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**Review Article**

# In Vivo Study of Anti- Inflammatory & Antioxidant Activity of Methanolic Fruit Extract of *Gardenia Latifolia*

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**Abstract:**

Evaluation of the Anti-inflammatory & Antioxidant exercise of the methanolic extract of Gardenia latifolia used to be performed making use of the formalin-caused rat paw oedema model using diclofenac sodium because the reference drug. Imply alterations in paw oedema thickness of animals treated with the tested compounds from induction of irritation used to be measured, at the side of the inhibition percentage of oedema by way of the validated extracts at 2 dose degree 100mg/kg and 200mg/kg. Outcome proven that all the verified extract 200mg/kg was found extra lively.

**Keywords:** Inflammation, Gardenia latifolia, Antioxidant, Formalin-induced paw edema, Phytochemical analysis

## Introduction

An uncontrolled and persistent inflammation may act as etiological factor for many chronic illnesses [1]. Non-steroidal anti-inflammatory drugs (NSAIDS) are widely used in the treatment of pain and inflammation. About 34-46% of the users of NSAIDS usually sustain some gastrointestinal damage due to inhibition of the protective cyclooxygenase enzyme in gastric mucosa [2]. Herbal medicine is still the mainstay of therapy for about 75-80% of the whole population in developing countries for primary health care [3]. This is because of better cultural acceptability, affordability, better compatibility with the human body and fewer or no side effects, in addition, the last few years have seen a major increase in the use of herbal remedies in developed countries [4]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation, has demonstrated the safety and efficient value of traditional medicine [5]. World Health Organization encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries because of the great potential they hold in combating various diseases [6]. Many Indian ethno botanic traditions propose a rich repertory of medicinal plants used by the population for the treatment, management

and/or control of different types of pain [7]. However, there were not enough scientific investigations on the anti-inflammatory and analgesic activities conferred to these plants. One such plant from Indian flora *G. latifolia*. *G. latifolia* (Rubiaceae) is commonly known as Indian boxwood or Ceylon boxwood, is a densely foliaceous small tree that occurs throughout the greater parts of Indian common in deciduous forests along the streams. The stem bark and fruits are reported to be used in the treatment of varius ailments such as snake bite, skin diseases, stomach pais, caries in humans and ephemeral fever in live stocks [8,9]. Fruits are used for making perfumes [10]. The bark and wood gave beta sestorol, hederegenin, Me-esters of oleanic and gypsogenic acids. Root gave gardenins. Saponins from bark decreased formation of histamine and may find use in asthma (market drug is expectorant and weak spasmolytic, but was not found effective in asthma). The stem bark contains hederagenin, D-mannitol, sitosterol and siaresinolic, episiaresinolic, oleanolic andspinosic acid [11, 12]. Hence, in the present study, the antioxidant and anti-inflammatory activities of methanolic extracts of *G. latifolia* were evaluated using DPPH radical method and formalin-induced paw edema methods. Total phenolic and flavonoids contents of the crude extracts were also determined.

**Different parts of *Gardenia Latifolia***

## Materials and Methods

**Plant materials**: Gardenia latifolia fruit were gathered in January 2019 from Bheem betika Mandideep (M.P.).

- **Selection (**Based on the plant's availability and Folk use)
- **Drying** (dried in the sun but under the shade)
- **Storage** (kept in plastic bags and tightly closed and powdered as required)

**Chemicals:** Analytical grade purchased from the Himedia lab was the entire Chemical used for the study. Pvt. Limited

**Extraction by maceration procedure**

47 gram dried fruit powder Gardenia latifolia was obtained with methanol solvents using 48-hour maceration, 400C filtered and dried use vacuum evaporator.

By using the following formula, the percentage yield of each sample was calculated**:**

$$\text{Percentage output} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

**Phytochemical Screening**

**Phytochemical screening:** Phytochemical examinations were performed according to the normal techniques for all the extracts.

| S.no | Phytoconstituent | Determination method of Phytoconstituent | Appearance |
|---|---|---|---|
| **1.** | **Alkaloid** | **Mayer's Test** | yellow precipitate formation |
| | | **Wagner's Test** | Brown / reddish formation of precipitate |
| | | **Dragendroff's Test** | formation of Crimson precipitate shows the existence of alkaloids |
| | | **Hager's Test** | Yellow precipitate formation |
| **2.** | **Carbohydrates** | **Molisch's Test** | formation of the violet ring at the junction indicates the existence of carbohydrates |
| | | **Benedict 's test** | orange crimson precipitate shows decreased sugar**.** |
| | | **Fehling's Test** | formation of pink precipitate shows the presence of sugar reduction. |
| **3.** | **glycosides** | **Modified Borntrager's Test** | ammonia layer, rose-purple color formation shows the presence of anthranol glycosides. |
| | | **Legal's Test** | crimson to pink blood color indicates the presence of cardiac glycosides. |
| **4.** | **saponins** | **Froth Test** | foam layer formation indicates the existence of saponin |
| | | **Foam Test** | foam produced persists for ten minutes, it indicates the presence of saponins |
| **5.** | **phytosterols** | **Salkowski's Test** | golden yellow color shows the presence of triterpenes. |
| | | **Libermann Burchard's test** | Brown ring formation at the junction shows the presence of phytosterol |
| **6.** | **phenols** | **Ferric Chloride Test** | formation of bluish black color represents the presence of phenols |
| **7.** | **tannins** | **Gelatin Test** | formation of white precipitate shows the presence of tannins |
| **8.** | **flavonoids** | **Alkaline Reagent Test** | Intense yellow color formation, which becomes colorless when mixed with diluted acid, shows the presence of flavonoids |
| | | **Lead acetate Test** | Flavonoids are indicated by precipitating yellow color formation. |
| **9.** | **Proteins and Aminoacids** | **Xanthoproteic Test:** | formation of yellow colors indicates the existence of proteins. |
| | | **Ninhydrin Test** | Blue color formation indicates the existence of amino acid. |
| **10.** | **Diterpenes** | Copper acetate Test | Smart green color formation shows that diterpenes exist |

**Total Phenolic content estimation**

**Principle:** The full phenolic content of the excerpt was determined by the modified Folin-Ciocalteu method.

**Preparation of Standard:** In 10 ml of methanol, 10 mg of gallic acid was taken and 5-25 µg / ml of various aliquots were generated in methanol.

**Preparation of Extract:** With 10 ml of methanol and filter, 10 mg of dried plant material extract was obtained. Two ml of this extract (1mg / ml) was used to estimate Phenol.

**Procedure:** Mixed with 1 ml Folin-Ciocalteu reagent (previously diluted with 1:10 v / v distilled water) and 1 ml (7.5g / l) of 2 ml of each extract or normal sodium carbonate. The mixture was vortexed for 15s and allowed to stand at 40 ° C for 15min color growth. The absorption was assessed at 765 nm using a spectrophotometer.

**Total flavonoids content estimation**

**Principle:** The total flavonoid content was determined using the aluminum chloride method.

**Preparation of standard:** The total flavonoid content was determined using the aluminum chloride method**.**

**Preparation of extract:** With 10 ml of methanol and filter, 10 mg of dried plant material extract was obtained. The flavonoid estimation was based on three ml (1mg / ml) of this extract.

**Procedure:** 1 ml of 2 percent AlCl3 methanol solution was added to 3 ml of extract or standard and allowed to stand at room temperature for 15 minutes; 420 nm of absorption was evaluated.

**Antioxidant activity of extract using DPPH method**

The spectrophotometer was used to measure DPPH scavenging recreation. Stock resolution (6 mg in 100 ml of methanol) was prepared so that 1.5 ml of it in 1.5 ml of methanol was preliminary absorbed.

Decrease in absorbance at unique consciousness in the presence of pattern extract (10-100 µg / ml) was once popular after 15 minutes. 1.5 ml of DPPH resolution was drawn and the amount of methanol produced up to 3 ml was absorbed instantly at 517 nm for study manipulation. . 1.5 ml of DPPH and 1.5 ml of one-size-fits-all scan sample were placed in a series of volumetric flasks and the final quantity of methanol was adjusted to 3 ml. Three sample samples were drawn and processed in a comparable manner. 1.5 ml of DPPH and 1.5 ml of one-size-fits-all scan sample were placed in a series of volumetric flasks and the final quantity of methanol was adjusted to 3 ml. Three sample samples were drawn and processed in a comparable manner.

Scavenged percentage = [ (Abs check – Abs sample)/Abs check] = 100%

***In-vivo* Anti-inflammatory Activity**

**Materials and methods:-**

**Animals:-**

Wistar rats (150–200 g) were housed in a group (n=6) under a typical 12 h mild / dark cycle and controlled temperature and humidity conditions (25±2 ° C, 55–65%). Rats have typical libitum for rodent chows and water advertisements. Rats had been acclimatized 7 days sooner than the tests to laboratory circumstances. All the experiments were carried out between 08.00 and 15.00 h in a noise-free room. For each set of tests, separate crew (n=6) of rats was once used. The animal reviews were endorsed by the Institutional Animal Ethics Committee (IAEC) to manipulate and supervise experimental animals through the Atmosphere and Forest Ministry, the Government of India, New Delhi, India.

**Drugs & Chemicals**

In the current research, Diclofenac Sodium (Themis Pharmaceuticals, Mumbai), Carrageenine (Sigma Chemical Co, St Louis, MO, USA) were used.

**Toxicity study**

Preliminary studies on rats (n=6) were performed. Gardenia latifolia hydroalcoholic extract was administered orally in unique

doses to determine the variety of doses causing 0 and 100 percent animal mortality.Acute oral toxicity used to be carried out in accordance with the Financial Cooperation and Development Company (OECD) procedure (68). Animals were maintained fasting and best water was delivered, extract was provided p. O. A 1000 and 2000 mg / kg / p. O. in 500 doses. Six rat organisations (n=6) and animals were administered orally for 4 days and held on mortality statements as good as any behavioral modifications to analyze a possible anti-inflammatory impact .

**Experimental designs**

Group –1: Control
Group –2: Diclofenac Sodium (Standard)
Group –3: Methanolic fruit extract of *Gardenia latifolia* (MEGL) (100mg/kg, p.o.)
Group –4: Methanolic fruit of *Gardenia latifolia* (MEGL) (200mg/kg, p.o.)

***Carrageenan*-induced paw edema model**

The animals were split into four groups of six animals each and fasted 24 hours before being trained.( Group 1 was treated as manage Group 1 was handled as management (formalin (0.2 ml of 2% v / v newly prepared formalin resolution prepared in distilled water), crew 2 was treated with Diclofenac Sodium 30 mg / kg, p. O. Crew 3 dealt with Gardenia latifolia (MEGL) Methanolic leaves extract (100mg / kg, p.o.). Team 4 was treated with Gardenia latifolia (MEGL) Methanolic leaves extract (100mg / kg, p.o.). The quantity was assessed sooner than injecting the formalin and using a vernier caliper (accuracy) after injecting the formalin daily at a specified moment for seven successive days.

**Statistical Analysis**

Using graph pad prism for home windows, all analysis was performed. All statistical assessments are expressed as ± commonplace mean mistake (SEM) implies. Knowledge was evaluated in one manner by ANOVA, the location appropriate to $p<0.05$ used to be considered statistically tremendous compared to the car, followed by Dunnett's scan.

**Results and Discussion**

In present mission leaves of *Gardenia latifolia* was once accrued from Bheem betika Mandideep (M.P.). The crude drug was solar dried and coarsely powdered. The powdered drug used to be extracted, from methanolic solvent utilising maceration method. The extracts dried and their Extractive price and bioactive compound has been decided.

**Determination of Percentage Yield**

**% Yield of *Gardenia latifolia***

| S. No. | Parts | % Yield of methanolic extract (W/W) |
|---|---|---|
| 1. | Leaves | 8.908 |

**Phytochemical screening of extract**

A tiny portion of the dried specimens were subjected to phytochemical scanning using Kokate58 methods for experimenting with alkaloids, glycosides, saponins, flavonoids and steroids for extracts of all samples separately. Small quantities of each and every extract are properly suspended in the sterile distilled water to create 1 mg per ml awareness.

**Photographs of Phytochemical screening of extract**

**Phytochemical screening of Gardenia latifolia leaves extract**

| S. No. | Constituents | Test | Methanolic extract |
|---|---|---|---|
| **1.** | **Alkaloids** | Dragendroff's test Hager's test | -ve<br>-ve |
| **2.** | **Glycosides** | Legal's test | -ve |
| **3.** | **Flavonoids** | Lead acetateAlkaline test | +ve<br>+ve |
| **4.** | **Phenolic** | $Fecl_3$ | +ve |
| **5.** | **Proteins And Amino acids** | Xanthoproteic test | +ve |
| **6.** | **Carbohydrates** | Fehling's test | +ve |
| **7.** | **Saponins** | Foam test | +ve |
| **8.** | **Diterpenes** | Copper acetate test | +ve |
| **9.** | **Tannins** | Gelatin Test | -ve |

**Results of Estimation of Total Phenolic Contents**

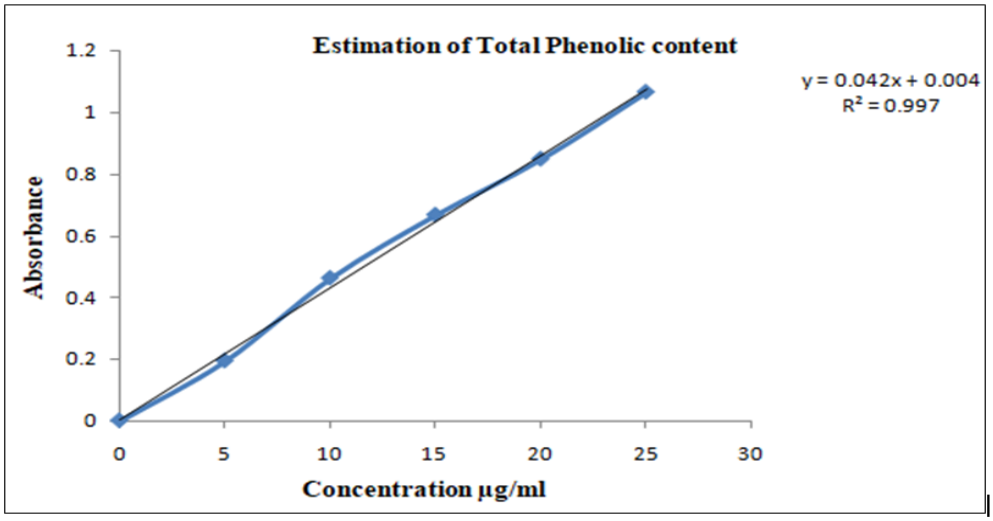
**Total Phenolic content estimation (TPC)**

The content of total phenolic compounds (TPC) content was expressed as mg/100 mg of dry extract equivalent Gallic acid sample using the equation extracted from the calibration curve: Y = 0.042X+0.004, R2 = 0.997, where X is the equivalent gallic acid (GAE) and Y is the absorbance.

**Gallic acid Calibration Curve :**

**Preparation of Gallic acid calibration curve**

| S. No. | Concentration | Absorbance |
|---|---|---|
| **0** | 0 | 0 |
| **1** | 5 | 0.194 |
| **2** | 10 | 0.422 |
| **3** | 15 | 0.637 |
| **4** | 20 | 0.848 |
| **5** | 25 | 1.035 |




**Graph of Estimation of Total Phenolic content**

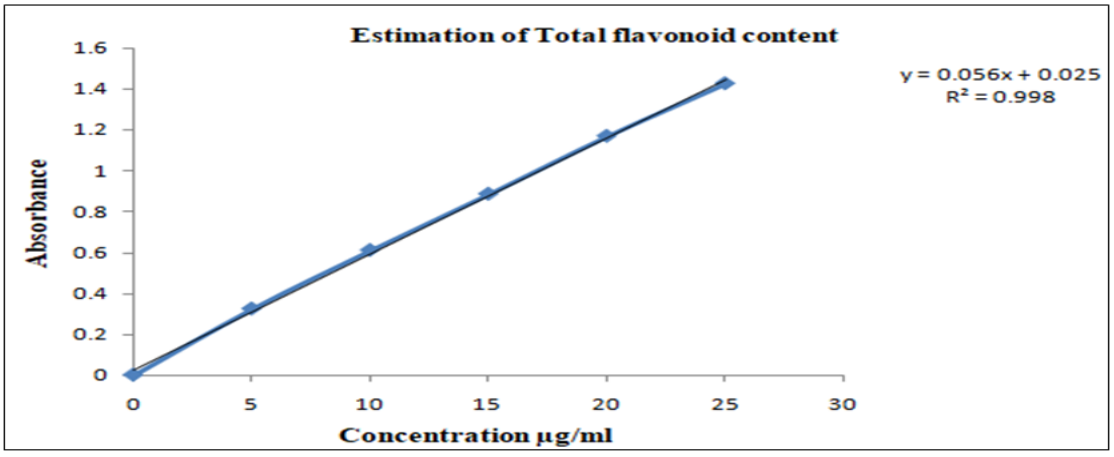
**Total flavonoid content estimation (TFC)**

The content of total flavonoid compounds (TFC) content was expressed as mg/100 mg of quercetin equivalent of the dry plant sample using the calibration curve equation: Y = 0.056X+0.025, R2 = 0.998, where X is the quercetin equivalent (QE) and Y is the absorbance.

**Calibration Curve of Quercetin**

**Preparation of calibration curve of Quercetin**

| S. No. | Concentration | Absorbance |
|---|---|---|
| **0** | 0 | 0 |
| **1** | 5 | 0.324 |
| **2** | 10 | 0.61 |
| **3** | 15 | 0.885 |
| **4** | 20 | 1.168 |
| **5** | 25 | 1.425 |




**Graph of Estimation of Total Flavonoid content**

**Total phenolic and total flavonoid content of *Gardenia latifolia***

| S. No. | Extracts | Total Phenol (GAE) (mg/100mg) | Total flavanoid (QE) (mg/100mg) |
|---|---|---|---|
| **1.** | **Methanolic** | 0.77 | 0.60 |

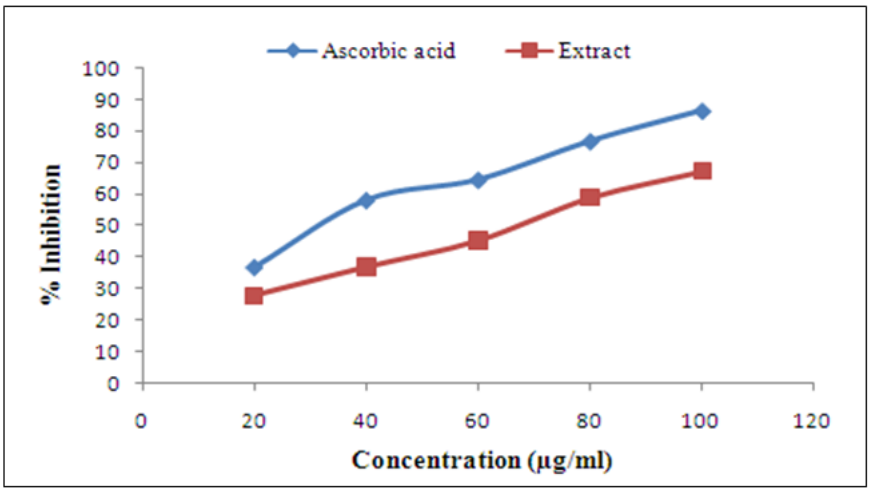
**Antioxidant activity results using DPPH**

**Absorbances of ascorbic acid and extract using DPPH method**

| S. No. | Concentration | Absorbances | |
|---|---|---|---|
| | | Ascorbic acid | Extract |
| 2 | 20 | 0.098 | 0.112 |
| 3 | 40 | 0.065 | 0.098 |
| 4 | 60 | 0.055 | 0.085 |
| 5 | 80 | 0.036 | 0.064 |
| 6 | 100 | 0.021 | 0.051 |

**% Inhibition of ascorbic acid and extract using DPPH method**

| S. No. | Concentration | % Inhibition | |
|---|---|---|---|
| | | Ascorbic acid | Extract |
| 1 | 10 | 36.77 | 27.74 |
| 2 | 20 | 58.06 | 36.77 |
| 3 | 40 | 64.51 | 45.16 |
| 4 | 60 | 76.77 | 58.70 |
| 5 | 80 | 86.45 | 67.09 |
| 6 | 100 | 36.77 | 27.74 |
| IC 50 | | 35.44 | 65.80 |



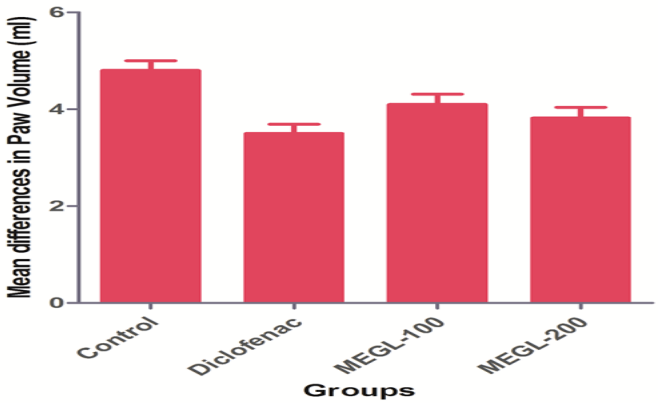

**% Inhibition of ascorbic acid and extract using DPPH method**

The located $IC_{50}$ worth showed that Ascorbic acid exhibited best possible antioxidant exercise (35.44ppm) as in comparison with methanolic extract (The $IC_{50}$ worth of the ascorbic acid (65.80ppm).

**Results of *In-vivo* Anti-inflammatory Activity**

Effect of different extracts on paw edema induced by formalin in rats

| Treatment | Dose (mg/kg) | Mean differences in Paw Volume (ml) | Percentage of Inhibition (%) |
|---|---|---|---|
| Control | 0.2 ml of 2% v/v | 4.80±0.20 | -- |
| Diclofenac | 30 | 3.50±0.19* | 96.0 |
| MEGL | 100 | 4.10±0.21 | 84.00 |
| MEGL | 200 | 3.82±0.22 | 87.00 |




**Effect of different extracts on paw oedema induced by formalin in rats**

**Conclusion**

Evaluation of the anti-inflammatory exercise of the methanolic extract of Gardenia *latifolia* used to be performed making use of the formalin-caused rat paw oedema model using diclofenac sodium because the reference drug. Imply alterations in paw oedema thickness of animals treated with the tested compounds from induction of irritation used to be measured, at the side of the inhibition percentage of oedema by way of the validated extracts at 2 dose degree 100mg/kg and 200mg/kg. Outcome proven that all the verified extract 200mg/kg was found extra lively.

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