

**ANTINOCICEPTIVE ACTIVITY OF *Clerodendrone multiflorum* LEAVES, STEMS
(Verbenaceae)**

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Summary

Present study reports effect of petroleum ether, chloroform, ethyl acetate and methanolic extracts of leaves and stems of *Clerodendrone multiflorum* Linn. (Fabaceae) on nociceptive response using hot plate method and writhing test in mice. All the extracts of *Clerodendrone multiflorum* showed significant central and peripheral analgesic activity in hot plate method and acetic acid-induced writhing test, respectively, at the dose of 50 mg/kg intraperitoneally. Methanolic extract of stems of *Clerodendrone multiflorum* showed highest increase in reaction time in hot plate method and more inhibitory effect on writhing induced by acetic acid. Pentazocin and paracetamol was taken as standard for hot plate and writhing model respectively.

Keywords, *Clerodendrone multiflorum*, analgesic activity, pentazocine, hot plate method, acetic acid induced writhing method.

Introduction

Clerodendrum phlomidis Linn is a large bush or small tree belonging to the family verbenaceae.¹ It is widely distributed throughout India in the drier parts, Baluchistan and Ceylon.² This plant is commonly known as " *Arni*" in Hindi. The Juice of leaves is used as an alternative and bitter tonic. The decoction of its roots is used as an astringent and demulscent in gonorrhoea.³ The whole plant has known to possess hypoglycemic effect.⁴ Secondary metabolites such as β -sitosterol, lupeol acetate, scutellarein, D-mannitol, ceryl alcohol and flavanoids have been isolated from *Clerodendrum phlomidis*. The certain flavonoids were isolated from dried roots and flower as, rhamnopyrosyl (1-2)-2-D-glucopyranosyl-7-O-naringin-4-O- α and D-glucopyranoside-5-methyl ether 2,4-trihydroxy, 6-methoxy chalcone-4, 4- α -D Diglucoside, pectolinarigenin, hispidulin, apigenin. From leaves steroid (24 s) ethylcholesta – 5, 22, 35- trine- 3 β – ol, flavonoids as scutellarein and pectolinarigenin (4', 6- dimethyl scutellarein) were isolated. Ceryl alcohol, clerodendrin, clerosterol and clerodendrin were isolated from roots.^{5, 6, 7}

The plant is also investigated for its antidiarrhoeal⁸ and psychopharmacological activity,⁹ the present study was carried out in an experimental animal model to substantiate the folklore claim for analgesic activity.

Materials And Method:

Plant material and Preparation of extracts:

The stems and leaves of *Clerodendron multiflorum* (Verbenaceae) were collected from Ahmednagar district, Maharashtra (India) in August 2009. The plant specimen was authenticated from Botanical Survey of India, Pune (Voucher specimen no. CKS1). Dried and coarsely powdered stem and leaf parts (500g each) of *Clerodendron multiflorum* were separately subjected to successive extraction using petroleum ether, chloroform, ethyl acetate and methanol in Soxhlet extractor. The extracts of various parts were concentrated by vacuum distillation and then dried in open air.

Animals

Healthy wistar albino mice of either sex and of approximately the same age, weighing about 25-30 gm were used for study. They were housed in polypropylene cages maintained under standard condition (12hour light/12 hour dark cycle; 30±4 C, 36-60 humidity).

Analgesic activity

Hot plate test:

Central analgesic activity was evaluated using hot plate method as per described by Woolfe and MacDonald.¹⁰ Mice were divided into ten groups of six animals each. The first group served as control and received only vehicle, second group was administered standard drug pentazocine (50 mg/kg, i.p.). The animals of third to sixth group were treated with petroleum ether, chloroform, ethyl acetate and methanol extracts (50 mg/kg, i.p.) of stems and animals of seventh to tenth group were treated with petroleum ether, chloroform, ethyl acetate and methanol extracts (50 mg/kg, i.p.) of leaves of *Clerodendron multiflorum* respectively. Mice were placed individually on the hot plate maintained at 55 °C ± 1 °C and latency of nociceptive response such as licking, flicking of a hind limb or jumping was noted. The readings were taken at 0, 30, 60, 90, 120, 150 and 180 min after administration of extracts. The experiment was terminated 20 second after their placement on the hot plate to avoid damage to the paws.

Writhing test:

Peripheral analgesic activity was evaluated using acetic acid-induced writhing test.¹¹ Mice were divided into ten groups of six animals each. The animals received petroleum ether extract or chloroform extract or ethyl acetate extract or methanol extracts (50 mg/kg, i.p.) of stems or petroleum ether extract or chloroform extract or ethyl acetate extract or methanol extracts (50 mg/kg, i.p.) of leaves of *Clerodendron multiflorum* or standard drug paracetamol (50 mg/kg, i.p.) or vehicle, 30 min before intraperitoneal injection of 0.1 ml of 0.6 % solution of acetic acid. Mice were placed individually into glass beakers after administration of acetic acid and five minutes were allowed to elapse. The mice were then observed for the period of 30 minutes and then number of writhes recorded for each animal.

Petroleum ether (60-80°C), chloroform, ethyl acetate and methanol extracts of *Clerodendron multiflorum* were suspended into minimum volume of DMF and then volume is adjusted with water for injection, and administered intraperitoneally in a constant volume (8 ml/kg). All drug solutions were prepared immediately before starting the experiment.

Chemicals:

The following drugs were used: pentazocine lactate injection (Ranbaxy, Ahmedabad), paracetamol injection (Heilenlab, Goa), acetic acid (AR Grade, PCL, Pune), petroleum ether (60-80°C), chloroform, ethyl acetate and methanol (AR Grade, PCL, Pune).

Statistical significance:

The results were analyzed for statistical significance using students't' test. $P < 0.05$ and $P < 0.0001$ were considered as significant.

Results and Discussion**Hot plate test:**

All the extracts of *Clerodendron multiflorum* showed significant analgesic activity at 50 mg/kg, i.p. dose (Table 1). Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, methanolic extract of stems of *Clerodendron multiflorum* showed highest increase in reaction time.

Writhing test:

All the extracts of *Clerodendron multiflorum* at dose of 50 mg/kg, i.p., significantly attenuated the number of writhing and stretching induced by intraperitoneal 0.6% acetic acid (Table 2). Methanolic extract of stems of *Clerodendron multiflorum* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts as well as standard drug paracetamol.

In the present study, all the extracts showed significant ($P < 0.05$ and $P < 0.0001$) analgesic activity but among all the extracts, methanolic extract of stems of *Clerodendron multiflorum* showed highest increase in reaction time. Thermic painful stimuli are known to be selective to centrally active drugs¹². Prostaglandins and bradykinins were suggested to play an important role in analgesia.^{13, 14} Flavonoids and tannins are reported to inhibit prostaglandin synthesis.¹⁵ A number of flavonoids and tannins have been reported to produce analgesic activity.¹⁶ As phytochemical tests showed presence of flavonoids and tannins in methanolic extract of leaf of *Clerodendron multiflorum*¹⁸, they might suppress the formation of prostaglandin and bradykinins or antagonize their action and exert its activity.

Peripheral analgesic activity was assessed by acetic acid-induced writhing test, which showed significant ($P < 0.05$ and $P < 0.0001$) suppression of writhing by all the extracts, but methanolic extract of stems of *Clerodendron multiflorum* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts and standard drug paracetamol (Table 2). It was observed that onset of writhing was delayed and duration of writhing was shortened. Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response¹⁷. The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals.

Overall we can say that stems of *Clerodendron multiflorum* possess better antinociceptive activity as compared to leaves.

Table1: Antinociceptive activity of various extracts of leaves and stems of *Clerodendron multiflorum*

Treatments	Latency to lick the paw (sec ± SEM)						
	Predrug reaction time(min)	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	3.6±0.5	3.8±0.88	4.0±0.44	3.8±0.37	3.8±0.20	3.57±0.58	4.45±0.37
Pentazocin	10.5±0.49	12.09±0.81#	13.0±0.67*	18.0±0.58*	17.5±0.74	13.5±0.65	10.54±0.47
PES	11.87±0.81	9.42±0.67*	12.27±0.81	12.99±0.62#	13.17±0.29	5.13±0.84	1.81±0.62
CHS	5.69±0.82	13.01±0.25*	13.12±0.49	12.08±1.25	10.84±0.83#	6.25±1.09	6.45±0.94
EAS	4.49±0.84	11.9±0.64*	18.00±2.01	17.18±0.87	16.80±0.26	11.98±.83	12.81±0.34
MES	2.86±0.56	13.89±0.76	18.83±0.85*	19.39±0.58#	20±1.42	19.46±0.99	16.43±0.65
PEL	5.88±0.87	13.33±0.72	7.29±0.67	8.90±0.93	12.31±0.74#	10.31±0.59	15.78±0.29
CHL	10.24±0.78	13.38±0.47#	12.21±0.68	13.7±0.59	18.36±0.47	15.28±0.56	10.52±0.83
EAL	6.02±0.49*	10.25±0.39	13.11±0.78	11.20±0.71	10.57±0.43*	10.12±0.28#	8.02±0.86
MEL	5.15±0.68	13.20±0.45	9.22±0.70	11.02±0.97	15.83±1.21	17.21±0.76	13.42±0.23*

All the values are expressed as mean ± SEM; n=6, #P<0.05, *P<0.0001, significant compared to control. All the extracts and pentazocine were given intraperitoneally at 50 mg/kg dose.

Table 2: Effect of various extracts of *Clerodendron multiflorum* L. on acetic acid-induced writhing in mice.

Treatment	Number of writhing
Vehicle	63.0±0.94
Paracetamol	9.95±0.75*
PES	15.03±0.78
CHS	31.94±1.02*
EAS	48.81±0.97*
MES	8.02±0.37#
PEL	47.53±1.23#
CHL	50.81±0.69#
EAL	56.7±1.76#
MEL	45.99±0.91*

All the values are expressed as mean ± SEM; n=6, #P<0.05, *P<0.0001 significant compared to control. All the extracts and paracetamol were given intraperitoneally at 50 mg/kg dose.

PEL-petroleum ether extract, CHL-chloroform extract, EAL- ethyl acetate extract, MEL-methanol extract of *Clerodendron multiflorum* leaves and PES-petroleum ether extract, CHS-chloroform extract, EAS- ethyl acetate extract, MES-methanol extract of *Clerodendron multiflorum* stems respectively.

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