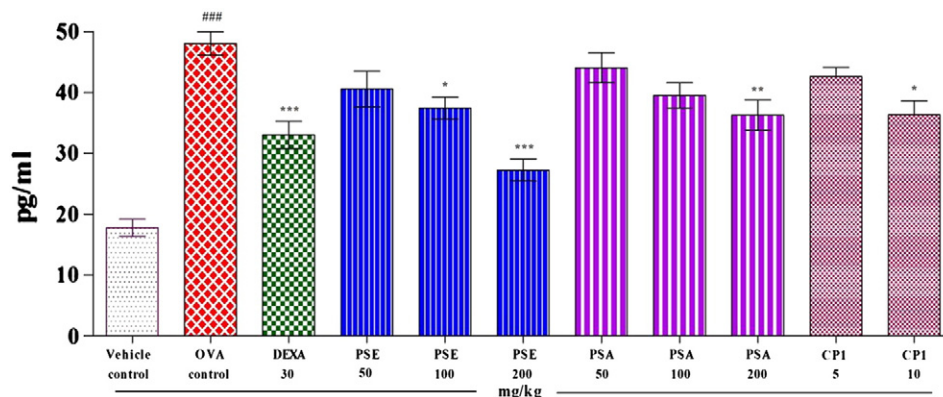


Fig. 7. Effect of *P. scandens* extract and isolated compound (PSE, PSA and CP1) at various tested doses (mg/kg) on the extent of suppression of inflammatory biomarker IL-13 (pg/ml) in BALF of OVA sensitized balb/c mice (except control). Each column and vertical bar represents the mean $\pm$ SEM for six mice. ###p < 0.001 vs. Control; *p < 0.05, **p < 0.01, ***p < 0.001 vs. OVA control; One Way ANOVA followed by Tukey's multiple comparison test.

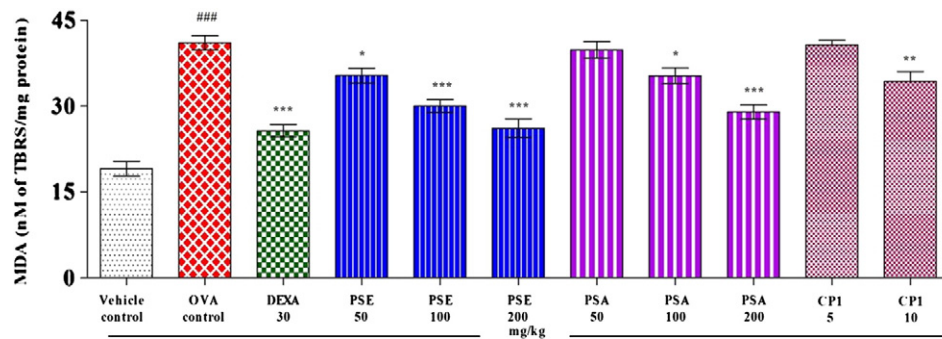


Fig. 8. Effect of *P. scandens* extract and isolated compound (PSE, PSA and CP1) at various tested doses (mg/kg) on the production of malonyldialdehyde (nM of TBARS/mg protein) in mice lung tissue of OVA sensitized balb/c mice (except control). Each column and vertical bar represents the mean $\pm$ SEM for six mice. ###p < 0.001 vs. Control; *p < 0.05, **p < 0.01, ***p < 0.001 vs. OVA control; One Way ANOVA followed by Tukey's multiple comparison test.

asthmatic phenotype, which remains an area of some controversy, has been extensively dissected using ovalbumin challenge models. Also of particular interest have been the studies that have identified the role of eosinophils in the afferent limb of the allergic response [50]. The other factors such as generation of inflammatory cells, inflammatory mediators and free radicals immensely contribute towards the symptoms of asthma.

In our study, it has been found that a significant increase in the number of total cell count and differential count in BALF of OVA sensitized animal's model. Total cell count is an important parameter in asthma. The results revealed that PSE shows significant reduction ($p < 0.001$) in total cell count among all the tested dose level. In differential count increased number of eosinophils and neutrophils has been observed. The increased number of eosinophils shows the phenomenon of eosinophilic infiltration and the increased number of neutrophils leads to activation of IL-8 which induces sputum during allergic asthmatic condition. The result revealed that PSE at 200 mg/kg extract proved to be more potent in reducing the eosinophil and neutrophil count when compared to all the other extracts and isolated CP1. *P. scandens* having potential in attenuating the proliferation and transmigration of eosinophils and neutrophils in the lung. Eosinophils play a major role in allergic asthma by producing EPO which leads to generate free radicals (including HOBr) and by adhering to the lung tissue which contribute to inflammation and generation of other leukocyte dependant mediator. In short eosinophils amplify the inflammatory response generated by T cells [54,55]. From the results it could be suggested that *P. scandens* dampens the inflammation by its action on eosinophils.

Increased levels of nitrite and nitrate in lung tissue lead to formation of NO which causes oxidative stress. The combination of increased oxidative stress and NO may lead to the formation of the potent peroxynitrite radical that may result in nitrosylation of proteins in the

airways [14]. The increased level of formation of nitrite and nitrate in the BALF of OVA sensitized mice was significantly ($p < 0.001$) inhibited by PSE at 200 mg/kg dose level among all the tested dose level and showed most potent inhibitory activity of nitrite and nitrate free radicals, further strengthening the antioxidant mechanism. Similar to the deleterious effects of ROS, RNS too has been strongly implicated in the inflammatory response in asthmatic individuals [13]. Agents which could inhibit iNOS activity or scavenge the NO could be, thus, considered as a viable adjuvant in the treatment of asthma [14]. Similarly, the estimation of TNF- α , IL-6 and IL-13 has performed. The increased level of TNF- α , IL-6 and IL-13 was observed in the BALF of OVA sensitized mice which was significantly ($p < 0.001$) decreased by PSE at 200 mg/kg demonstrated uniform inhibition of TNF- α level, and a corresponding ability to decrease the IL-6 and IL-13 levels in the collected BALF. In the previous study it was observed that the plant extracts contain flavonoids and poly-phenolic compound. Apart from possessing anti-oxidative effects, flavonoids and poly-phenolic compounds inhibit release of histamine and other preformed granule associated mediators by inhibiting the activation of basophils and mast cells [56]. Flavonoids and poly-phenolic compounds also inhibit synthesis of IL-4, IL-13, and CD40 ligand but initiate generation of new phospholipid-derived mediators. Vascular changes are one of the major components for asthmatic pathogenesis. The changes include an increase in vascular permeability, vascular dilation/enlargement, and vasculogenesis/angiogenesis [57]. Flavonoids and their related poly-phenolic compounds have been shown to not only modulate expression of hypoxia-inducible factor (HIF-1), vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), and epidermal growth factor receptor but also inhibit nuclear factor-kappaB (NF- κ B), phosphoinositide 3-kinase (PI3K/Akt), and ERK1/2 signaling pathways [58,59]. These observations have suggested that flavonoids as well as their related compounds inhibit certain

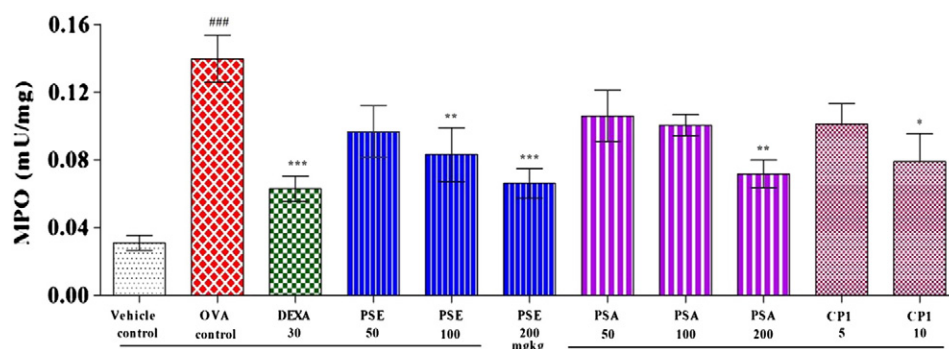


Fig. 9. Effect of *P. scandens* extract and isolated compound (PSE, PSA and CP1) at various tested doses (mg/kg) on the production of myeloperoxidase (mU/mg protein) in mice lung tissue of OVA sensitized balb/c mice (except control). Each column and vertical bar represents the mean $\pm$ SEM for six mice. ###p < 0.001 vs. Control; *p < 0.05, **p < 0.01, ***p < 0.001 vs. OVA control; One Way ANOVA followed by Tukey's multiple comparison test.

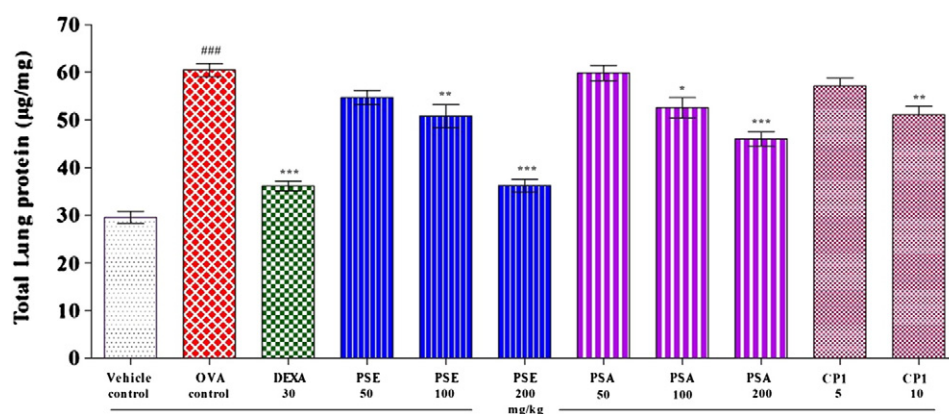


Fig. 10. Effect of *P. scandens* extract and isolated compound (PSE, PSA and CP1) at various tested doses (mg/kg) on the production of total lung protein (µg/mg) in mice lung tissue of OVA sensitized balb/c mice (except control). Each column and vertical bar represents the mean $\pm$ SEM for six mice. ###p < 0.001 vs. Control; *p < 0.05, **p < 0.01, ***p < 0.001 vs. OVA control; One Way ANOVA followed by Tukey's multiple comparison test.

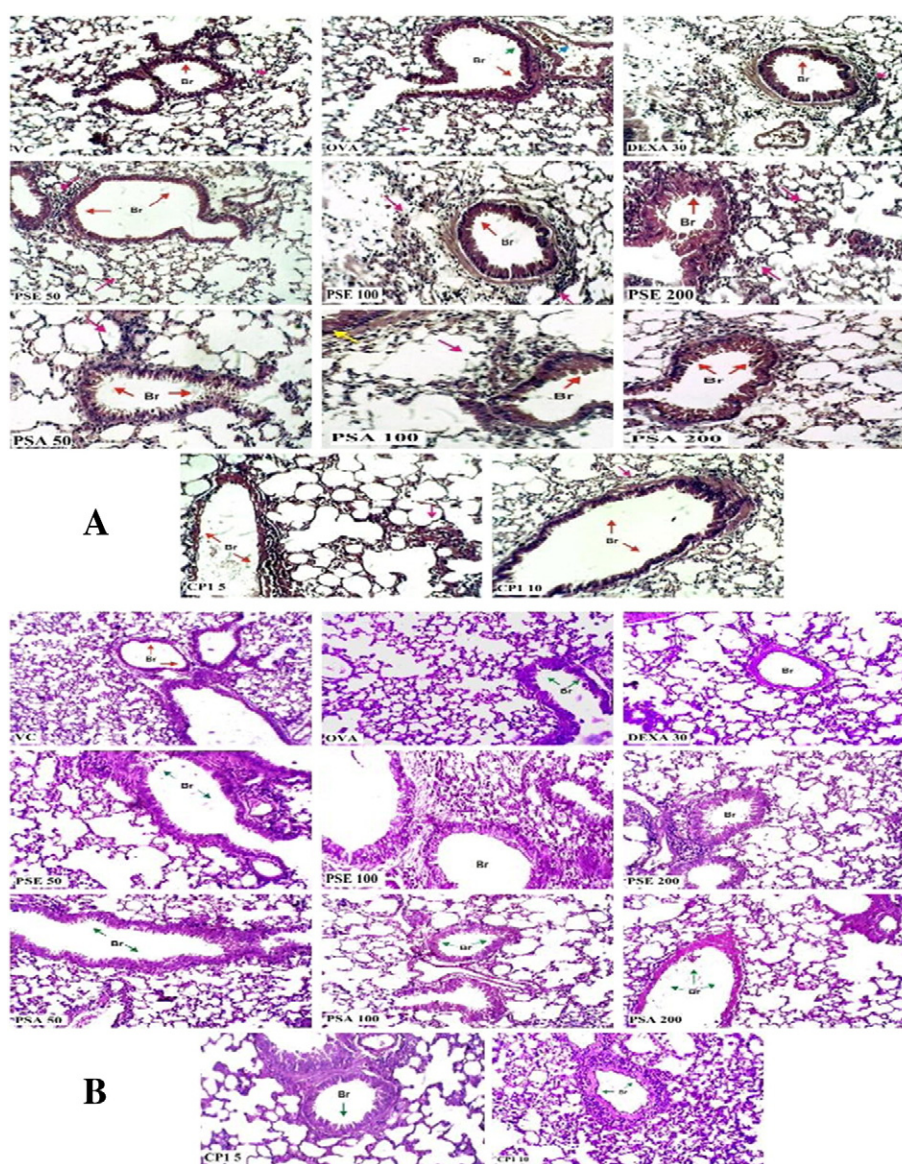


Fig. 11. Histological examination of lung tissues was performed 48 h after the final OVA challenge. Lung tissues were fixed, sectioned at 4 µm thicknesses, and stained with H&E solution (A) and periodic acid–Schiff (PAS) (B). Scale bars equal 50 µm. Vehicle control (VC), OVA control, DEXA 10 mg/kg, PSE 50 mg/kg, PSE 100 mg/kg, PSE 200 mg/kg, PSA 50 mg/kg, PSA 100 mg/kg, PSA 200 mg/kg. Red arrows, pink arrows, green arrows, blue arrows and yellow arrows denote active goblet cell, inflammatory cell infiltrate, metaplastic goblet cells, vascular cuffing and sub epithelial fibrosis, respectively in H and E stain. Whereas red arrows, green arrows and green dotted arrows denote active goblet cell, metaplastic goblet cells, and mild metaplastic goblet cells, respectively in PAS stain. Treatment of DEXA or PSE or PSA or CP1 was performed 1 h before the challenge. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

steps of angiogenesis that are cell migration, microcapillary tube formation, and MMP expression [60]. These findings suggest that flavonoids are antiallergic and anti-inflammatory agents effective in treating/preventing of asthma.

The different parameters estimated with the lung homogenate of balb/c are MPO, MDA and TLP. Eosinophils and neutrophils play a major role by the presence of MPO, which is considered as marker for inflammation. MPO is hemeprotein catalyses in the formation of HOCl from H₂O₂. HOCl contributes directly to the tissue dysfunction and destruction of protein along with the other free radicals. The elevated level of MPO has been observed in lung tissue of OVA sensitized mice which was significantly ($p < 0.001$) decreased by PSE at 200 mg/kg that showed most significant reduction ($p < 0.001$) in the elevated MPO level. In asthmatic patients there is increased neutrophil derived MPO in blood and it is also being considered as biomarker for the prognoses of the disease. The final action of ROS along with RNS is the production of MPO which indicates the cellular damage leading to the altered cellular function within the inflamed lung [13]. The estimation of LPO in the form of MDA is thus considered to be a reliable marker for ROS [38]. In the present study result revealed that there was significant ($p < 0.001$) increase in MDA levels in the lungs of the OVA sensitized mice and PSE at 200 mg/kg showed a significant ($p < 0.001$) decrease in elevated MDA levels, when compared to all the extracts and isolated CP1. The elevated protein level was observed in lung tissue of OVA sensitized mice which was significantly ($p < 0.001$) decreased by PSE at 200 mg/kg dose level that showed most significant reduction ($p < 0.001$) in the elevated TLP. This might be due to the inhibitory activity of the extract on NOS. NOS and high oxidative stress lead to the formation of a potent peroxy radical which causes nitrosylation of proteins. The effect of *P. scandens* extract on LPO, TLP, NO and MPO implicates the antioxidant potential and the reduction of WBC, eosinophils and neutrophils (inflammatory cells) indicates the anti-inflammatory and immunomodulatory activity [by suppression of innate (reduction in eosinophils, neutrophils, MPO and mast cell stabilization of plant) and probably by adaptive immune response].

This has been further strengthened by histopathological results obtained from OVA sensitized model. After pre-sensitization of balb/c mice on 0 and 7th day by OVA (50 µg) and alum (1 mg) solution intraperitoneally they were challenged with 2.5% (w/v) OVA aerosol through a nebulizer (inhalation) on 14th and 21st day. This has resulted in structural changes in the airways of asthmatic bronchi which included epithelial fragility, goblet cell hyperplasia, goblet cell metaplasia, enlarged submucosal mucus glands, angiogenesis, increased matrix deposition in the airway wall, increased airway smooth muscle mass, wall thickening and abnormalities in elastin. Two types of staining methods were used for light microscopic evaluation of histology of lung tissue. The first slide was stained with H&E, and second with PAS. The slides stained with H&E were analyzed for tissue structure and morphometric features such as the thickness of the epithelium, sub epithelial smooth muscle layers and small airways [61]. The slides stained with periodic acid–Schiff (PAS) were analyzed for goblet cell arrangement feature including goblet cell hyperplasia, hypertrophy and metaplastic condition of the bronchi [62]. The histology of lung tissue stained with (H&E) and (PAS) reveals that PSE 200 mg/kg was more effective and shows restoration of bronchi.

Above result revealed that the isolated compound CP1 (5-oxoundecyl-3-hydroxypentanoate) is least effective in ovalbumin-induced AHR in balb/c mice. This antiasthmatic activity exerted by plants due to the presence of multiple constituents and at certain specific concentration these could act synergistically and in additive fashion till the concentration attains a threshold dose, after which the very same constituents could antagonize the therapeutic effect. However exact mechanism is beyond the scope of the present study. The study also has certain other limitations such as the use of single model, the use of allergens, etc. Although, mouse allergic asthma model mimics clinically important human disease [34]. It has certain limitations such as difficulty to detect

AHR (characteristic feature of asthma), and lack of a true sustained model of active chronic disease inflammation [63,34]. Further study, using other models (IgE responder rats like Brown Norway rats, human specific allergen like ragweed and pollen) are suggested to further validate the therapeutic efficacy in allergic asthma. Nevertheless, the present study has strongly demonstrated the antiasthmatic activity in allergic animal models used in the present study by *P. scandens*.

6. Conclusion

In conclusion, this study is the first to provide experimental evidence demonstrating that *P. scandens* inhibits ovalbumin-induced AHR in murine model of asthma. Our results indicated clearly that *P. scandens* ethanolic extract treatment reduces the accumulation of eosinophils and other inflammatory cells (neutrophils, lymphocytes, and macrophages), nitrite, nitrate, TNF- α , IL-6 and IL-13 are estimated in BALF; in the BALF and lung tissues of an OVA-induced murine asthma model; that MDA, MPO, TLP levels were unregulated; and that *P. scandens* reduced significantly the severity of airway inflammation and the accompanying oxidative stress. Our results suggested that *P. scandens* is promising candidate as an adjuvant therapy for AHR patients. It virtues further more work towards the isolation of phytoconstituents from *P. scandens* L.

Conflict of interest statement

The author(s) declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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