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Research Article

ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF *CLAUSENA DENTATA* (WILLD.) ROEM

RAJU KAMARAJ[1], ANNAMALAI MADURAM[2]*

[1]Department of Pharmacognosy, SRM College of Pharmacy, SRM University, Chennai, Tamil Nadu, India. [2]Department of Pharmacology, Sri Sathya Sai Medical College & Research Institute, Kancheepuram, Tamil Nadu, India. Email: maduramraj@gmail.com

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ABSTRACT

Objectives: The present study was undertaken to study the analgesic and anti-inflammatory activity of various extracts of *Clausena dentata*. *Clausena* (Rutaceae) is a genus of about 23 species of unarmed trees and shrubs. The stem bark of *C. dentata* is used in veterinary medicine for the treatment of wounds and sprains. Even though *C. dentata* has a lot of potential medical uses, the study of pharmacological properties is very scarce.

Methods: The plant *C. dentata* was collected in May 1999 from Kadagaman, near Tiruvannamalai, Tamil Nadu, India, and authenticated by Centre for Advanced Study in Botany, University of Madras, Chennai. The dry powder of stem bark was extracted with hexane, chloroform, and methanol. The extracts were subjected to thin-layer chromatography and phytochemical screening. Further, the extracts were tested for analgesic by the tail flick and anti-inflammatory by carrageenan-induced rat paw edema using plethysmometer.

Results: Qualitative chemical tests revealed the presence of various phytochemicals such as alkaloids, glycosides, carbohydrate, proteins and amino acids, phytosterols, and volatile oil. The hexane, chloroform, and methanol extracts exhibited significant analgesic activity as compared to control group (Tween 80, 1%). The anti-inflammatory activity of *C. dentata* extracts revealed that the percent inhibition of carrageenan-induced rat hind paw edema was highly significant as compared to control group after 4 h of injection.

Conclusion: The presence of coumarin and alkaloids all the extracts promising properties of analgesic and anti-inflammatory.

Keywords: *Clausena dentata*, Coumarin, Extracts, Analgesic, Anti-inflammatory, Tail flick, Carrageenan, Plethysmometer.

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INTRODUCTION

The family *Rutaceae* consists of about 140 genera and 1300 species. These plants are aromatic trees, shrubs, and a few herbs and are distributed throughout the warm and temperate regions of the world, being most abundant in South Africa and Australia. The aroma of the plant is due to the universal occurrence of lysigenous oil cavities in the leaves and other young organs. A number of plants of Rutaceae are of medicinal value and furnish several drugs and pharmaceutical products.

Casino (Rutaceae) is a genus of about 23 species of unarmed trees and shrubs mainly grow in the Indo-Malayan with a few in China, Africa, and Australia. 10 species are known to grow in India, of which five are of economic importance. The stem bark of *Clausena dentata* is used in veterinary medicine for the treatment of wounds and sprains [1]. The dried powdered rootstock is also used by the Kols, the tribes in Chotanagpur region, India, for decayed teeth. In Cambodia, the stem is considered a bitter tonic and astringent [2]. The infusion is given for colic pain with diarrhea. *C. dentata* is used for digestion and as diuretic. Even though *C. dentata* has a lot of potential medical uses, the study of pharmacological properties is very scarce [3,4]. Plants and plant-derived products are practiced from ancient times in various traditional and folk medicines to cure such pathological conditions. Nevertheless, proper justification with a scientific background is continually being researched for their medicinal use [5]. Considering the importance of the plant, the present study was undertaken with the following objective: To carry out the analgesic, antipyretic, and anti-inflammatory activities of various extracts of *C. dentata*.

METHODS

Plant collection

The plant *C. dentata* (Willd.) Roem. was collected in May 1999 from Kadagaman, near Tiruvannamalai, Tamil Nadu, India, and authenticated by the Department of Pharmacognosy, Sri Ramachandra College of Pharmacy, Porur, Chennai. Subsequently, the identification was confirmed as *C. dentata* at Centre for Advanced Study in Botany, University of Madras, Chennai. A voucher specimen of the plant has been deposited at the herbarium. The collected plant material was free from disease and also free of contamination of other plants.

Preparation of extracts

The dry powder of stem bark (2.5 kg) was first soaked, at room temperature, in hexane (1:4 w/v) for 24 h. The extract was suction filtered using Whatman filter paper. This was repeated for two more days and similar extracts were pooled together and concentrated at 40°C under reduced pressure using Buchi R-153 Rotavapor [6,7]. The residual plant material was extracted successively with chloroform and methanol in the same manner as follows for hexane.

Thin-layer chromatography (TLC)

Pre-coated silica gel thin-layer chromatogram sheet (E.Merck) was used for TLC. The crude extracts at 2 cm from the edge of the sheet. The chromatogram was developed with a mixture of suitable solvent system and dried at room temperature. The spots were visualized with ultraviolet light at 254 and 346 nm. The dried TLC plates were then sprayed with 10% H_2SO_4 and heated at 110°C for 5 min [8]. Alternatively, the developed TLC plates were placed in iodine chamber. The R_f values of the colored spots were recorded.

Qualitative phytochemical screening

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. The extracts were subjected to test for alkaloids, glycosides, carbohydrate, proteins and amino acids, phytosterols, fixed oils and fats, gums and mucilages, and volatile oil.

Analgesic activity of various extracts of *C. dentata*

The hexane, chloroform, and methanol extracts of *C. dentata* were screened for analgesic activity by tail-flick method. Requirements: Analgesiometer (Techno), animal-albino mice either sex weighing (25–30 g) standard drug - Paracetamol [9] (100 mg/kg body weight). The study was approved by the Institutional Animal Ethics Committee.

Procedure

The tail-flick method was followed in the evaluation of analgesic activity. Albino mice of either sex weighing between 25 and 30 g were randomly distributed into 13 groups consisting of six animals in each group. The basal reaction time was noted in all groups by placing the tip of the tail over the electric wire of analgesiometer. The withdrawal of the tail from heat (flicking response) was taken as the basal reaction. The first group served as control and the animals were administered orally with vehicle (Tween 80, 1%). The 2nd, 3rd, and 4th groups of mice were administered orally, 25, 50, and 100 mg/kg body weight of paracetamol, respectively. The animals of the 5th, 6th, and 7th groups were treated with hexane extract (50,100, and 150 mg/kg body weight) orally. The animals of the 8th, 9th, and 10th groups were treated with the chloroform extract (50,100, and 150 mg/kg body weight) orally. The animals of the 11th, 12th, and 13th groups were treated with methanol extracts orally, 50,100, and 150 mg/kg body weight, respectively. The reaction time was noted at 15, 30, 45, and 60 min of time intervals after the drug administration, percent protection against tail flicking was calculated, allowing maximum tolerability time 10 s was considered as 100%.

Anti-inflammatory activity of various extracts of *C. dentata*

The hexane, chloroform, and methanol extracts of *C. dentata* were screened for anti-inflammatory activity by carrageenan-induced rate paw edema method. The requirements were plethysmometer and female albino rats weighing 180–200 g. Standard drug was diclofenac sodium [10] (5, 10, and 20 mg/kg body weight).

Procedure

Carrageenan-induced rate paw edema method

The animals were housed under standard environmental conditions and were fed with standard diet and water. Anti-inflammatory activity was evaluated by carrageenan-induced rat paw edema method. Albino rats of either sex weighing between (180 and 200 g) were divided into 17 groups of six animals each. The first group served as the control and received vehicle (Tween 80, 1%), the 2nd, 3rd, and 4th groups of animals were administered orally with standard drug diclofenac sodium (5, 10, and 20 mg/kg body weight) [11]. The animals of the 5th, 6th, 7th, and 8 groups were treated with hexane extract (50, 100, 150, and 200 mg/kg body weight). The animals of 9th, 10th, 11th, 12th, and 13th groups were treated with chloroform extract (50, 100, 150, 200, and 250 mg/kg body weight). The animal of the 14, 15, 16, and 17 groups was treated with methanol extract (50, 100, 150, and 200 mg/kg body weight) of *C. dentata*, orally.

A mark was made on both the hind paws just below the tibiotarsal junction in the mercury column of plethysmograph up to the mark to ensure constant paw volume. After 30 min, an inflammatory edema was induced in the left hind paw by injection of 0.1 Ml of 1% carrageenan in normal saline in the plant tissue of the paw of all the animals. The right paw served as reference to non-inflamed paw for comparison. The initial paw volume was measured plethysmographycally within 30 s of the injection. The relative increase in the paw volume was measured in control, standard, and treated groups, 4 h after carrageenan injection the percent increase [12,13] in the paw volume in animals treated with standard drug and the three extracts of *C. dentata* were compared with the increase in paw volume of untreated control animals.

Thus, percent inhibition of paw volume in treated animals was used for calculating the percent inhibition of edema of the control group using the formula.

% inhibition = C-S) ×100/C

Where

C = mean relative changes in the paw volume of the test.

S = mean relative changes in the paw volume of the control.

RESULTS

TLC

The TLC profile of hexane, chloroform, and methanol extracts of *C. dentata* reveals that the presence of coumarin and alkaloids.

Qualitative phytochemical screening

Qualitative chemical tests revealed the presence of various phytochemicals in hexane, chloroform, and methanol extracts of *C. dentata* (Table 1). The methanol extract showed positive test for alkaloids. All the extracts contained carbohydrates, glycosides, amino acids, proteins, and volatile oils. Ferric chloride test showed the presence of phenolic compounds, in all the extracts. Saponins, phytosteroids, fixed oils, and fats were absent.

Analgesic activity of various extracts of *C. dentata*

The results of analgesic activity of crude extracts of *C. dentata* (Table 2) revealed that hexane, chloroform, and methanol extracts exhibited significant analgesic activity as compared to control group (Tween 80, 1%). Percent protection against tail flicking was calculated, allowing maximum tolerability time 10 s was considered as 100%.

Table 1: Qualitative phytochemical screening of various extracts of *C. dentata*

Phytochemical test	Hexane	Chloroform	Methanol
Alkaloids			
Mayer's reagent	-	-	+
Wagner's reagent	-	-	+
Hager's reagent	-	-	+
Dragendorff's reagent	-	-	+
Carbohydrates and glycosides			
Molisch's test	+	+	+
Fehling's test	+	+	+
Barfoed's test	+	+	+
Benedict's test	+	+	+
Borntrager's test	+	+	+
Legal's test	+	+	+
Saponins			
Foam test	-	-	-
Proteins and amino acids			
Millon's reagent	+	+	+
Biuret reagent	+	+	+
Ninhydrin reagent	+	+	+
Phytosteroids			
Liebermann–Burchard's test	-	-	-
Fixed oils and fats			
Spot test	-	-	-
Saponification test	-	-	-
Phenolic compounds and flavonoids			
Ferric chloride test			
Gelatin test	+	+	+
Lead acetate test	+	-	-
Alkaline reagent	+	-	-
Magnesium and hydrochloric acid	+	-	-
Reduction	+	-	-
Gums and mucilages	+	_	-
alcohol 95% test			
Volatile oils			
Steam distillation	+	+	+

-: Negative, +: Positive, *C. dentate: Clausena dentata*

Table 2: Analgesic activity of various extracts of *C. dentata*

Extract of *C. dentata*/drug	Dose mg/kg	Mean time (in sec.) ± SD, 95% confidence interval mean (lower-upper)				(ED_{50}) mg/kg	% Protection
		15 min	30 min	45 min	60 min		
Control (Tween 80, 1%)	-	3.500±0.089	3.5±0.089	3.400±0.089	3.400±0.089		-
Paracetamol	25	3.5*±0.0816	3.8*±0.103	4.21±0.116	4.60±0.089	57.5	46
	50	4.91±0.2483 (1.67-1.15)	5.56±0.273 (2.35-1.78)	6.08±0.43 (3.13-2.22)	7.2±0.260 (4.07-3.52)		72
	100	6.150±0.105 (2.77-2.52)	6.150±0.105 (2.77-2.52)	8.200±0.089 (4.91-4.68)	9.2±0.089 (5.91-5.68)		92
Hexane extract	50	3.5*±0.116	3.8*±0.116	4.01±0.147	4.16±0.103	97.5	41.6
	100	4.05±0.1516 (0.715-5.1)	4.35±0.137 (1.00-0.697)	4.63±0.121 (1.371-1.094)	5.11±0.147 (1.87-1.55)		51
	150	5.1±0.0894 (1.415-1.184)	5.4±0.0894 (1.67-1.42)	7.0±0.0894 (3.5-3.28)	7.6±0.0894 (3.81-3.58)		76
Chloroform extract	50	3.50*±0.081	3.50*±0.051	3.51*±0.041	3.71*±0.075	112.5	37.1
	100	3.73±0.051 (0.330-0.136)	3.93±0.103 (1.44-1.050)	4.65±0.187 (0.944-0.622)	4.18±0.147 (557-0.308)		41.8
	150	4.50±0.089 (−1.71-1.1484)	4.8±0.089 (−2.01-1.78)	5.1±0.089 (−3.71-3.48)	6.7±0.089 (4.31-4.08)		67
Methanol extract	50	3.51*±0.1169	3.70*±0.089	3.80*±0.075	3.88*±0.075	105	38
	100	3.93±0.051 (0.530-0.336	4.11±0.75 (0.723-0.509)	4.40±0.089 (1.115-0.884)	4.71±0.213 (1.542-1.090)		47
	150	4.80±0.089 (1.415-1.184)	5.05±0.1048 (1.67-1.42)	6.80±0.089 (3.50-3.28)	7.10±0.089 (3.81-3.58)		71

Values are expressed as mean±SD. (n=6) p<0.01 compared to control (Tween 80, 1%), (unpaired t-test). *No significant. *C. dentate: Clausena dentate*

Table 3: Anti-inflammatory activity of various extracts of *C. dentata*

Extract of *C. dentata*/ drug	Dose mg/kg	Paw value mean±SD 1 h	t	95% confidence internal mean		Paw volume mean±SD 4 h	t	95% confidence interval mean		%	ED_{50} mg/kg body wt.
				Lower	Upper			Lower	Upper		
Control (Tween	-	0.169±0.0015	10.68	0.045	0.029	0.170±0.0011	444.28	0.031	0.034		
Diclofenac sodium	5	0.206±0.0083	10.68	0.046	0.028	0.137±0.0013	444.28	0.031	0.034	19	13.2
	10	0.022±0.0010	-	0.054	0.051	0.104±0.0008	112.77	0.064	0.067	38	
	20	0.231±0.0015	93.10	0.064	0.060	0.045±0.0001	256.86	0.123	0.126	73.5	
Hexane extract	50	0.182±0.0005	18.59	0.014	0.011	0.138±0.0010	49.50	0.030	0.033	18	137.5
	100	0.182±0.0015	7.66	0.023	0.012	0.107±0.0001	129.66	0.062	0.064	37	
	150	0.181±0.0054	7.42	0.023	0.011	0.073±0.0018	107.66	0.095	0.091	55.2	
	200	0.180±0.0040	7.82	0.018	0.0099	0.044±0.0002	255.35	0.124	0.126	73	
Chloroform extract	50	0.149±0.0045	10.47	0.015	0.025	0.140±0.0001	61.00	0.028	0.031	14	245
	100	0.152±0.0038	10.22	0.013	0.021	0.122±0.0018	53.05	0.045	0.050	29	
	150	0.148±0.0036	13.26	0.017	0.024	0.105±0.0001	133.54	0.063	0.066	38.2	
	200	0.145±0.0023	21.45	0.022	0.027	0.097±0.0010	113.10	0.071	0.074	43	
	250	0.143±0.0021	20.50	0.021	0.025	0.081±0.0011	110.10	0.069	0.073	52	
Methanol extract	50	0.169±0.0035	0.05	0.003	0.003	0.137±0.0004	57.41	0.028	0.030	17	145
	100	0.170±0.0011	0.63	0.004	0.002	0.109±0.0007	108.90	0.059	0.062	35	
	150	0.169±0.0116	0.65	0.004	0.002	0.080±0.0089	111.91	0.052	0.061	52.9	
	200	0.167±0.0112	0.65	0.004	0.002	0.049±0.0082	112.93	0.053	0.062	71	

Values are expressed as mean±SD. (n=6) p<0.001 compared to control (Tween 80%, 1%), (Unpaired t-test), SD: Standard deviation

Standard drug paracetamol showed maximum protection (92%) at 100 mg/kg body weight with ED_{50} value of 57.5 mg/kg body weight. The hexane, chloroform, and methanol extracts 76%, 67%, and 71% of protection, respectively. The ED_{50} values were 97.5, 112.5, and 105 mg/kg body weight for hexane, chloroform, and methanol extracts, respectively.

Anti-inflammatory activity of various extracts of *C. dentata*

Medicinal plants have been used in folk medicine for the treatment of many inflammatory diseases since ages with lesser side effects. Plants contain many useful constituents that might provide a direction for the development of novel drugs [14]. Carrageenan-induced rat hind paw edema assay (an *in vivo* model) was carried out for the study of mediators found in developing edema associated with inflammation. The extract used for experimental study with its anti-inflammatory property was able to prevent the inflammation. The result of anti-inflammatory activity of *C. dentata* extracts (Table 3) revealed that the percent inhibition of carrageenan-induced rat hind paw edema was highly significant as compared to control group after 4 h of injection. The percentage inhibition of edema was high in animals treated with standard drug diclofenac sodium (20 mg/kg body weight), (73.5%). The ED_{50} value was found as 13.2 mg/kg body weight. Among the *C. dentata* extracts, hexane extract showed maximum percent of 73% inhibition at (200 mg/kg body weight). The ED_{50} value was 135.5 mg/kg body weight. The methanol extract showed 71% inhibition (200 mg/kg body weight). The ED_{50} value was found to be 145 mg/kg body weight. The chloroform extract showed only 51.1% inhibition (250 mg/kg body weight) (For 200 mg of chloroform extract only 43% of inhibition was obtained; hence, 250 mg was used). The ED_{50} value was found to be 245 mg/kg body weight.

DICUSSION

Currently, available analgesic drugs such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases due to

their adverse effects. In this respect, new compounds with improved pain management capacity and fewer side effects are being sought with urgency [15]. Analgesic activity of hexane, chloroform, and methanol extracts of *C. dentata* was carried out by tail-flick method. All the three extracts showed significant analgesic effect compared to the control group. However, the tail flicking time was less, as compared to standard drug paracetamol. The analgesic activity of *C. dentata* extracts may be due to the presence coumarin as the active molecules. The analgesic activity of coumarin has been established by O' Kennedy and Thrones [16]. The presence of coumarins, 3-(1,1-dimethylallyl) xanthyletin, in *C. dentata* may be responsible for the anti-inflammatory activity [17].

The ED_{50} value was found to be 57.5, 97.5, 105, and 112.5 mg/kg body weight for paracetamol, hexane, chloroform, and methanol extracts of *C. dentata*, respectively.

Inflammation has different phases; the first phase is caused by an increase in vascular permeability, the second one by the infiltrate of leukocytes, and the third one by granuloma formation. We determined anti-inflammatory activity using inhibition of carrageenan-induced inflammation, which is one of the most feasible methods to screen anti-inflammatory agents [18]. In rheumatoid arthritis, an autoimmune disease, several factors contribute to the deformity of joints; most important of these are the inflammatory destruction and remodeling of the articulating surfaces. The inflammation is a response of vascularized tissue of the body to injury involving the infiltration of cells, production of mediations, and release of hydrolytic enzymes. One of the causes of inflammation is the free radicals, which causes peroxidation of membrane lipids, aging, atherosclerosis, delayed wound healing, oxygen toxicity, liver disorders, etc. [19]. Various coumarins have been reported to possess anti-inflammatory activity as shown in carrageenan-induced inflammation and cotton pellet granuloma tests. Carrageenan stimulates the release of several inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins [20]. NSAID block the synthesis of prostaglandins by inhibiting cyclooxygenase (Cox). Cox and 5-lipoxygenase (5-LO) catalyze peroxidation of arachidonic acid and polyphenols such as coumarins and flavonoids might be expected to interfere with this process [21]. The presence of coumarins, 3-(1,1-dimethylallyl)xanthyletin, in *C. dentata* may be responsible for the anti-inflammatory activity [17]. The mechanism may be similar to that of NSAID. Hexane extract of *C. dentate* contained more amount of coumarin than other extracts, as evidenced by HPTLC studies, due to that it showed the maximum percentage of inhibition among the extracts. The ED_{50} value of hexane extract was 137.5 mg/kg body weight, followed by methanol extract 145 mg/kg body weight and chloroform extract 245 mg/kg body weight. Since the methanol extract contained alkaloids also, it may be responsible for potentiating the anti-inflammatory effect than the chloroform extract.

CONCLUSION

C. dentata (Willd). Roem. (Rutaceae) a plant of immense medicinal value was taken for detailed study for qualitative phytochemical, analgesic, and anti-inflammatory investigations. The dry powder of stem bark was extracted successively with three solvents, namely, hexane, chloroform, and methanol. The extracts were subjected to a qualitative phytochemical screening. All the extracts contained carbohydrates, glycosides, amino acids, proteins, and volatile oils. The extracts were tested for analgesic and anti-inflammatory activities. The carrageenan-induced inflammation was challenged by various extracts of *C. dentata*. Hexane extract showed maximum percentage of inhibition among the extracts. Analgesic activity was carried out by the tail-flick method. Hexane extract showed maximum percentage of protection. This may be due to high content of coumarins.

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AUTHORS CONTRIBUTIONS

1st author has contributed to conception and design, acquisition of data to this study. 2nd and corresponding author has contributed for analysis, interpretation of data, drafting the article and gave the final approval for the version to submit.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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