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 anoma of the plant is due to the universal occurrence of hygienic 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 India. 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 [2,3]. 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 [4]. 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 v/w) 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 366 nm. The dried TLC plates were then sprayed with 10% H2SO4 and heated at 110°C for 5 min [8]. Alternatively, the developed TLC plates were placed in iodine chamber. The Rf 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, carbohydrates, proteins and amino acids, phytosterols, fixed oils and fats, gums and mucilages, and volatile oil.
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 standard diet and water. Anti-inflammatory activity was evaluated by carrageenan-induced rate 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 the animals were administered orally with vehicle (TWEEN 80, 1%). The 2nd, 3rd, and 4th groups of mice were administered orally, 50, 100, and 150 mg/kg body weight of paracetamol, respectively. The animals of the 5th, 6th, and 7th groups were treated with methanol 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 tailflicking 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                           | -      | -          | +        |
| Wagner’s reagent                    | -      | -          | +        |
| Hager’s reagent                     | -      | -          | +        |
| Dragenorf’s reagent                 | -      | -          | +        |
| Carbohydrates and glycosides        | -      | -          | +        |
| Molsch’s test                       | +      | +          | +        |
| Fehling’s test                      | +      | +          | -        |
| Barfoed’s test                      | +      | +          | -        |
| Benedict’s test                     | +      | +          | -        |
| Borntrager’s test                   | +      | +          | -        |
| Legal’s test                        | +      | +          | -        |
| Saponins                            | +      | +          | -        |
| Foam test                           | -      | -          | -        |
| Proteins and amino acids            | -      | -          | -        |
| Milk’s reagent                      | +      | +          | +        |
| Biuret reagent                      | +      | +          | +        |
| Ninhydrin reagent                   | +      | +          | +        |
| Phytosteroids                       | +      | +          | +        |
| Liebermann’s test                   | -      | -          | -        |
| 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                    | +      | +          | +        |

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.

**Analytical 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 flick 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                           | -      | -          | +        |
| Wagner’s reagent                    | -      | -          | +        |
| Hager’s reagent                     | -      | -          | +        |
| Dragenorf’s reagent                 | -      | -          | +        |
| Carbohydrates and glycosides        | -      | -          | +        |
| Molsch’s test                       | +      | +          | +        |
| Fehling’s test                      | +      | +          | -        |
| Barfoed’s test                      | +      | +          | -        |
| Benedict’s test                     | +      | +          | -        |
| Borntrager’s test                   | +      | +          | -        |
| Legal’s test                        | +      | +          | -        |
| Saponins                            | +      | +          | -        |
| Foam test                           | -      | -          | -        |
| Proteins and amino acids            | -      | -          | -        |
| Milk’s reagent                      | +      | +          | +        |
| Biuret reagent                      | +      | +          | +        |
| Ninhydrin reagent                   | +      | +          | +        |
| Phytosteroids                       | +      | +          | +        |
| Liebermann’s test                   | -      | -          | -        |
| 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                    | +      | +          | +        |

- : Negative, +: Positive, *C. dentata: Clausena dentata*
The anti-inflammatory activity of various extracts of *C. dentata* was examined using the rat hind paw edema assay. The results are presented in Table 2. The hexane, chloroform, and methanol extracts showed significant anti-inflammatory activity with percentage inhibition values of 73%, 67%, and 71% at 200 mg/kg body weight, respectively. The standard drug paracetamol showed maximum inhibition (92%) at 100 mg/kg body weight.

### 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 (lower-upper) | (ED_{50}) mg/kg | % Protection |
|-----------------------------|------------|---------------------------------------------------------------|-----------------|--------------|
| Control (Tween 80, 1%)      | -          | 3.50±0.089                                                   | 3.40±0.089      | -            |
| Paracetamol                 | 25         | 3.5±0.081                                                   | 4.21±0.116      | 4.6±0.089    | 57.5 | 46 |
|                            | 50         | 4.91±0.2483                                                 | 6.08±0.43       | 7.2±0.260    | 72  |
|                            | 100        | 6.15±0.105                                                  | 8.20±0.089      | 9.2±0.089    | 92  |
| Hexane extract              | 50         | 3.5±0.116                                                   | 4.01±0.147      | 4.16±0.103   | 97.5 | 41.6 |
|                            | 100        | 4.05±0.1516                                                 | 4.63±0.121      | 5.11±0.147   | 51  |
| Chloroform extract          | 50         | 3.5±0.116                                                   | 4.01±0.147      | 4.16±0.103   | 97.5 | 41.6 |
|                            | 100        | 4.05±0.1516                                                 | 4.63±0.121      | 5.11±0.147   | 51  |
| Methanol extract            | 50         | 3.5±0.081                                                   | 4.01±0.147      | 4.16±0.103   | 97.5 | 41.6 |
|                            | 100        | 4.05±0.1516                                                 | 4.63±0.121      | 5.11±0.147   | 51  |

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

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

| Extract of *C. dentata*/ drug | Dose mg/kg | Mean volume mean±SD 4 h | Lower | Upper | % | ED_{50} mg/kg body wt. | Lower | Upper |
|-------------------------------|------------|-------------------------|-------|-------|---|------------------------|-------|-------|
| Control (Tween 80, 1%)        | -          | 0.16±0.0015              | 0.045 | 0.029 |   | 0.170±0.0011           | 0.031 | 0.034 |
| Diclofenac sodium             | 5          | 0.206±0.0083             | 0.046 | 0.028 |   | 0.137±0.0013           | 0.044 | 0.031 |
|                              | 10         | 0.022±0.0010             | 0.104±0.0008 | 0.112±0.007 | 0.64 | 0.123 | 0.036 |
|                              | 20         | 0.231±0.0015             | 0.064 | 0.060 |   | 0.045±0.0001           | 0.256 | 0.723 |
| Hexane extract                | 50         | 0.182±0.0005             | 0.014 | 0.011 |   | 0.138±0.0010           | 0.495 | 0.030 |
|                              | 100        | 0.182±0.0015             | 0.023 | 0.012 |   | 0.107±0.0007           | 0.129 | 0.062 |
|                              | 150        | 0.181±0.0054             | 0.023 | 0.011 |   | 0.073±0.0018           | 0.107 | 0.095 |
|                              | 200        | 0.180±0.0040             | 0.018 | 0.009 |   | 0.044±0.0002           | 0.255 | 0.124 |
| Chloroform extract            | 50         | 0.149±0.0045             | 0.015 | 0.025 |   | 0.140±0.0001           | 0.610 | 0.028 |
|                              | 100        | 0.152±0.0038             | 0.013 | 0.021 |   | 0.122±0.0018           | 0.535 | 0.045 |
|                              | 150        | 0.145±0.0036             | 0.013 | 0.021 |   | 0.105±0.0011           | 0.135 | 0.036 |
|                              | 200        | 0.145±0.0033             | 0.022 | 0.027 |   | 0.097±0.0010           | 0.113 | 0.071 |
|                              | 250        | 0.143±0.0021             | 0.024 | 0.025 |   | 0.081±0.0011           | 0.110 | 0.069 |
| Methanol extract              | 50         | 0.169±0.0035             | 0.003 | 0.003 |   | 0.137±0.0004           | 0.571 | 0.028 |
|                              | 100        | 0.170±0.0011             | 0.004 | 0.002 |   | 0.109±0.0007           | 0.108 | 0.059 |
|                              | 150        | 0.169±0.0016             | 0.004 | 0.002 |   | 0.080±0.0089           | 0.111 | 0.052 |
|                              | 200        | 0.167±0.0012             | 0.004 | 0.002 |   | 0.049±0.0082           | 0.112 | 0.053 |

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 showed 76%, 67%, and 71% 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 chloroform extract showed 71% inhibition (200 mg/kg body weight). The ED_{50} value was found to be 145 mg/kg body weight. The methanol extract showed only 51% inhibition (250 mg/kg body weight) (For 200 mg of chloroform extract only 43% of inhibition was observed; hence, 250 mg was used). The ED_{50} value was found to be 245 mg/kg body weight.

**DISCUSSION**

Currently, available analgesic drugs such as opiates and non-steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases due to their side effects.
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 ED₅₀ 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 mediators, 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 S-lipoxigenase (S-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. dentata 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 ED₅₀ 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 (Wild). 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.

ACKNOWLEDGMENT

The authors acknowledge the Prof. N. Raman, Centre for Advanced Studies in Botany, University of Madras, for continuous support throughout the project.

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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