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Anti-inflammatory and analgesic activity of *Clerodendrum splendens*

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ABSTRACT

Clerodendrum splendens is a twining evergreen climber, commonly known as Bharangi. The shrub is native to tropical Western Africa, is commonly cultivated in gardens for its brilliant scarlet flower in many parts of India. *Clerodendrum* species are rich source of flavanoids and diterpenoids compounds hence plant extracts are traditionally used as antiasthmatic and anti-inflammatory. No scientific work is yet reported on *Clerodendrum splendens*, hence present work was undertaken to establish analagesic and anti-inflammatory potential of the stem, leaves and flower extracts. Stem, leaves and flowers of the plant was collected, authenticated, shade dried and extracted successively with various solvents. Analgesic activity was assessed by hot plate method and acetic acid induced writing test in swiss albino mice and anti inflammatory activity was assessed by carrageenan-induced hind paw edema method in rats at 50 mg/kg, i.p. dose. Ethyl acetate extract of leaves showed most significant analgesic and anti-inflammatory activity.

Keywords: *Clerodendrum splendens*, antihistaminic, analgesic, anti-inflammatory.

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INTRODUCTION

Clerodendrum splendens is a twining evergreen climber, commonly known as Bharangi. The shrub is native to tropical Western Africa and leaves, roots and stem bark extracts of plant are used extensively in traditional medicine for treating many diseases in African folk medicine. It is commonly cultivated in gardens for its brilliant scarlet flower in many parts of India, additionally Steroidal compounds like 24β-ethylcholesta-5,22,25-triene-3-β ol, clerosterol and cycloartenol have been isolated from leaves of *C. splendens*[1]. β-Amyrin and Clerodolone have been isolated from the aerial parts of the plant[2]. The flavonoids apigenin and hispudilin including it glycoside have been isolated from the leaf extract of the plant[3]. Traditionally the *Clerodendrum* species are rich source of flavonoids and diterpenoids compounds hence plant extracts are used for the treatment of asthma. Inflammation of lungs and airways is one of the symptoms in asthma. Anti-inflammatory and analgesic drugs play a significant role in the treatment of asthma by providing relief from the inflammation and pain of the airways. Hence, objective of the present study was to check the analgesic and anti-inflammatory potential of *Clerodendrum splendens* and validate the ethno-medicinal claims.

MATERIAL AND METHOD

Plant material

Plant parts of *Clerodendrum splendens* G. Don was collected from Nashik district of Maharashtra in February 2011 and later on authenticated by Mr. A. Bennjamin, Botanical Survey of India, Pune, where herbarium voucher specimen No. BSI/WRC/Tech/2015 SBP-1 has been deposited.

Extraction and isolation

Dried and powdered leaves, stem and flower of *C. splendens* were extracted successively with various solvents viz. petroleum ether, chloroform, ethyl acetate and methanol in Soxhlet extractor. Extracts were concentrated by vacuum distillation and then dried in open air to produce respective extracts. All the extracts were vacuum dried and various extractive values such petroleum ether extract, chloroform extract, ethyl acetate extract and methanol extract of leaves was 8.4%, 6.0%, 5.7% and 3.5% w/w, petroleum ether extract, chloroform extract, methanol soluble of *Clerodendrum splendens* stem was found to be 8.86, 3.78, 4.1% w/w and petroleum ether extract and methanol extract of flower was found to be 6.86, 5.7% w/w respectively.

Animals

Male albino mice (Swiss strain) weighing 25-28 g were housed under standard laboratory conditions, in groups of six each and used for analgesic activity. Colony-bred adult Wistar rats (150–200 g) were used for anti-inflammatory activity. The animal had free access to food and water, standard pelleted laboratory animal diet (Godrej Agrovet Ltd. Mumbai, India) was provided *ad libitum* during acclimation and study period. The animals were kept at temperature 20° – 25° C and relative humidity 30 – 70 %. The cycle of 12-hour light and 12 hour dark was maintained). The ethical committee of the institute approved the protocol of the study having resolution no. COP/IAEC/2012-13/03.

Drugs and Chemicals

The following drugs and chemicals were used. Drugs: Pentazocine lactate injection (Ranbaxy, India), paracetamol injection (Heilenlab, India) and naloxone hydrochloride injection (Samarth Life Sciences Pvt. Ltd., India) purchased from a commercial source. Chemicals: Petroleum ether extract of stem, chloroform extract of stem, methanol extract of stem, petroleum ether extract of flowers, methanol extract of flowers and petroleum ether extract of leaves, ethyl acetate extract of leaves, methanol extract of leaves of *Clerodendrum splendens* and 5% Tween 80 (Seppic) solution in distilled water as vehicle.

ASSESSMENT OF ANALGESIC ACTIVITY

Hot plate test

Central nociceptive activity was evaluated using the hot plate method[4-5]. Mice were divided into 10 groups of six animals each. The first group served as control and received only vehicle (10% Tween 80 in distilled water), and the second group was administered standard drug pentazocine (10 mg/kg, i.p.). The animals of third to tenth groups were treated with petroleum ether extract of leaf, ethyl acetate extract of leaf, methanol extract of leaf, petroleum ether extract of stem, chloroform extract of stem, methanol extract of stem, petroleum ether extract of flower and methanol extract of flower of *Clerodendrum splendens* (50 mg/kg, i.p., each), respectively. All the extracts and standard drug were dissolved into the vehicle. The mice were placed individually on the hot plate maintained at 55 ± 0.2^{o}C, and latency of nociceptive response such as licking, flicking of the hind limbs or jumping was noted. The readings were taken at 0, 30, 60, 90, 120 and 150 min after treatment. The experiment was terminated 20 sec after their placement on the hot plate to avoid damage to the paws.

Acetic acid-induced writhing test

Peripheral nociceptive activity was evaluated using acetic acid-induced writhing test[5-6]. Mice were divided into ten groups of six animals each. The first group served as control and was

treated with vehicle only (10% Tween 80 in distilled water). The second group was administered standard drug paracetamol (50 mg/kg, i.p.). The animals of third to tenth groups were treated with petroleum ether extract of leaf, ethyl acetate extract of leaf, methanol extract of leaf, petroleum ether extract of stem, chloroform extract of stem, methanol extract of stem, petroleum ether extract of flower and methanol extract of flower of *Clerodendrum splendens* (50 mg/kg, i.p., each), respectively, 30 min before intra-peritoneal injection of 0.6% solution of acetic acid (10 ml/kg). All the extracts and standard drug were dissolved in the vehicle. After acetic acid injection, the mice were observed for the number of writhing responses for the period of 30 min for each animal.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using carrageenan-induced hind paw edema method[5,7]. Wistar rats of either sex were divided into ten groups of six animals each. The first group served as control and received only vehicle (10% Tween 80 in distilled water), and the second group was administered standard drug ibuprofen (50 mg/kg, i.p.). The animals of the third to tenth groups were treated with petroleum ether extract of leaf, ethyl acetate extract of leaf, methanol extract of leaf, petroleum ether extract of stem, chloroform extract of stem, methanol extract of stem, petroleum ether extract of flower and methanol extract of flower of Clerodendrum splendens (50 mg/kg, i.p., each), respectively. All the extracts and standard drug were dissolved in the vehicle. After 30 min of the above treatments, 0.05 ml of 1%w/v carrageenan in saline was injected into the subplantar tissue of the left hind paw of the animals. The degree of paw edema of all the groups was measured plethysmometrically at 0, 30, 60, 90 and 120 min after the administration of carrageenan to each group; 0 min readings are the initial paw volume of animals.

Statistical analysis

All the data is presented as mean ± SEM. Data was analyzed by one-way ANOVA followed by Dunnett's test. Prism Graph pad 3 was used for statistical analysis. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Analgesic activity

Hot plate test

Ethyl acetate extract (50 mg/kg, i.p.) of leaves of *Clerodendrum splendens* showed significant increase in reaction time as compared to control and other extracts. It showed promising activity

comparable to the standard drug pentazocine (10 mg/kg, i.p.). Petroleum ether extract of leaves, stem and flowers also showed significant activity (Table 1). The hot plate test is the specific central antinociceptive test[8]. Ethyl acetate extract (50 mg/kg, i.p.) of leaves and petroleum ether extract of leaves, stem and flowers of *Clerodendrum splendens* showed significant results in this test, so there may be involvement of opioid receptors. The opioid agents exert their analgesic action via the supraspinal (μ_1, κ_3, δ_1, σ_2) and spinal (μ_2, κ_1, δ_2) receptors[9].

Table 1: Effect of various extracts of *Clerodendrum splendens* (50 mg/kg, i.p.) on thermic stimulus-induced pain (Hot plate test)

Treatment	Reaction time (Sec)					
	Time after treatment (min)					
	0	30	60	90	120	150
Control	2.3±0.3	2.8±0.2	2.4±0.3	2.6±0.4	2.1±0.1	2.8±0.2
Pentazocine (10 mg/kg, i.p.)	2.8±0.4	7.4±0.3*	11.9±0.4*	10.2±0.3*	6.1±0.2*	3.4±0.3
PE-L	2.2±0.2	4.9±0.3*	7.9±0.3*	8.2±0.2*	5.8±0.3*	3.2±0.3
EA-L	2.2±0.2	5.0±0.3*	8.1±0.5*	8.9±0.2*	6.0±0.1*	3.3±0.3
MT-L	2.2±0.5	4.2±0.2*	7.0±0.5*	6.8±0.3*	4.3±0.3	3.4±0.1
PE-S	2.3±0.2	4.9±0.3*	7.8±0.4*	8.1±0.2*	5.3±0.3	3.3±0.4
CL-S	2.4±0.3	4.0±0.4	6.4±0.3*	6.7±0.1*	4.1±0.4	3.3±0.1
MT-S	2.2±0.4	4.3±0.5	7.0±0.2*	7.2±0.1*	4.8±0.3	3.2±0.4
PE-F	2.2±0.2	4.4±0.4	7.2±0.1*	7.8±0.4*	5.2±0.3	3.3±0.3
MT-F	2.2±0.4	4.3±0.5	7.0±0.2*	7.2±0.1*	4.8±0.3	3.2±0.4

All the values are expressed as mean ± SEM; n = 6; *P < 0.05 significant compared to control.

Where, PE-S – Petroleum ether extract of stem; CL-S – Chloroform extract of stem; MT-S – Methanol extract of stem; PE-F- Petroleum ether extract of flowers; MT-F- Methanol extract of flowers; PE-L – Petroleum ether extract of leaves; EA-L – Ethyl acetate extract of leaves; MT-L – Methanol extract of leaves.

Acetic acid-induced writhing test

Ethyl acetate extract (50 mg/kg, i.p.) of leaves of *Clerodendrum splendens* produced significant inhibition of writhing reaction induced by acetic acid compared to control group. Petroleum ether extract of leaves, stem and flowers also showed significant activity (Table 2).

Table 2: Effect of various extracts of *Clerodendrum splendens* (50 mg/kg, i.p.) on acetic acid-induced writhing on mice.

Treatment	Number of writings
Control	61.17±1.2
Paracetamol (50 mg/kg, i.p.)	28.33±2.4*
PE-L	38.14±1.4*
EA-L	35.1±1.2*
MT-L	52.12±1.7

PE-S	40.2±3.2*
CL-S	57.5±1.8
MT-S	50.12±2.1
PE-F	43.12±1.3*
MT-F	47.1±2.0

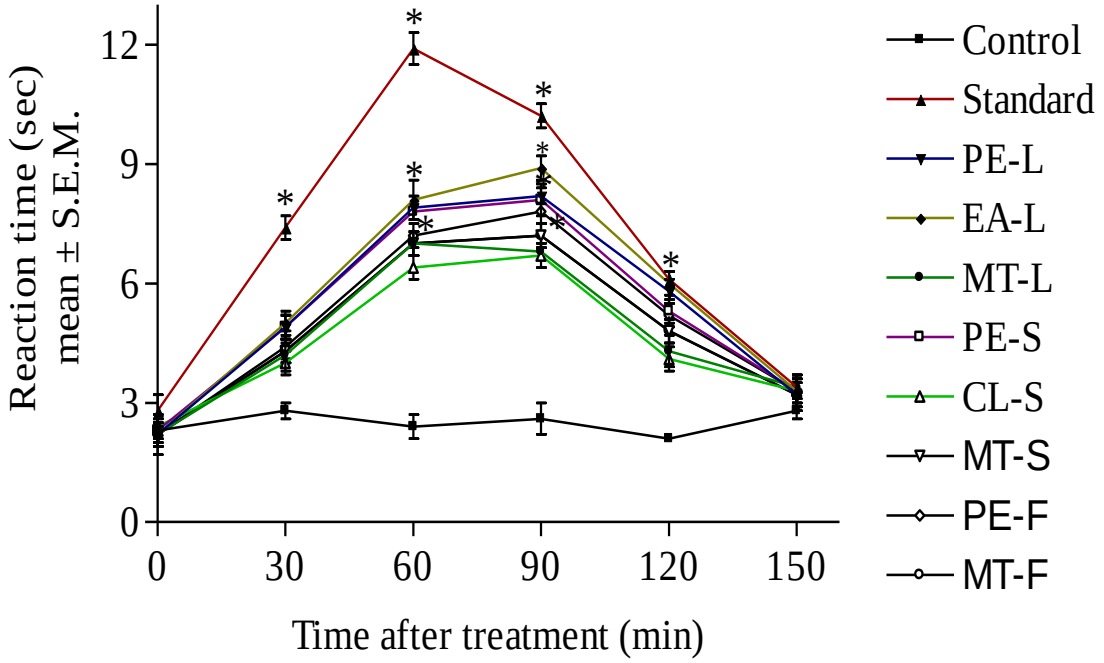

Figure 1: Effect of various extracts of *Clerodendrum splendens* (50 mg/kg, i.p.) on thermic stimulus-induced pain (Hot plate test).

All the values are expressed as mean ± SEM; n = 6; *P < 0.05 significant compared to control.

Where, PE-S – Petroleum ether extract of stem; CL-S – Chloroform extract of stem; MT-S – Methanol extract of stem; PE-F- Petroleum ether extract of flowers; MT-F- Methanol extract of flowers; PE-L – Petroleum ether extract of leaves; EA-L – Ethyl acetate extract of leaves; MT-L – Methanol extract of leaves.

Anti-inflammatory activity

In the acute inflammation model i.e. carrageenan-induced rat paw edema method, ethyl acetate extract of leaf, petroleum ether extract of leaf, stem and flower (50 mg/kg, i.p.) produced significant (P<0.05) inhibition of paw edema as compared to the control. The activity of extracts was compared with standard drug ibuprofen (50 mg/kg, i.p.). Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 h[10]. In case of analgesia, prostaglandins and bradykinins were

suggested to play an important role in the pain process[11]. Some sterols and triterpenes are responsible for anti-inflammatory and analgesic activity[12]. Ethyl acetate extract of leaf and Petroleum ether extracts of leaf, stem and flower of *Clerodendrum splendens* showed significant anti-inflammatory activity. Airway obstruction/bronchoconstriction or airway hyper-responsiveness in asthma are believed to be a direct consequence of airway wall inflammation[13-14]. This proposed mechanism is consistent with previous findings that anti-inflammatory plant principles have shown to act through control of adrenocorticoid hormone and immunosuppression, respectively[12,15].

Table 3: Effect of various extracts of *Clerodendrum splendens* on carrageenan-induced rat paw edema.

Treatment	Mean increase in paw volume ml ± SEM Time in min				
	0	**30**	**60**	**90**	**120**
Carrageenan (Control)	0.24±0.01	0.48±0.03	0.78±0.09	0.85±0.12	0.89±0.14
Ibuprofen (50 mg/kg, i.p.)	0.24±0.03	0.31±0.07*	0.30±0.07*	0.27±0.06*	0.26±0.13*
PE-L	0.25±0.01	0.36±0.05	0.36±0.09*	0.34±0.06*	0.30±0.05*
EA-L	0.24±0.05	0.34±0.05	0.34±0.01*	0.30±0.02*	0.28±0.06*
MT-L	0.25±0.07	0.40±0.09	0.47±0.07	0.49±0.01	0.40±0.09
PE-S	0.24±0.14	0.35±0.09	0.35±0.07*	0.32±0.09*	0.30±0.12*
CL-S	0.25±0.07	0.41±0.02	0.48±0.01	0.51±0.12	0.42±0.13*
MT-S	0.25±0.09	0.36±0.07	0.35±0.09*	0.35±0.07*	0.32±0.11*
PE-F	0.25±0.05	0.35±0.09	0.35±0.01*	0.34±0.07*	0.31±0.06*
MT-F	0.25±0.13	0.37±0.17	0.36±0.09*	0.35±0.02*	0.32±0.04*

All the values are expressed as mean ± SEM; n = 6; *P < 0.05 significant compared to control.

Where, PE-S – Petroleum ether extract of stem; CL-S – Chloroform extract of stem; MT-S – Methanol extract of stem; PE-F- Petroleum ether extract of flowers; MT-F- Methanol extract of flowers; PE-L – Petroleum ether extract of leaves; EA-L – Ethyl acetate extract of leaves; MT-L – Methanol extract of leaves.

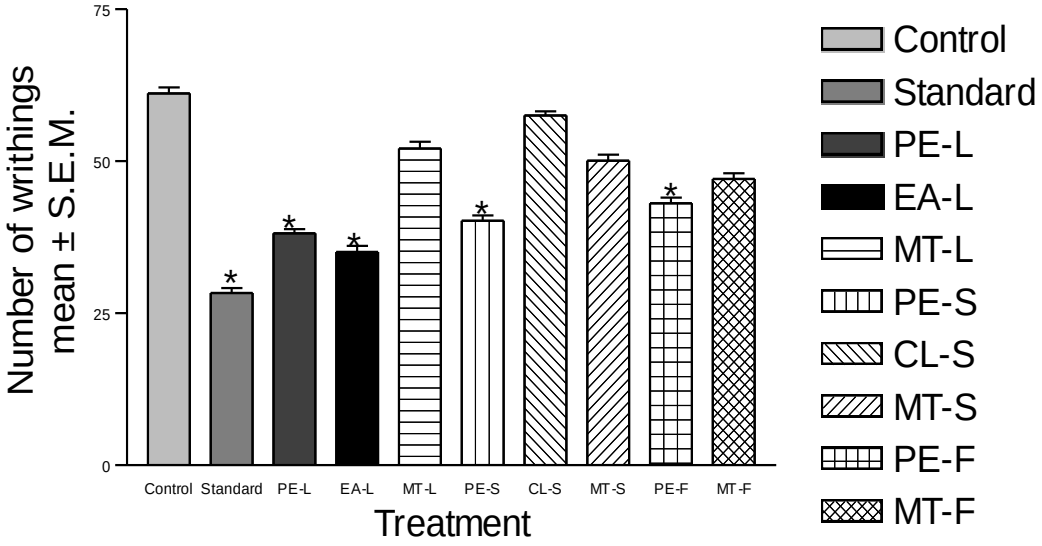

Figure 2: Effect of various extracts of *Clerodendrum splendens* (50 mg/kg, i.p.) on acetic acid-induced writhing on mice.

All the values are expressed as mean ± SEM; n = 6; *P < 0.05 significant compared to control.

Where, PE-S – Petroleum ether extract of stem; CL-S – Chloroform extract of stem; MT-S – Methanol extract of stem; PE-F- Petroleum ether extract of flowers; MT-F- Methanol extract of flowers; PE-L – Petroleum ether extract of leaves; EA-L – Ethyl acetate extract of leaves; MT-L – Methanol extract of leaves.

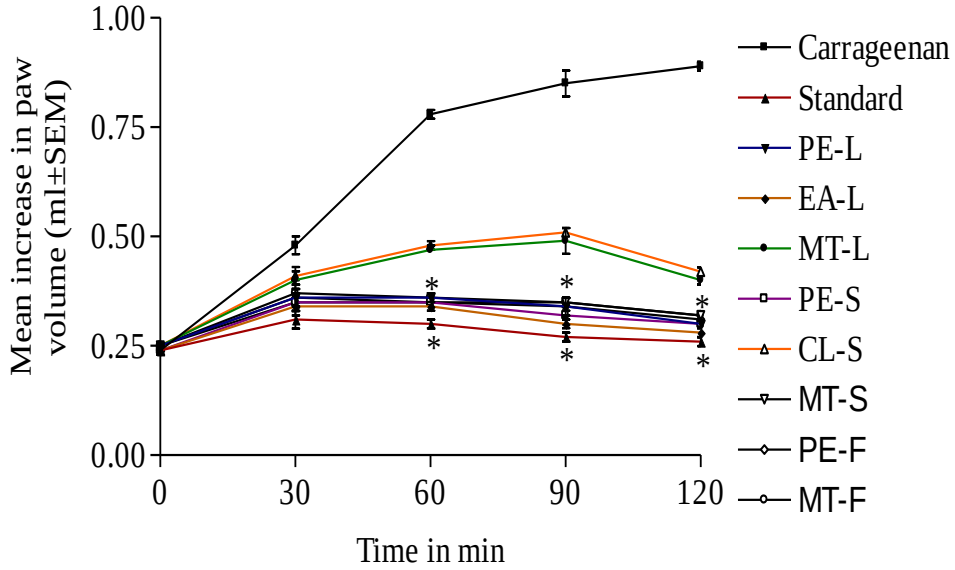

Figure 3: Effect of various extracts of *Clerodendrum splendens* (50 mg/kg, i.p.) on carrageenan-induced rat paw edema.

All values are expressed as mean ± SEM; n=6, * P<0.05 significant compared to control.

Where, PE-S – Petroleum ether extract of stem; CL-S – Chloroform extract of stem; MT-S – Methanol extract of stem; PE-F- Petroleum ether extract of flowers; MT-F- Methanol extract of flowers; PE-L – Petroleum ether extract of leaves; EA-L – Ethyl acetate extract of leaves; MT-L – Methanol extract of leaves.

CONCLUSION

Ethyl acetate extract of leaves of *Clerodendrum splendens* showed most significant analgesic and anti-inflammatory activity and the constituents present in ethyl acetate extract might be responsible for the activity.

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