Methanolic Extract of Leathery Murdah, *Terminalia coriacea* (Roxb.) Wight and Arn. Leaves Exhibits Anti-Inflammatory Activity in Acute and Chronic Models

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Significance of the Study

- Anti-inflammatory activity of methanolic extract of *Terminalia coriacea* was evaluated in acute and chronic inflammation models. The methanolic extract of *T. coriacea* leaves exhibited antitransudative and antiproliferative effects and also reduced edema induced by carrageenan. Thus, *T. coriacea* and its components could be potential agents for the treatment of inflammation.

Keywords

Anti-inflammatory activity · *Terminalia coriacea* · Carrageenan · Granuloma

Abstract

**Objective:** The aim of the present study was to evaluate anti-inflammatory activity of methanolic extract of *Terminalia coriacea*. **Materials and Methods:** A methanolic extract of *T. coriacea* leaves was studied in albino Wistar rats with carrageenan-induced paw edema, an acute model, and cotton pellet-induced granuloma, a chronic model, at 3 oral test doses (125, 250, and 500 mg/kg). Aspirin 100 mg/kg was used as a positive control. Paw volume and wet and dry weights of cotton pellets were determined. The data were analyzed by one-way ANOVA followed by Dunnett’s multiple comparison test. **Results:** The test extract at doses of 125 and 250 mg/kg decreased paw volume and wet and dry weights of cotton pellets. The highest test dose (500 mg/kg) displayed a response comparable to that of the standard drug (*p* < 0.01) on paw volume. The extract produced similar (*p* < 0.05) decrease in wet weight of the cotton pellet at 125 and 250 mg/kg, whereas the effect of 500 mg/kg of the extract was comparable to that of aspirin 100 mg/kg (*p* < 0.01). The extract of *T. coriacea* at 500 mg/kg induced the most significant (*p* < 0.01) effect on wet weight of granulomatous tissue. **Conclusion:** The methanolic extract of *T. coriacea* leaves successfully decreased paw edema as well as dry and wet weights of granulomatous tissue in both acute and chronic inflammatory models thus confirming the anti-edematogenic, antitransudative, and antiproliferative properties of *T. coriacea*.

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Introduction

Leathery Murdah, Terminalia coriacea (Roxb.) Wight and Arn. belonging to the Combretaceae family is found in dry and warm parts of Andhra Pradesh and Tamil Nadu in India. In traditional medicine, the plant is used as a cardiac stimulant and to treat atomic diarrhea, callous ulcers, and inflammatory conditions [1, 2]. Recent pharmacological investigations on the methanolic extract of T. coriacea leaves revealed antinociceptive [3], wound healing [4], anticonvulsant [5], anti-ulcer [6], and hepatoprotective potentials [7]. Phytochemical studies had revealed the presence of bioactive compounds such as flavonoids like apigenin, kaempferol, luteolin, myricetin, quercetin, rutin [6], 1H-inden-1-one, 2,3-dihydro-3,3,5,6-tetramethyl; levoglucosan; neophytadiene; phytol; hexadecanoic acid; n-hexadecanoic acid; stigmasterol; β-sitosterol [8]; betulic acid, arjunic acid, arjunicolic acid, arjunetin, ellagic acid [9], gallic acid, procyandin B1, B2, and B3, resveratrol, tyrosine [10]; raffinose; 1,2-benzene dicarboxylic acid; undecanoic acid; (2-propyl-1,3-dioxolan-2-yl)acetic acid, 2,2-dimethyl propane, and octadecatrienoic acid [11]. Based on the rich phytochemical nature and traditional use in inflammation, the present study was conducted to evaluate anti-inflammatory activity of methanolic extract of T. coriacea in acute and chronic inflammatory models.

Material and Methods

Plant Material and Extraction

(Fresh leaves of Leathery Murdah, T. coriacea (Roxb.) Wight and Arn. (synonyms – T. alata, T. crenilata, T. elliptica, and T. tomentosa) belonging to the Combretaceae family were collected from Talakonda forest, Tirumala Hills, Tirupati, Andhra Pradesh, India. The plant material was authenticated by a plant taxonomist, Dr. P. V. Prasanna of the Botanical Survey of India, Deccan Regional Centre, Hyderabad (Ministry of Environment and Forests, Government of India). The specimen deposited in the herbarium was assigned voucher No. BSID 882. Leaves of T. coriacea were washed, dried in the shade for 7 days, and then weighed. Approximately 1 kg of dried leaves were ground into a coarse powder using a mechanical grinder and passed through a No. 40 sieve to get the powder of desired coarseness, and then preserved in an airtight container. Approximately, 120 g of the powder was macerated in 600 mL of methanol (solvent) at a ratio of 1:5 and allowed to stand for 72 h and later boiled for 5 h [3]. The solution was filtered to obtain a decoction which was concentrated to a syrupy liquid in a water bath. The concentrated methanolic extract was then weighed. Distilled water was used to prepare a solution for the screening of anti-inflammatory activity.

Animals

A total of 60 adult male albino Wistar rats weighing 150–200 g were used for the assessment of anti-inflammatory activity. The rats were fed standard pellet diet with free access to drinking water.

Five groups of 6 rats were used in both experiments. The Institutional Animal Ethics Committee, Anwarul Uloom College of Pharmacy, Hyderabad, India, approved the study.

Acute Oral Toxicity and Selection of Test Doses

The reported maximum oral safe dose is 20,000 mg/kg [6]. Three test doses 125, 250, and 500 mg/kg were selected for the assessment of anti-inflammatory activity, with the highest test dose being 1/40th of the maximum oral safe dose. These 3 doses were used previously for the evaluation of antinociceptive [3], anti-epileptic [5] and gastroprotective activities [6].

Carrageenan-Induced Paw Edema Model

Acute inflammation in experimental animals was induced by the carrageenan-induced edema model [12, 13]. Five groups, each containing 6 animals, were used in this experiment. The rats in the negative control group were injected normal saline (0.9% w/v NaCl; mL/kg), and aspirin at a dose of 100 mg/kg was administered to the positive control group. Three test groups received the extract at doses of 125, 250, and 500 mg/kg. Subplantar injection of 0.1 mL of 1% suspension of carrageenan in normal saline was used to produce acute inflammation in the left hind paw of the rats 1 h after oral administration of the respective treatments. Paw volume was measured using a plethysmometer (UGO Basile S.R.L. Samtik Instrument, Italy) before (baseline) and after treatment with carrageenan at 1, 2, 3, and 4 h [14]. Mean increase in paw volume was calculated, and percentage inhibition was determined using the formula of Olajide et al. [15].

\[
\text{Percentage inhibition} = \frac{\left( \frac{C_t - C_0}{C_t} \right)_{\text{control}} - \left( \frac{C_t - C_0}{C_t} \right)_{\text{treated}}}{\left( \frac{C_t - C_0}{C_t} \right)_{\text{control}}} \times 100,
\]

where \(C_t = \text{mean paw volume for each group at time } t\) and \(C_0 = \text{mean paw volume for each group before carrageenan injection}\).

Cotton Pellet-Induced Granuloma Model

The cotton pellet-induced granuloma model was adopted to induce chronic inflammation in experimental animals. The methods described by Meier et al. [16] and Niemeegers et al. [17] were used with slight modifications. Thirty rats were divided into 5 groups, each containing 6 animals. Rats in the negative control group received normal saline orally (0.9% w/v NaCl; mL/kg), the positive group received aspirin orally (100 mg/kg, p.o.), while the 3 test groups were treated with the extract at doses of 125, 250, and 500 mg/kg orally for 5 consecutive days. On day 1, a sterilized cotton pellet weighing 30 mg was introduced subcutaneously into the dorsal part of rats under ether anesthesia by the procedure described by Shaik Mossadeq et al. [18]. On the 6th day, the animals were subjected to light ether anesthesia, and the wet cotton pellets were removed and weighed to measure the antitransudative effect. These pellets were later dried for 2 h at 60°C in a hot air oven and weighed again to determine the dry weights of the cotton pellets, which correspond to the antiproliferative effect. Percentage inhibition was calculated using the formula of Okoli et al. [19].

\[
\text{Percentage inhibition} = \frac{\text{Tc} - \text{Tt}}{\text{Tc}} \times 100,
\]

where \(\text{Tc} = \text{weight of granuloma tissue of the control group and } \text{Tt} = \text{weight of granuloma tissue of the treated group}\).
Statistical Analysis
The data were analyzed by one-way analysis of variance followed by Dunnett’s multiple comparison post hoc test using GraphPad Prism version 6 for Windows (GraphPad Software, San Diego, CA, USA). The values were expressed means ± SEM. \( p < 0.05 \) was considered significant.

Results

Yield of the Extract
The yield of the extract was found to be approximately 16 g with respect to the initial weight of the powdered material.

Carrageenan-Induced Paw Edema Model
The subplantar injection of carrageenan produced edema in negative control rats. The mean paw volume in negative control ranged from 0.36 ± 0.00 to 0.3 ± 0.00 (1–4 h). Pretreatment with the extract at all the test doses produced consistently significant inhibition from 1 to 4 h. A mild decrease (0.30 ± 0.00; \( p < 0.05 \)) in paw volume was noticed after treatment with the extract at 125 and 250 mg/kg at the end of 1 h (Table 1). However, the effects of the extract at 500 mg/kg and aspirin were analogous (\( p < 0.01 \)) from 1 h: the mean paw volume was found to be 0.26 ± 0.00 in both groups. Following treatment with the extract at 500 mg/kg and aspirin, the mean paw volume at 3 and 4 h were found to be similar (0.24 ± 0.00 and 0.18 ± 0.00; \( p < 0.01 \)). The test extract at 500 mg/kg exhibited a progressive decline in paw edema as that of the standard drug at all time points tested after carrageenan injection (Table 1). These observations point toward the anti-edematogenic property of \( T. \) coriacea leaves.

Cotton Pellet-Induced Granuloma Model
The methanolic extract of \( T. \) coriacea leaves after oral administration at doses of 125 and 250 mg/kg significantly decreased the mean weight of wet granuloma to 0.19 ± 0.01 (19.26% inhibition) and 0.20 ± 0.01 (19.78% inhibition), respectively (\( p < 0.05 \)). Aspirin and the extract at 500 mg/kg produced comparable and significant (\( p < 0.01 \)) reduction (0.18 ± 0.02 – 20.57% inhibition and 0.17 ± 0.00 – 26.84% inhibition) compared to the negative control (0.26 ± 0.00) (Fig. 1a). This indicates the antitransduorative potential of the extract. Similarly, oral administration of the extract at doses of 125, 250, and 500 mg/kg led to a significant (\( p < 0.05 \)) decrease in the mean weight of dry cotton pellets to 0.15 ± 0.00 versus 0.19 ± 0.00 in the negative control group. The percentage inhibition for the 3 test doses was found to be 23.82, 26.51, and 28.78% respectively. The most significant decrease in the weight of dry granulomatous tissue was produced by acetylsalicylic acid (0.13 ± 0.00) with 32.19% inhibition (\( p < 0.01 \)) (Fig. 1b). This highlights the effect of the leaf extract on the proliferative stage of inflammation.

Discussion
In this study, the methanolic extract of \( T. \) coriacea leaves showed significant anti-inflammatory activity at all test doses. This biological action was found to be dose dependent and as effective as the positive control (aspirin). The therapeutic potential of the extract could be attributed to the antioxidant activity and its flavonoid content (apigenin, kaempferol, luteolin, myricetin, quercetin, and rutin) [6]. Flavonoids have been reported to possess a wide range of biological actions [20]. In the inflammatory process, the enzymes cyclooxygenase and lipoxygenase...

### Table 1. Effect of oral administration of the leaf extract of \( T. \) coriacea and acetylsalicylic acid (ASA) on paw volume (means ± SEM) in the carrageenan-induced paw edema model

| Treatment        | 0 h (baseline) | 1 h  | 2 h  | 3 h  | 4 h  |
|------------------|---------------|------|------|------|------|
| Saline 1 mL      | 0.44±0.00     | 0.36±0.00 | 0.38±0.01 | 0.35±0.01 | 0.30±0.00 |
| ASA 100 mg/kg    | 0.18±0.01     | 0.26±0.00*** | 0.25±0.01** | 0.24±0.00** | 0.18±0.00** |
| TCLME 125 mg/kg  | 0.40±0.00     | 0.30±0.00*  | 0.28±0.01** | 0.26±0.01** | 0.22±0.00** |
| 250 mg/kg        | 0.36±0.00**   | 0.30±0.01*  | 0.24±0.00** | 0.26±0.00** | 0.20±0.00** |
| 500 mg/kg        | 0.26±0.00**   | 0.26±0.01** | 0.22±0.00** | 0.24±0.00** | 0.18±0.00** |

\( n = 6. \) TCLME, methanolic extract of \( T. \) coriacea leaves. \* \( p < 0.05 \), \** \( p < 0.01 \), vs. control (saline); one-way analysis of variance followed by Dunnett’s multiple comparison post hoc test using GraphPad Prism.
ase play important roles causing the release of arachidonic acid, used subsequently by neutrophils containing lipoxygenase to produce chemotactic compounds and simultaneously provoking the release of cytokines [21].

The cyclooxygenase and 5-lipoxygenase pathways that lead to the release of arachidonic acid are inhibited by certain phenolic compounds like quercetin [21, 22]. Prostaglandin is the end product of the cyclooxygenase pathway, and it is involved in various immunologic responses [22]. The synthesis of such an eicosanoid is also inhibited by flavonoids. Moreover, some of the flavonoids also produce vasodilation, which is essential in antinociceptive and anti-inflammatory processes [23]. These compounds also inhibit the action of phospholipase A₂ and C in inflammation besides inhibiting the L-arginine/NO pathway and blocking the protein kinase C pathway [24, 25]. They are capable of modulating the induction of nitric oxide synthase type-2 (NOS-2) by indirect inhibition of the cyclooxygenase and/or lipoxygenase pathways and nuclear factor-κB [26, 27]. They produce anti-inflammatory actions by effectively blocking NO synthesis. The NO inhibition has been found to produce antinociceptive and anti-inflammatory effects [26, 28, 29]. Flavonoids are also capable of inhibiting neutrophil degranulation. This directly diminishes the release of arachidonic acid by neutrophils and other immune cells [30]. The antioxidant and anti-inflammatory properties of the leaf extract give further insights into its healing effects on gastric ulcer [6] and wounds.

Some of the non-flavonoid anti-inflammatory compounds present in leaf extract of *T. coriacea* include 1H-inden-1-one,2,3-dihydro-3,3,5,6-tetramethyl; neophytadiene; 7,11,15-trimethyl-3-methylene-1-hexadecene; methyl palmitate; palmitic acid, and stigmasterol. The latter 2 compounds are reported to occur at the highest concentrations [11]. These phytochemicals could also play important roles in the anti-inflammatory actions of *T. coriacea*.

### Conclusion

The observations of the present study, such as decreases in paw volume and wet and dry weights of granulomatous tissue in acute and chronic models of inflammation, suggest that the methanolic leaf extract of *T. coriacea* possesses anti-inflammatory activity particularly at doses of 250 and 500 mg/kg. However, further studies are required to confirm the actual compounds responsible and the mode of action.

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### Disclosure Statement

The authors have no conflict of interest to disclose.
Anti-Inflammatory Potential of *T. coriacea* Leaves

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