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# Evaluation of Anti-inflammatory activity of *Jacquemontia caerulea* Leaf Methanolic Extract in Wistar rats

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## ABSTRACT

**Objective:** To evaluate anti-inflammatory activity of methanolic extract of obtained from the leaves of plant, *Jacquemontia caerulea* in Wistar rats.

**Methods:** Fresh leaves of *Jacquemontia caerulea* (Family: Convolvulaceae) were extracted using methanol and subjected to phytochemical analysis using standard procedures. The anti-inflammatory nature of the methanolic leaf extract (JCLME) was assessed on Wistar rats, with Indomethacin serving as the standard reference drug. Two established methods were employed: Paw edema method by using Carrageenan and Cotton pellet granuloma method.

**Results:** Phytochemical analysis of methanolic extract was found to have phytoconstituents such as saponins, flavonoids, glycosides, steroids, proteins, amino acids, phenols, and tannins. The anti-inflammatory evaluation showed that JCLME produced anti-inflammatory effect. In the paw edema model by Carrageenan, JCLME demonstrated remarkable ($p<0.05$) inhibition of paw edema with reduction of 23% and 30% at dose of 250 mg/kg and 500 mg/kg, respectively. Similar result was observed in the Cotton pellet granuloma method.

**Conclusion:** The leaf methanolic extract of *Jacquemontia caerulea* contains a high concentration of various phytochemicals and exhibits significant ($p<0.05$) anti-inflammatory effect in both Carrageenan-induced paw edema and Cotton pellet granuloma model in Wistar rats.

**Keywords:** *Jacquemontia caerulea,* Phytochemicals, Paw edema granuloma model, Cotton pellet, Indomethacin.

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## Introduction

*Jacquemontia caerulea*, commonly known as sky blue cluster vine is a flowering plant from family *Convolvulaceae* and is widely distributed across tropical and subtropical regions, including areas of Africa, Asia and America. In traditional medicine, various parts of Jacquemontia caerulea have been utilized for their purported medicinal properties, ranging from wound healing to respiratory ailments. Phytochemical analysis of plants is crucial for understanding their potential therapeutic benefits and thus the JCLME showed presence of various secondary metabolites such as alkaloids, flavonoids, tannins, saponins and phenolic compounds, which are known to have many biological activities including anti-inflammatory effect. These secondary metabolites serve as the basis for the pharmacological investigation of medicinal plants. Inflammation is a compound biological process that plays an essential role in defense mechanism of body against noxious stimuli. However, excessive or extended inflammation may cause tissue damage and lead to various chronic diseases such as arthritis, cardiovascular diseases and cancer. Therefore, the exploration for natural anti-inflammatory agents has gained substantial attention in recent time.

The present study is carried out to investigate the potential anti-inflammatory effect of JCLME using an animal model. Wistar rats, widely used experimental model in biomedical research, are employed to evaluate the anti-inflammatory activity of extract. The solvent, methanol is choosen for extraction because of its ability to efficiently extract wide range of phytochemicals from plant materials. Understanding the phytochemical composition of *Jacquemontia caerulea* leaves and elucidating its anti-inflammatory potential could provide valuable insights into its medicinal properties. Furthermore, if proven effective, the methanolic extract of *Jacquemontia caerulea* leaves will be a promising biological source for development of anti-inflammatory agents with potential therapeutic applications. Therefore, this study holds significance in bridging the traditional use of Jacquemontia caerulea with scientific evidence, paving the way for its pharmacological exploration and eventual integration into modern healthcare practices.

*Jacquemontia caerulea* is a member of the morning glory family Convolvulacea, which includes 55 genera and 1650 species, widely cultivated for their colourful flowers and heart-form leaves [7]. This family is distributed in both topical and temperate regions of the world. Convolvulacea includes many commercial uses, edible crops, ornamentals, medicinal herbs, and significant weeds.[8] *Jacquemontia caerulea* is an attractive ornamental, flourishing twining climber. Chemically strains of this family include phytoconstituents as; alkaloids, flavonoids, terpenoids, and coumarins [9]. Also known as cluster vine, this perennial plant possesses a woody base and has the potential to adopt a shrubby growth form. It primarily thrives in sandy littoral beachfront and maritime hammock environments [10]. The plant blooms from September to June, with vining upper stems adorned with relatively tomentose leaves [11] Its flowers are bell or wheel-shaped, ranging from blue to white, with a relatively short corolla tube. [12]


The juice extracted from the leaves and roots of *Jacquemontia caerulea* is traditionally consumed as a remedy to treat bites from green mamba snakes. Additionally, an infusion made from the leaves is used to wash wounds, highlighting the plant's potential medicinal applications in traditional practices. Fruit decoction is utilized in the management of constipation, flatulence, and liver complaints. Seeds of this family have carminative and purgative properties. *Jacquemontia caerulea* has been used topically in traditional medicine to address various skin conditions. It is often applied as a poultice or used in creams or ointments to treat rashes, insect bites, and skin irritations [13]. Extracts from *Jacquemontia caerulea* have shown antioxidant potential. Antioxidants play a crucial role in counteracting detrimental free radicals within the body, providing protection against oxidative stress, a condition linked to numerous diseases. The core foundation for the therapeutic effectiveness of plants lies in the phytochemicals they contain and their antioxidative capacities. Comprehensive phytochemical analyses on diverse components of the *Jacquemontia caerulea* plant have revealed the existence of a range of phytoconstituents and compounds, encompassing alkaloids, flavonoids, and saponins. These substances serve as secondary metabolites and also function as defense mechanisms. Flavonoids dissolve the extracellular proteins and form protein complexes. Alkaloids possess antibacterial, antioxidants, antifungal, analgesic, anxiolytic, anticoagulant, and anticancer activities [14].

## Materials and Methodology

The *Jacquemontia caerulea* leaves were obtained from an authorized plant supplier and was authenticated by the Department of Botany at Sri Venkateshwara University in Tirupati-517 502, Andhra Pradesh, India. The leaves were shade dried for 15 days and were coarsely crushed using a blender. The plant material was taken up for extraction using methanol and water of 80% respectively. The extraction was carried out by a soxhlet extraction process followed by distillation and subsequently stored in the refrigerator [15].

### Preparation of extract

300g of leaves of *Jacquemontia caerulea* were collected, washed thoroughly, and dried in the shade. Leaves were made to coarse powder and subjected to soxhlet extractor with methanol solvent for extraction procedure. The extract obtained was then subjected to distillation and heated in a water bath for semisolid consistency then placed in the refrigerator [16].


*Fig.4.2.1. Soxhlet extraction*

*Fig. 4.2.2 Powdered extract of Jacquemontia caerulea*

### Experimental animals

Adult Wistar rats of both gender, weighing between 200-250 g were procured from animal facility at Shadan College of Pharmacy in Peerancheruvu, Hyderabad. Upon arrival, the rats were subjected to one-week acclimatization period under standard environmental conditions, with 12-hour light dark cycle at temperature of 22 ± 2°C, 55 ± 5% relative humidity and were provided with ad libitum access to standard pellet diet and water. The Institutional Animal Ethics Committee (IAEC) had approved the experimental protocol from Shadan Institute of medical sciences and care was taken as per guidelines of CPCSEA, Department of Animal Welfare Government of India.

## Methods

### Preliminary Phytochemical Screening

Phytochemical screening of Jacquemontia caerulea methanolic leaf extract was conducted to assess the presence or absence of steroids, flavonoids, saponins, glycosides, carbohydrates, oils, phenols, etc. The tests for these active principles were carried out using standard procedures [17-19].

### Evaluation of Anti-inflammatory activity of *JCLME*

### 1. Carrageenan Induced Paw Edema Method

30 Wistar rats of both gender weighing between 150-200 grams were divided into five groups containing 6 in each group.

### Animal grouping

Group 1: Control (n=6)
Group 2: Test 1 (n=6), treated by using methanolic extract of *Jacquemontia caerulea* (250 mg/kg, p. o)
Group 3: Test 2 (n=6), treated by using methanolic extract of *Jacquemontia caerulea* (500 mg/kg p. o)
Group 4: Standard group, treated with indomethacin (1mg/kg p. o)

### Procedure

The methodology outlined by Winter et al. was employed for evaluating Anti-inflammatory effect of JCLME (1962). A total of 30 Wistar rats were selected and divided into five groups, each comprising six rats. Group 1 served as the control and were administered 1 ml of distilled water orally.

Rats in groups 2 and 3 were given methanolic extract of Jacquemontia caerulea by oral route (250 and 500mg/kg, respectively) while those in group 4 were given indomethacin (standard, 1 mg/kg, p.o.). 1 hour post-administration of the respective treatments, 0.05 ml of 1% carrageenan suspension was injected into the sub-plantar surface of left hind paw to induce edema. Paw volume was measured initially and subsequently at 1, 2, and 4 hours following the carrageenan injection utilizing a plethysmometer [20].

**2. Cotton Pellet Granuloma Method**

30 Wistar rats of both gender weighing between 150-200 grams were divided into five groups containing 6 in each group.

ANIMAL GROUPING:

Group 1: Control (n=6)

Group 2: Test 1 (n=6), treated with methanolic leaf extract of *Jacquemontia caerulea* (250 mg/kg, p.o)

Group 3: Test 2 (n=6), treated with methanolic leaf extract of *Jacquemontia caerulea* (500 mg/kg, p.o.)

Group.4: Standard group (Indomethacin, 10 mg/kg, p.o)

**Procedure**

30 rats were divided into five groups comprising of six rats in each group. Under ether anesthesia and with precautions, autoclaved cotton pellets were bilaterally subcutaneously implanted above scapula region. Since day 1 of pellet implantation, Jacquemontia caerulea extract (250 & 500 mg/kg) and 1 ml of distilled water (DW) were orally administered daily for 7 consecutive days. On day 8, rats were euthanized and the pellets along with the granuloma tissue were extracted and dried in an oven at 60°C until a constant weight is obtained [21]. The % inhibition of inflammation was evaluated based on edema volume inhibition [22] by using the following formula:

% inhibition of inflammation = $\underline{1\text{-}V_t}/V_c \times 100$,

Where Vt is the average paw edema volume of treated group or positive control group, and Vc is the paw volume of the control group that received only vehicle.

**STATISTICAL ANALYSIS**

The data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Mean values ± standard error of mean (SEM) was calculated for each group. Statistical significance was considered at p value < 0.05. The analysis was conducted using GraphPad Prism version 10.1.

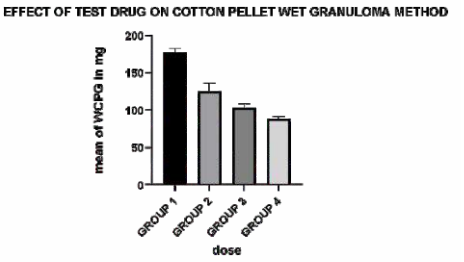
## Results

| Phytoconstituents | Test performed | Inference |
|---|---|---|
| Carbohydrates | i. Molisch's test<br>ii. Benedict's reagent<br>iii. Bontrager's test | Present<br>Present<br>Absent |
| Glycosides | i. Fehling's test<br>ii. Legal's test | Present<br>Present |
| Proteins and free amino acids | i. Millon's test<br>ii. Biurett test<br>iii. Ninhydrin test | Present<br>Absent<br>Present |
| Steroids | i. Salkowski reaction<br>ii. Liebermann- Burchard reaction | Present<br>Present |
| Flavonoids | i. Shinoda test<br>ii. Lead acetate test | Present<br>Present |
| Tannins | i. Lead acetate solution<br>ii. Potassium dichromate | Absent<br>Present |
| Phenolic compounds | i. Lead acetate solution<br>ii. Potassium dichromate | Absent<br>Present |
| Alkaloids | i. Mayer's test<br>ii. Hager's test<br>iii. Wagner's test | Present<br>Present<br>Present |
| Fixed oils and fats<br>Gums and mucilage's | i. Filter paper test<br>ii. Saponification test | Present<br>Present<br>Present |

***Effect Of Jacquemontia Caerulea On Cotton Pellet Wet Granuloma Method***

| S. No | Groups | Dose (mg/kg) | Mean of wet granuloma method | Average % inhibition of inflammation |
|---|---|---|---|---|
| 1. | Control | 10 ml/kg | 177.4±2.20 | 29% |
| 2. | JCE- 1 | 250mg/kg p. o | 125.0±4.61 | 29% |
| 3. | JCE- 2 | 500mg/kg p. o | 103.1±2.11 | 41% |
| 4. | Indomethacin | 10mg/kg p. o | 88.45±1.13* | 50% |

The data was analyzed by using one-way ANOVA followed by Dunnett's multiple comparison test. Mean values ± standard error of the mean (SEM) was calculated for all groups. Statistical significance was found at a p-value of < 0.05.

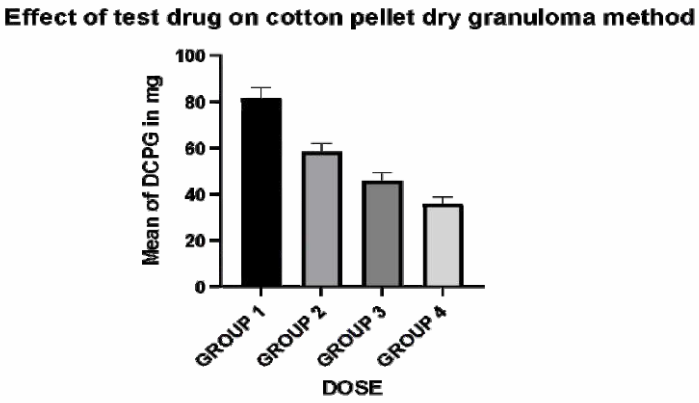



***Note: Group 1: Control, Group 2: JCLME-1, Group 3: JCLME-2, Group 4: Standard (Indomethacin)***
***JCE-Jacquemontia caerulea Leaf Methanolic Extract***
***WCPG-Wet Cotton Pellet Granuloma***

***Table No.3-Effect of Jacquemontia Caerulea On Cotton Pellet Dry Granuloma Method***

| S. No | Groups | Dose (mg/kg) | Mean weight of dry cotton pellet | Average % inhibition of inflammation |
|---|---|---|---|---|
| 1. | Control | 10ml/kg | 81.60±1.83 | 28% |
| 2. | JCLME-1 | 250mg/kg | 58.38±1.44* | 28% |
| 3. | JCLME-2 | 500mg/kg | 45.93±1.39** | 43% |
| 4. | Indomethacin | 10mg/kg | 35.95±1.11* | 56% |

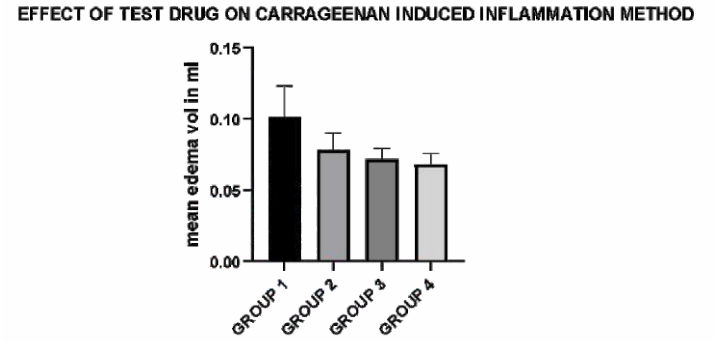
The data analysis was done by using one-way ANOVA followed by Dunnett's multiple comparison test. Mean values ± standard error of the mean (SEM) was computed for all groups. A p-value of < 0.05 was deemed statistically significant.

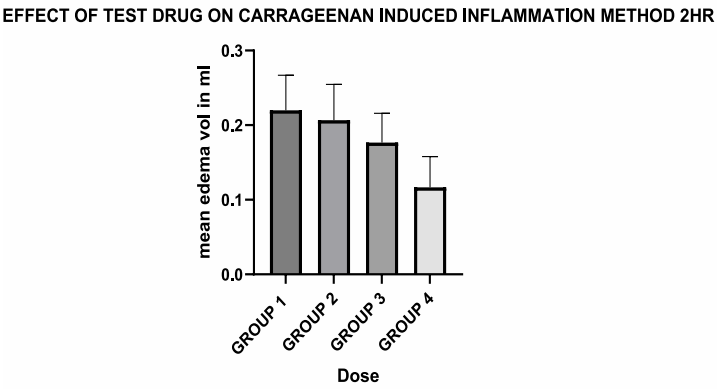


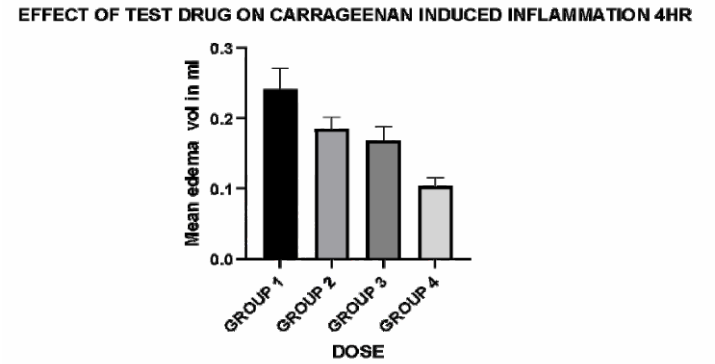

***Note: Group 1: Control, Group 2: JCLME-1, Group 3: JCLME-2, Group 4: Standard (Indomethacin)***
***JCE-Jacquemontia caerulea Leaf Methanolic Extract***
***DCPG-dry cotton pellet granuloma***

*Table No.4- Effect of Jacquemontia Caerulea against Carrageenan Induced Inflammation Method*

| S. No | Groups | Dose (mg/kg) | Mean edema volume | | | Average % inhibition | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1hr | 2hr | 4hr | 1hr | 2hr | 4hr |
| 1. | Control | 10ml/kg | 0.101±0.008 | 0.220±0.019 | 0.241±0.011 | 22% | 6% | 23% |
| 2. | JCLME-1 | 250 mg/kg p. o | 0.078± 0.004* | 0.206± 0.019* | 0.185± 0.006* | 22% | 6% | 23% |
| 3. | JCLME-2 | 500 mg/kg p. o | 0.071± 0.003* | 0.176± 0.016* | 0.168± 0.007* | 29% | 20% | 30% |
| 4. | Indomethacin | 10 mg/kg p. o | 0.068± 0.003* | 0.116± 3.343* | 0.103± 0.004* | 32% | 47% | 57% |




**Note: Group 1: Control, Group 2: JCLME-1, Group 3: JCLME-2, Group 4: Standard (Indomethacin) JCLME-Jacquemontia caerulea Leaf Methanolic Extract**

## Discussion

The present study was carried out to find out the anti-inflammatory potential of methanolic extract obtained from Jacquemontia caerulea leaves in Wistar rats. Phytochemical screening revealed the presence of various phytoconstituents including alkaloids, flavonoids, tannins, saponins and phenolic compounds. These phytoconstituents exhibit many biological activities and are associated with therapeutic properties of medicinal plants. The anti-inflammatory effect of methanolic extract was done by using carrageenan-induced paw edema model in Wistar rats. Carrageenan-induced inflammation is commonly used to assess the anti-inflammatory efficacy of herbal products as well as pharmacological agents. In this study, the extract demonstrated significant inhibition of paw edema in comparison with control group, thus showing its potential anti-inflammatory effect. The anti-inflammatory activity of Jacquemontia caerulea extract could be due to the presence of compounds in plants like flavonoids and phenols which possess antioxidant as well as anti-inflammatory properties. These phytoconstituents act through various mechanisms, including inhibition of pro-inflammatory mediators, suppression of inflammatory cytokines, and modulation of signalling pathways involved in the inflammatory process. The anti-inflammatory effect of JCLME was comparable to that of indomethacin, a nonsteroidal anti-inflammatory drug (NSAID) which is commonly used as a standard reference in preclinical studies. This suggests that JCLME exhibits potent anti-inflammatory activity comparable to conventional medications, albeit with potentially fewer adverse effects. The study thus provides scientific evidence supporting use of *Jacquemontia caerulea* in folk medicine for the management of inflammatory conditions. The presence of phytoconstituents responsible for the observed anti-inflammatory effect needs further investigation through isolation, purification, and characterization techniques focussing on elucidating the underlying mechanisms of action and assessing the safety profile of extract through acute and chronic toxicity studies. Therefore, the methanolic extract of Jacquemontia caerulea leaves exhibits promising anti-inflammatory activity in Wistar rats, validating its potential as herbal therapeutic agent for treating inflammatory disorders. Further research aimed at harnessing the therapeutic potential

of *Jacquemontia caerulea* extract which could lead to development of new anti-inflammatory drugs with improved efficacy and safety profiles.

## Conclusion

The findings of this study thus conclude the potential therapeutic value of the methanolic extract derived from *Jacquemontia caerulea* leaves as an anti-inflammatory agent. By preliminary phytochemical screening, the presence of various phytoconstituents known for their anti-inflammatory properties was confirmed. Subsequent evaluation was done by utilizing carrageenan-induced paw edema model in Wistar rats which demonstrated significant inhibition of inflammation by extract on comparing with effect of standard anti-inflammatory drug, indomethacin. The results not only validate traditional use of Jacquemontia caerulea in folk medicine but also provide scientific evidence supporting its anti-inflammatory efficacy. The observed activity may be due to the presence of secondary metabolites like flavonoids and phenols which exhibit antioxidant and anti-inflammatory effects through various molecular mechanisms. The promising anti-inflammatory activity of Jacquemontia caerulea extract highlights its potential as a natural alternative to conventional anti-inflammatory drugs, with the added advantage of potentially fewer adverse effects. However, further research is to be done to find out specific mechanisms of action, identify active compounds responsible for the observed effects, and evaluate the safety profile of the extract through rigorous toxicity studies. Continued exploration of this botanical resource may lead to discovery of new therapeutic interventions relating to inflammatory conditions, referring to unmet need for safer and more effective treatment options.

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## CONFLICT OF INTEREST

All authors approve the final manuscript with no conflicts of interests.

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