



Research Article

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ANALGESIC ACTIVITY OF *LEPIDAGATHIS CRISTATA* WILLD FLOWER EXTRACTS

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ABSTRACT

Aim of the study was to screen the *Lepidagathis cristata* Willd, flower extracts for analgesic activity. In the present study the analgesic activity of flower extracts was performed. The methanolic, ethyl acetate, chloroform extracts were prepared and were used for analgesic activity in two dose level of 200 and 400mg/kg body weight in two screening methods, Hot Plate (n=5) and Tail Immersion method (n=5). The flower extracts showed significant analgesic activity. The plant extracts did not exhibit any mortality up to the dose level 4000mg/kg. The methanol, chloroform and ethyl acetate extracts of flower was evaluated for analgesic activity. The flower ethyl acetate extract of *Lepidagathis cristata* showed 47% and 57.1% activity at 200 and 400mg/kg.b.wt, after 30 min by Eddy's Hot plate Method respectively. The flower chloroform extract showed 43.7% and 44.7% protection at 200, 400mg/kg respectively. The flower methanol, chloroform and ethyl acetate extracts showed dose dependent analgesic activity in thermal models. The flower ethylacetate extract has maximum analgesic activity with 57.1% (p < 0.001⁵).

Keywords: *Lepidagathis Cristata*, Analgesic, Hot plate, Tail Immersion, Methanol, Ethyl acetate, Chloroform.

INTRODUCTION

The plant *lepidigathis cristata* willd is as a common herb in the eastern plains of Karnataka, India. The leaves, roots, flowers, seeds and the whole plant of various species of *Lepidigathis cristata* are medicinally useful. The roots of the herb are particularly considered in stomachic and dyspepsia, leaves are used for fever and flower ash is used for burns¹. *Lepidigathis cristata* willd (Family: Acanthaceae) locally known as nakkapidi, lankapidi by yanadi tribal people at Andhra Pradesh, India was selected for present study². This Yanadi tribal population is much widely distributed in Cuddapah, Chittoor, Nellore and Prakasham districts and use this plant for burns, wounds and the tuber ash mixed with coconut oil and used as a lotion. In other parts of India is known as Kollchechutar (Bombay), karappanundu (Malayalam), Bhuyaterada (Marathi) and Otdhompo (Santhal). It is a perennial, prostrate, small herb with woody root stock. Leaves are alternate, elliptic-oblong, clothed with silky pubescence. Flowers are blue, solitary or in pairs with long pedicels, axillary. Fruit is globose capsule^{3,4}. It is common weed of open places, roadsides, grasslands and scrub jungles.

MATERIAL AND METHODS

The plant was collected from Sheshachala hills near by Rajiv Gandhi Institute of Medical Sciences Kadapa, Andhra Pradesh, India. The plant was identified by Assistant professor Dr. Madhusudana Reddy Department of Botany, Yogivemana University, Kadapa, Andhra Pradesh, India. The specimen of the plant was stored in the department of pharmacology (specimen No. TPCP/LC/09/2011). The collected whole plant was dried at room temperature under shade for fifteen days then the flowers are separated manually. The separated plant material was powdered by using mechanical mixer and sieved (mesh size 40) material was used for extraction.

Extraction was carried out by using soxhlet of 1000ml capacity round bottom flask. The extractions of flower part were carried with methanol, ethyl acetate and chloroform until the solvent becomes colorless in the soxhlet and the extracts were concentrated under reduced pressure. The solvent free substances were mixed with 1%v/v tween 80 and used for the experimentation⁵.

Phytochemical Screening

The phytochemical tests^{6,7} were performed to *Lepidagathis cristata* flower chloroform, ethyl acetate and methanolic extracts and the results are depicted in the Table 1.

Acute Toxicity Studies

Acute toxicity study was carried out using female Albino Rats (150-200g) by up and down/staircase method as per OECD guidelines. All the extracts were orally administered to different groups of rats at the doses of 50, 300, 1000, 2000 and 5000mg/kg body weight respectively. Animals were observed for 48h to study the general behavior of animals, sign of discomfort and nervous manifestation. All the extract was found devoid of mortality of animals at the dose of 2000mg/kg body weight. Hence the 1/10th (200mg/kg, p.o.) and 1/5th (400mg/kg, p.o.) of the dose was selected for the screening of analgesic and anti-inflammatory activity.

Pharmacological Screening

The albino mice (20-25g) of either sex were used for the study. They were acclimatized to normal laboratory conditions for one week under 12h light and dark cycle and the given pellet diet and U.V. purified filtered water. The extracts were administered orally and the dose was selected between the minimum effective dose and maximum non lethal dose. All the experimentations were performed according to the protocols and

recommendations of the institutional animal ethics committee (IAEC) with protocol and the reference number Rc.No.413/Acad/2011-2012.

Tail Immersion Method

Albino mice were randomly divided into twenty groups of 5 mice each and fasted for 12h. About 3-5cm of the tail of each mice was dipped into a water bath containing warm water maintained at the temperature of 56±2°C and the time taken for a mice to withdraw the tail known as the pain reaction time (PRT) was recorded for all the mice, in 0.01s units using a stopwatch. The cut off time was 10s. Group I served as control and received normal saline (10ml/kg b.w), Group II received (12.5mg/kg standard drug). Group III to Group VIII received orally, the test substances at a dose of 200 and 400mg/kg b.w through oral route. PRT was recorded again as described earlier for all the mice at 0, 30, 60, 90, and 120. The withdrawal time of untreated animals is between 1 and 5.5s. The increase in mean reaction withdrawal time was calculated^{8,9}. (Table 2, 3 and Figure 1)

- Group-1: Normal saline Control
- Group-2: Standard control-12.5mg/kg (Diclofenac)
- Group-3: Flower Methanol Extract (FME) -200mg/Kg
- Group-4: Flower Methanol Extract (FME) -400mg/Kg
- Group-5: Flower Chloroform Extract (FCE) - 200mg/kg
- Group-6: Flower Chloroform Extract (FCE) - 400mg/kg
- Group-7: Flower Ethyl acetate Extract (FEE) - 200mg/kg

- Group-8: Flower Ethyl acetate Extract (FEE) - 400mg/kg

Eddy's Hot plate Method

Albino mice were randomly divided into eight groups of 5 mice each and fasted for 12h. Animals are individually placed on a hot plate maintained at constant temperature (55°C) and the reaction time of animals such as paw licking or jumping response is taken as end point. Analgesics increase reaction-time. The cut off time was 10s. Group I served as control and received normal saline (10ml/kg b.w), Group II received (12.5mg/kg standard drug)¹⁰. Group III to Group VIII received orally, the test substances at a dose of 200 and 400mg/kg b.w through oral route as mentioned above. The reaction time was recorded for all the mice at 0, 15, 30, 60, and 90^{11,12}. (Table 4)

RESULTS

The plant extracts did not exhibit any mortality up to the dose level 4000mg/kg. The methanol, chloroform and ethyl acetate extracts of flower was evaluated for analgesic activity.

Eddy's Hot Plate Method

The flower methanolic extract (FME) of *Lepidagathis cristata* showed 47% protection at 200mg/kg (p< 0.01) and 47.6% at 400mg/kg (p< 0.05). The flower chloroform extract (FCE) showed 36.8% at 400mg/kg and the flower ethyl acetate extract (FEE) showed 47% (p< 0.01) and 57.1% (p < 0.001) activity at 200 and 400mg/kg after 30min.

Table 1: Phytochemical screening

Extracts	Alkaloids	Glycosides	Phenolic compounds	Tannins	Resins	Volatile Oils	Carbohydrate
Leaf Chloroform Extract	+	+	-	-	+	+	-
Leaf Ethyl Acetate Extract	+	+	+	-	+	+	-
Leaf Methanol Extract	+	+	+	+	+	+	+

Table 2: Analgesic Activity by Tail Immersion Method

Group	Dose (mg/kg)	Reaction time in seconds				
		0min	30min	60min	90min	120min
Control		3.25±0.25	2.25±0.62	3.00±0.40	2.75±0.25	2.75±0.47
Diclofenac	12.5	2.25±0.25	2.50±0.28	3.75±0.75 ^b	4.25±0.25 ^c	2.75±0.25
FME	200	3.75±0.62	4.25±0.25	4.75±0.47	5.00±0.40	3.85±0.64
FME	400	3.00±0.40	4.30±0.40	4.50±0.28	4.90±0.25 ^b	3.75±0.47
FCE	200	2.25±0.25	3.50±0.25 ^b	4.00±0.40 ^a	3.00±0.40 ^a	2.75±0.25
FCE	400	2.50±0.28	4.00±0.25	4.50±0.25	3.50±0.28	2.90±0.28
FEE	200	2.75±0.47	3.25±0.25 ^a	3.75±0.47	4.25±0.25 ^a	3.25±0.40
FEE	400	2.75±0.47	3.75±0.47 ^b	4.10±0.25 ^b	4.75±0.40 ^b	3.50±0.62 ^c

Values are expressed in MEAN±S.E.M. for five animals each group. ANOVA followed by Tukeys multiple comparison tests. Values are statically ^ap<0.05, ^bp<0.01 and ^cp<0.001 when compared with 0 min interval

Table 3: Percentage protection of the *Lepidagathis cristata* extracts by Tail Immersion Method

Group	Dose	% protection			
		30min	60min	90min	120min
Control		-	-	-	-
Diclofenac	12.5	10	40	47	18.8
FME	200	11.7	22.2	25	25
FME	400	30.2	33.3	38.7	20
FCE	200	35	43.7	25	18
FCE	400	37	44.4	28.5	13.7
FEAE	200	15.3	26.6	35.2	15.3
FEAE	400	26.6	32	42	21.4

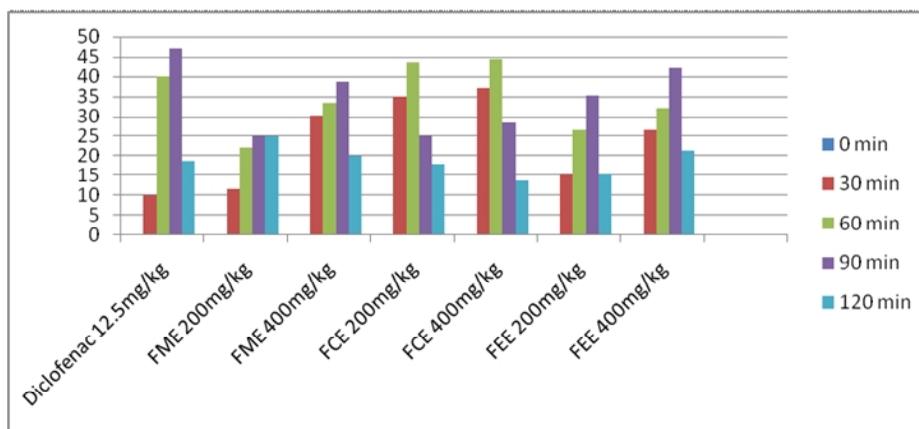


Figure 1: Percentage protection of the *Lepidagathis cristata* flower extracts by Tail Immersion Method

Table 4: Analgesic Activity by Eddy’s Hot Plate Method

Group	Dose (mg/kg)	Reaction time in seconds					
		0min	15min	30min	45min	60min	90min
Control		2.50±0.5	2.75±0.75	3.25±0.25	3.00±0.40	3.00±0.40	2.50±0.28
Diclofenac	12.5	2.75±0.25	6.25±0.25 ^c	6.50±0.5 ^b	4.60±0.40 ^b	4.50±0.47 ^b	4.00±0.91 ^a
FME	200	2.25±0.25	3.00±0.40	4.25±0.47 ^b	3.75±0.40	3.00±0.47 ^a	2.75±0.25
FME	400	2.75±0.47	4.00±0.40 ^a	5.25±0.62 ^a	4.75±0.25 ^b	3.75±0.25	3.25±0.47 ^b
FCE	200	3.00±0.40	4.00±0.57	3.50±0.28	4.50±0.28	3.75±0.47	3.75±0.62
FCE	400	3.00±0.40	4.00±0.40	4.75±0.28	4.50±0.64	3.75±0.47	3.40±0.40
FEE	200	3.00±0.40	4.50±0.64	5.75±0.94 ^c	3.75±0.62 ^a	3.50±0.28 ^a	3.25±0.47 ^a
FEE	400	2.25±0.62	4.75±0.75 ^c	5.25±0.94 ^c	4.25±0.47	3.50±0.50	2.75±0.25

Values are expressed in MEAN±S.E.M. for five animals each group. ANOVA followed by Tukeys multiple comparison tests. Values are statically ^ap<0.05, ^bp<0.01 and ^cp<0.001 when compared with 0 min interval

Table 5: Percentage protection of the *Lepidagathis cristata* extracts by Eddy’s Hot Plate Method

Group	Dose (mg/kg)	% protection				
		15min	30min	45min	60min	90min
Control	-	-	-	-	-	-
Diclofenac	12.5	56	57.6	40.2	38.8	31.2
FME	200	25	47	40	25	18.1
FME	400	31.2	47.6	42.1	26.6	15.3
FCE	200	25	14.28	33.3	20	20
FCE	400	25	36.84	33.2	14.28	11.7
FEE	200	33.3	47	20	14	7.6
FEE	400	52.6	57.1	47.5	35.7	18.1

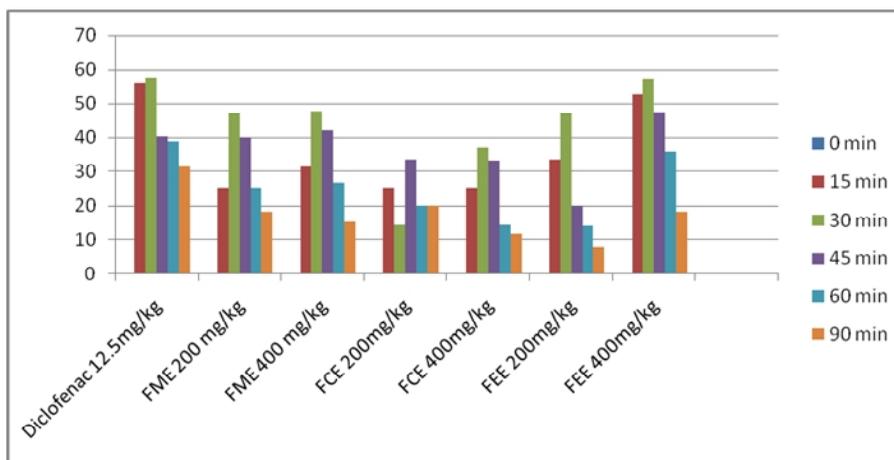


Figure 2: Percentage protection of the *Lepidagathis cristata* flower extracts by Eddy’s Hot Plate Method

Tail Immersion method

The FMC showed 38.7% ($p < 0.01$) protection at 90min with 400mg/kg. FCE was 43.7% and 44.7% ($p < 0.001$) at 200, 400mg/kg respectively. The FEE protected 35.2%, 42% at 200, 400mg/kg at 90min respectively. The flower ethyl acetate extract showed good analgesic activity with 57.1% protection at 30min with 400mg/kg body weight.

DISCUSSION

The plant extracts did not exhibit any mortality up to the dose level 2000mg/kg body weight hence doses are selected $1/10^{\text{th}}$ and $1/5^{\text{th}}$ that is 400 and 200mg/kg body weight p.o. The hot plate method and tail immersion method have been found to be suitable for evaluation of analgesics. The analgesics act centrally and peripherally. Peripherally acting analgesics block the impulse at chemoreceptor site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain and suppress the subject anxiety and apprehension. Pain and inflammation are an essential prelude to the repair process¹³. In the present study the evaluation of analgesic activity was highest with 57.1% protection and all the extracts of flower showed significance response when compared with 0 time interval and graded dose response was observed with all the extracts. The analgesic activity may be because of alkaloid or phenolic compounds, the phytochemical constituents and further research will establish the role of these phytopharmaceuticals.

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