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Open Access Research Article

Anti-Inflammatory, Analgesic and Antipyretic Activity of *Catunaregam spinosa* (Thumb.) Tirveng Extracts

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ABSTRACT

Catunaregam spinosa leaves have been ethnopharmacologically accounted for acquiring various pharmacological properties. The present study was undertaken to evaluate anti-inflammatory, analgesic and antipyretic potential of leaves of *C. spinosa.*The ethanolic extract was selected for this purpose based on phytochemical screening. Inflammation was inhibited at the dose of 200 mg/kg with percent inhibition of inflammation 32.06, 37.28 and 43.16 %, respectively, at 1, 3 and 5 h, while in egg albumin model % inhibition was found to be 47.81%. There was no significant analgesic activity seen in acetic acid induced writhing response method while significant effects were observed in the doses of 25 and 100 mg/kg on hot plate test. No antipyretic activity was shown by ethanolic extracts(25, 100 and 200 mg/kg) against Brewer's yeast induced pyrexia in rats.

Keywords: *Catunaregam spinosa*, Anti-inflammatory activity, Phytochemical screening, Ethanolic extract.

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INTRODUCTION

Ever since ancient times, in search of rescue for their disease, the people looked for drugs in nature. According to the World Health Organisation (WHO), more than 80% of the World's population relies on traditional herbal medicine for their primary health care needs. In last few years interest of researches in medicinal plants has increased significantly. Identification and validation of plant derived substances for the management of several diseases is the goal of current researchers. It is estimated that about 25% of the western medicines are directly or indirectly derived from plant sources[1].

Catunaregam spinosa (Thumb.) Tirveng is also known as a *Randia dumetorum* belongs to family Rubiaceae. It occurs in almost throughout India up to 4,000 ft attitude[2,3]. *C. spinosa* is known to show alexiteric and antipyretic activity with also significant in skin diseases, inflammations, ulcers, wounds and tumours like diseases[4]. It contains secondary metabolites like triterpenoidal saponins, tannins, essential oil, resin and veleric acid. A number of Phytoconstituents are reported like D-mannitol, 1-keto-3αhydroxy Oleanane, saponins, scopoletin, iridiodal and glycosides, such as garenoside, randioside and geniposide. Also, steroids and fatty acid such as caprlic acid, capric acid, tauric acid, myristic acid has been reported in literature[5,6]. The main aim of the current study is to to explore presence of phytochemical and secondary metabolites profile analysis of *C. spinosa* leaves and investigate the anti-inflammatory, analgesic and antipyretic activity of the plant.

METHODOLOGY

Collection of plant material and authentication

The leaves of the *C. spinosa* were collected and dried. The dried material was powdered in the coarse powder by mechanical grinder. The resulting coarse powder was used for the studies. The leaves of the plant were authenticated by the Dr. Dilip Gena, Botanist, Pteridophyte biology lab Department of Botany, Government College, Ajmer.

Extraction procedure for collected plant material

The extract of leaves of *Catunaregum spinosa* was obtained different types of solvents like, Methanol, Ethanol, Acetone, Petroleum ether, Benzene and Distilled water. 10 gm of powdered leaves of *C. spinosa* were subjected Soxhlet extraction and maceration method using different solvents[7].The macerated extract was kept on the shaker for 24 hours, later it was centrifuged and the supernatant was taken for the excess solvent evaporation in laboratory conditions. After evaporation of excess solvent the crude extract was stored in refrigerator till further analysis.

Phytochemical analysis of *Catunaregam spinosa*

Phytochemical tests for the identification of amino acids, carbohydrates, saponins, tannins, phytosterols, alkaloids, proteins, glycosides, flavanoids and phenolic compounds were carried out for all the extracts. The present investigation was planned with an objective to establish Pharmacognostic standards and to evaluate preliminary phytochemical data that can facilitate the authentication and the isolation of the desired constituent from the correct extract[8].

Characterization of extract using Fourier Transform Infrared Spectrophotometer

FTIR is the most powerful and applicable tool for identification of chemical bonds (functional groups), and their types present in sample. The wavelength which absorbed light in different variation is characteristic and predictable information for the chemical bond which can be seen in the annotated spectrum of FITR. By interpreting the infrared absorption spectrum, the chemical bonds present in molecule can be determined. Dried powder of plant sample for different solvents extracts were used in FTIR analysis with 100 mg KBr pellet as encapsulate in sample discs. The powdered plant part sample was loaded in FTIR spectroscope (Shimadzu, IR Affinity 1, Japan), with the Scanning range from 400 to 4000 cm^{-1} with a 4 cm^{-1}resolution[9,10].

Anti-inflammatory activity

Carrageenan induced rat paw edema

The anti-inflammatory activity of ethanolic extract of *C. spinosa* was determined by carrageenan induced inflammation test in hind paw of rats. Rats were fasted for 24 h before the commencement of experiment. Edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan in saline. Rats were divided into different treatment groups (n = 5) viz., group I (vehicle, 10 mL/kg), group II (indomethacin, 10 mg/kg), group III (Ethanolic extract, 100 mg/kg), group IV (Ethanolic extract, 200 mg/kg). The extracts and indomethacin (IM) were suspended in 1% CMC and administered orally by intragastric tube 1 h before the carrageenan injection. After the administration of the phlogistic agent, paw volume (in mm) of individual rat was measured at 1, 3 and 5 h[11].

Egg albumin induced rat paw edema

This method has been applied in the screening of antiinflammatory activity of African spices and herbs by some researchers. Egg albumin is reported to induce inflammation in the hind paw of rats and therefore, this model was employed to evaluate the anti-inflammatory activity of extracts. Acute inflammation was induced by injecting 0.1 ml/kg of fresh egg albumin into the plantar region of the hind paw of rats. Separate sets of rats (n = 5) were employed for control, standard and treatment groups as described in carrageenan induced inflammation experiment. The change in paw volume (mm) was measured up to 120 min, at 20 min intervals after egg albumin injection[12,13,14].

Analgesic activity

Analgesic activity was tested in mice (King Instt. Strain) weighing between 20-25 g with six number of animals in each group using Acetic acid induced writhing response and Hot plate response methods.

Acetic acid induced writhing response

Different groups of six mice each received orally normal distilled water (2 ml/kg) (i.e. control), Ethanolic extract of *C. spinosa* (25, 100 and 500 mg/kg) and Analgin (500 mg/kg)(i.e. standard). Thirty minutes later, 0.7% acetic acid (10 ml/kg) solution was injected intraperitoneally to all the animals in different groups. The stretching episodes of individual mice were counted for 30 min post-administration of acetic acid. Analgin in a dose of 500 mg/kg orally served as standard. The formula used for computing percent inhibition was: average writhes in the control group minus writhes in the drug group divided by writhes in the control group times 100. Compounds with less than 70% inhibition are considered to have minimal antinociceptive activity[15].

$$\% \text{ Inhibition} = [(Wc - Wt) \times 100] / Wc$$

Where, Wc = average number of writhes in control c group; Wt = average number of writhes in test t group

Hot-plate response in mice

Mice were retained on a hot plate having a stable temperature of 55 ± 1°C. The time taken for either paw licking or jumping was recorded. Each mouse was individually placed on the hot plate in order to find the animal's reaction to electrical heat-induced pain (licking of the forepaws and eventually jumping). The latency until mice showed first signs of discomfort (hind paw lifting, hind paw licking, or jumping) was recorded, before (baseline), and response was determined at 30, 60, 90,120, 150 and 180 min after the administration of *C. spinosa* (25, 100, and 500 mg/kg), and analgin (500 mg/kg) [16].

Anti-pyretic activity

The rats were made pyretic by a sub-cutaneous injection of 20% suspension of dried Brewer's yeast in normal saline in a dose of 1 ml/l00 g. The rectal temperature was recorded by introducing a clinical thermometer about 2 cm deep into the rectum for the duration of one min before and 4 h after yeast injection. Animals showing pyrexia were selected and the drugs were administered. The rectal temperature was recorded at hourly intervals for 5 h. Animals that indicated a rise in body temperature of at least 0.6 °C were considered eligible for carrying out further experiments.

RESULTS AND DISCUSSION

Phytochemical analysis of *Catunaregam spinosa*

The results of phytochemical analysis of leaves of *C. spinosa* are tabulated below in Table.1. The ethanolic extract was selected for further investigations based on the results obtained phytochemical analysis.

Table.1: Phytochemical analysis of different extracts of *Catunaregum spinosa* leaves

S. No.	Name of the constituent	(P)	(B)	(C)	(A)	(E)	(M)	(Aq)
1	Alkaloids	—	—	—	—	—	—	—
2	Glycosides	—	—	—	+	+	+	—
3	Carbohydrates	—	—	—	+	+	+	+
4	Phytosterols/ Triterpenoids	+	+	+	+	+	+	—
5	Proteins & Amino acids	—	—	—	—	—	—	+
6	Saponins	—	—	—	—	+	+	+
7	Flavonoids	—	—	—	+	+	+	—
8	Fixed oils & Fats	+	—	—	—	—	—	—
9	Gums/Mucilage	—	—	—	—	—	—	—
10	Volatile oil	+	—	—	—	—	—	—
11	Phenolics/Tannins	—	—	—	+	+	+	+

(P):Petroleum ether (60-80ºC); (B):Benzene; (C): Chloroform; (A): Acetone; *(M)*: Methanol; (E): Ethanol; (Aq): Aqueous; +: Positive; -: Negative

FTIR Spectral Analysis

The methanolic extract of *C. spinosa* leaves showed typical absorption bands at 3295 cm^{-1} for a hydroxyl (-OH) group 2829 cm^{-1}, 2413 cm^{-1} (for C-H stretching), 1386 cm^{-1} (for C-H bending), and at 1620 cm^{-1} for C=C group. The ethanolic extract illustrated the observation of absorption bands at 2919 cm^{-1} (for C-H stretching), 1433 cm^{-1} (for C-H bending) for C-H group and at 1709 cm^{-1}, for a carbonyl group (C=O).

Table.2: FTIR spectral peak values and functional groups obtained for the leaf extract

Extracts	Peak values	Functional groups
Methanol	1386	C-H bending
	1620	C=C group
	2413	C-H stretching
	2829	C-H stretching
	3295	-OH group
Ethanol	1433	C-H bending
	1614	C=C group
	1709	C=O carbonyl group
	2919	C-H stretching
	3301	-OH group

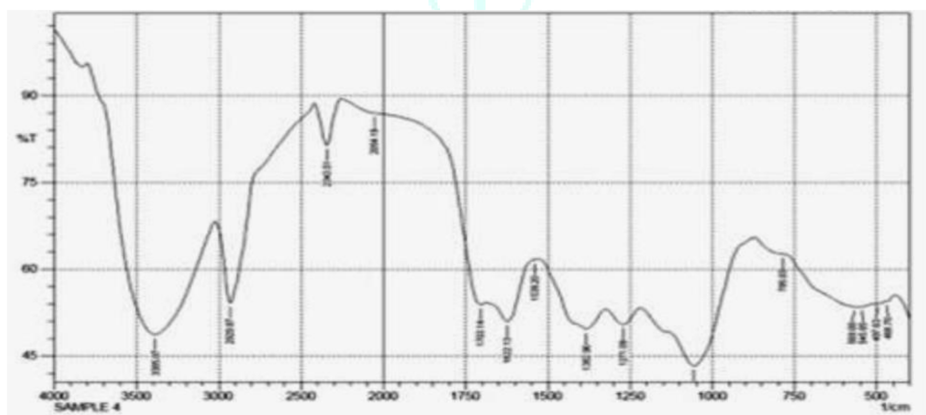

Figure.1: FTIR of *C. spinosa* Leaves in Methanol

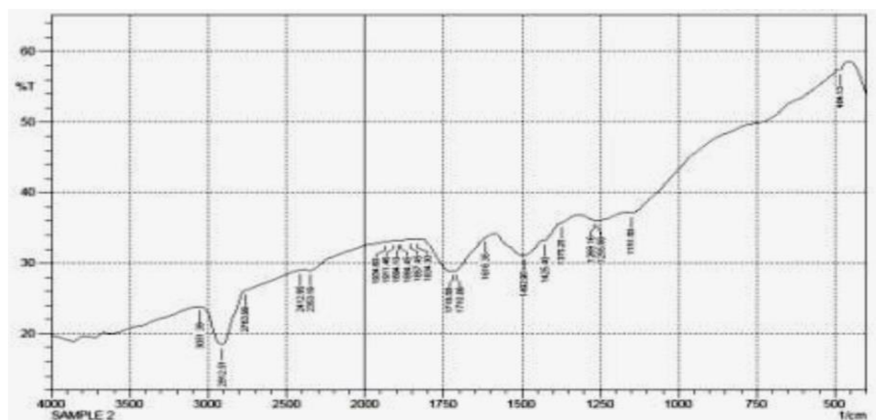

Figure.2: FTIR of *C. spinosa* Leaves in Ethanol

Anti-inflammatory activity

Anti-inflammatory activity against carrageenan- induced inflammation

Ethanolic exract of *C.spinosa* at the dose of 100 mg/kg inhibited only the initial phase of inflammation, while initial as well as lateral phases of inflammation were inhibited at the dose of 200 mg/kg with percent inhibition of inflammation 31.69, 35.11 and 41.62 %, respectively, at 1, 3 and 5 h. Standard drug, indomethacin attenuated the inflammation at 10 mg/kg with 18.97, 57.72 and 43.53% inhibition, respectively, at 1, 3 and 5 h (Table.3). These results were found to be dose dependent.

Anti-inflammatory activity against egg albumin- induced inflammation

To substantiate the anti-inflammatory activity of the extract, the efficacy was also studied against egg albumin-induced inflammation (Table.4). The ethanolic extract (100 mg/kg, *p.o.*) failed to exhibit anti-inflammatory effect ($p > 0.05$) at 20 and 40 min time interval (initial phase) but showed significant inhibition of inflammation at 60, 80, 100 and 120 min (lateral phase) after egg albumin administration as compared to control. On the other hand, the extract at higher dose (200 mg/kg, *p.o.*) significantly inhibited ($p < 0.05$) egg albumin-induced inflammation at all time interval (initial as well as lateral phase).

Table.3: Anti-inflammatory activity of Ethanolic extract of *C. spinosa* in carrageenan-induced inflammation model

Treatment	Dose (mg/kg)	Edema inhibition against carrageenan-induced inflammation (h)		
Measurement time interval →		1	3	5
Control		6.23 ± 0.13	7.45 ± 0.14	6.50 ± 0.19
Ethanolic extract	100	5.10 ± 0.21 [17.30]#	7.11 ± 0.15 [4.45]ns	6.33 ± 0.10 [3.35]ns
	200	4.26 ± 0.25 [32.06]#	4.83 ± 0.18 [37.28]#	1.79 ± 0.11 [43.16]#
Indomethacin	10	5.05 ± 0.17 [18.97] #	3.15 ± 0.15 [57.72]	3.67 ± 1.02 [43.53] #

Table.4: Anti-inflammatory activity of Ethanolic extract in egg- albumin induced inflammation model

Treatment	Dose (mg/kg)	Edema inhibition against egg albumin-induced inflammation (min)					
Measurement time interval →		20	40	60	80	100	120
Control		5.45 ± 0.12	5.96 ± 0.23	6.62 ± 0.17	7.67 ± 0.21	7.30 ± 0.33	7.14 ± 0.19
Ethanolic extract	100	5.28 ± 0.23 [3.11]ns	5.76 ± 0.26 [3.45] ns	6.31 ± 032 [4.60]*	5.27 ± 0.34 [31.30]#	4.86 ± 0.23 [33.29] #	5 32 ± 0.22 [25.59] #
	200	4.16 ± 0.15 [21.44] #	4.69 ± 0.16 [20.94] #	5.41 ± 0.16 [18.12] #	5.43 ± 0.27 [30.02] #	4.80 ± 0.21 [33.94] #	3.69 ± 0.11 [47.81] #
IM	100	4.99 ± 0.13 [8.41] #	5.27 ± 0.12 [11.58] #	5.53 ± 0.23 [16.43] #	5.76 ± 0.16 [24.96] #	3.93 ± 0.18 [46.15] #	3.25 ± 0.14 [54.48] #

Values represent the mean±SEM. of five animals for each group; Values in square brackets indicatethe percentage inhibition rate of inflammation. *p < 0.01 and #p < 0.001 indicate different levels of statistically significant values against control and ns stands for non significant. % Inhibition calculated using formula: paw edema volume control-sample/control.

Analgesic activity

The compound did not show significant analgesia on stretching episodes induced by acetic acid in any dose (Table.5), whereas, it showed significant effects in the doses of 25 and 100 mg/kg on hot plate test. The analgesia produced by the former doses was for a brief period (between 60 to 90 min) whereas, the effect produced by 100 mg/ kg was more prolonged and with an earlier onset comparable to the effect produced by Analgin in a dose of 500 mg/kg (Table.6).

Table.5: Effect of extract of *C. spinosa* on acetic acid induced stretching episodes in mice

(Values are Mean ± S.E., Number of observations in parenthesis)

Groups	Dose in mg/kg oral	Stretching episodes for 30 min	% Inhibitory activity	P-value
Control (6)	Distilled water	36.33 ± 5.77	---	---
Analgin (6)	500	3.16 ± 1.19	91.31	<0,001
Extract (6)	25	40.16 ± 8.19	---	ns
Extract (6)	100	46.16 ± 9.33	---	ns
Extract (6)	200	26.00 ± 4.46	28.44	Ns

Table.6: Analgestic Acttvity of extract of *C. spinosa* on Hot plate in Mice

(Figures are Mean ± S. E., Number of observations in parenthesis)

Groups	Dose in mg/kg oral	Initial response time (sec)	Response time in seconds after					
			30 min	60 min	90 min	120 min	150 min	180 min
Analgin (6)	500	5.05 ± 0.48	7.41 ± 1.21ns	14.46 ± 1.83 p<0.001	18.55 ± 1.24 p<0.001	19.14 ± 1.09 p<0.001	14.63 ± 1.87 p<0.001	7.55 ± 0.88 p<0.05
Extract (6)	25	5.01 ± 0.68	6.51 ± 0.45ns	8.80 ± 1.20 p<0.02	7.18 ± 0.29 p<0.02	6.86 ± 1.07ns	5.96 ± 0.74ns	6.51 ± 0.64ns
Extract (6)	100	5.11 ± 0.32	7.20 ± 0.53 p<0.001	7.38 ± 0.96 p<0.01	7.10 ± 1.06 p<0.01	6.75 ± 0.86 p<0.01	5.41 ± 0.91ns	6.20 ± 0.94 p<0.05
Extract (6)	200	5.21 ± 0.65	7.63 ± 0.98ns	6.11 ± 1.11ns	7.75 ± 1.06ns	6.48 ± 0.73ns	6.35 ± 0.41ns	5.43 ± 0.55ns

ns stands for non significant.

Antipyretic activity

There was no 'antipyretic effect observed in this model of experiment in any of the doses employed (Table.7).

Table.7: Effect of extract of *C. spinosa* On Brewer's Yeast Induced Pyrexia in Rats

(Values are Mean ± S. E., Number of animals in parenthesis)

Groups	Body Weight (in g)	Fasting rectal temp. (ºF)	Initial Pyrexia	Mean rectal temperature in ºF at				
				1st h	2nd h	3rd h	4th h	5th h
Control(6)	131 ± 6.57	99.45 ± 0.05	102.75 ± 0.29	102.95 ± 0.13	103.10 ± 0.17	103.70 ± 0.33	103.85 ± 0.20	103.26 ± 0.27
Paracetamol 500 mg/kg(6)	163.75 ± 8.50	99.30 ± 0.15	102.35 ± 0.24	100.85* ± 0.11	99.70* ± 0.21	100.20# ± 0.63	101.25# ± 0.25	101.80 ± 0.21ns
Extract 25 mg/kg (6)	148.75 ± 13.90	99.30 ± 0.23	102.80 ± 0.25	102.50 ± 3.28ns	102.00 ± 3.28ns	102.85 ± 0.26ns	103.85 ± 0.65ns	102.25 ± 0.41ns
Extract 100 mg/kg (6)	111.25 ± 9.43	99.05 ± 0.22	102.60 ± 0.29	103.00 ± 0.25ns	103.40 ± 0.14ns	102.00 ± 0.80ns	103.05 ± 0.17ns	103.60 ± 0.42ns
Extract 200 mg/kg(6)	125.00 ± 8.89	98.90 ± 0.17	101.85 ± 0.62	102.50 ± 0.97ns	102.20 ± 0.39ns	101.95 ± 1.01ns	102.50 ± 0.4ns	101.10 ± 2.03ns

CONCLUSION

The multifunctional properties of *C. spinosa* exposed in previous studies about its antioxidant and anticancer activities might be useful in explaining ethnopharmacological claim of its therapeutic potential in inflammation, pain and fever, showing no signs of oral toxicity.

The extract of *C. spinosa* showed significant anti-inflammatory effect both in exudative and proliferative phases of inflammation. In carrageenin induced edema, the effect produced by the dose of 100 mg/kg was little lesser than Indomethacin in the dose of 10 mg/kg with further higher increase in anti-inflammatory activity with higher dose of 200 mg/kg. In case of egg-albumin induced inflammation, dose of 200 mg/kg showed anti-inflammatory activity which was comparable to Indomethacin (10mg/kg).

The compound did not show significant analgesia in chemical writhing test whereas, on hot-plate, significant effect was observed in the doses of 25 and 100 mg/kg. The analgesia produced by the former doses was for a brief period (between 60 to 90 min) whereas, the effect produced by 100 mg/ kg was more prolonged and with an earlier onset comparable to the effect produced by Analgin in a dose of 500 mg/kg.

The compound is devoid of antipyretic effect in this model of experiment. The maximum anti-inflammatory and analgesic activities are almost around the dose of 200 mg/ kg which certainly may make it interesting to study further in detail therefore it could be clinically tried.

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