International Journal of Pharmaceutics and Drug Research
ISSN: 2347-6346
Available online at http://ijpdr.com

Original Research Article

IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAVES EXTRACT OF *GARDENIA RESINIFERA*

Archana Rai*, Surendra Kumar Jain, K. K. Badoniya
Truba Institute of Pharmacy, Bhopal (M.P.)

***Correspondence Info:**
Archana Rai
Truba Institute of Pharmacy, Bhopal (M.P.)
Email:
archanarai08081997@gmail.com

***Article History:**

Received: 23/10/2024
Revised: 02/11/2024
Accepted: 17/11/2024

ABSTRACT

This study evaluated the phytochemical composition and pharmacological activities of the ethanolic extract obtained from *Gardenia resinifera* leaves. The extraction process yielded 8.6% w/w of the ethanolic extract, indicating moderate efficiency. Phytochemical screening confirmed the presence of alkaloids (via Hager's and Wagner's tests), flavonoids, phenols, proteins, saponins, diterpenes, and tannins, indicating a diverse array of bioactive compounds. Quantitative analysis revealed the presence of 1.26 mg/100 mg of total flavonoids and 1.45 mg/100 mg of total phenols in the ethanolic extract. Antioxidant activity was assessed using the DPPH assay, with the extract exhibiting an IC_{50} value of 83.06 µg/mL, demonstrating moderate antioxidant potential compared to ascorbic acid (IC_{50} = 34.57 µg/mL). Additionally, in a carrageenan-induced paw edema model, the extract displayed dose-dependent anti-inflammatory effects, significantly reducing paw volume compared to the control group. This observed anti-inflammatory activity supports the traditional use of *Gardenia resinifera* in managing inflammatory conditions. These findings highlight *Gardenia resinifera* as a promising source of bioactive compounds with antioxidant and anti-inflammatory properties, prompting further research into its therapeutic applications.

Keywords: *Gardenia resinifera,* anti-inflammatory activity, leaves, ethanolic extract

INTRODUCTION

Gardenia resinifera is a tropical plant species known for its diverse pharmacological properties, including anti-inflammatory potential. Ethnobotanical studies have highlighted its traditional use in treating various inflammatory conditions in indigenous medicine systems (Singh *et al.,* 2010; Waterhouse, 2009). The plant belongs to the Rubiaceae family and is native to tropical and subtropical regions, particularly found in parts of Asia and Africa.

Phytochemical investigations of *Gardenia resinifera* have identified bioactive compounds such as iridoids, flavonoids, terpenoids, and phenolic compounds in its leaves. These constituents have been associated with antioxidant and anti-inflammatory activities, making *Gardenia resinifera* a subject of interest for scientific research aimed at validating its traditional medicinal uses (Sarris *et al.,* 2013).

Previous studies have reported on the antioxidant and anti-inflammatory properties of various *Gardenia* species, attributing these effects to the presence of specific phytochemicals. However, despite the promising pharmacological profile of

Gardenia resinifera, there remains a gap in scientific literature regarding its specific anti-inflammatory mechanisms and in-vivo efficacy. Therefore, this study aims to investigate the *in-vivo* anti-inflammatory activity of the ethanolic extract of *Gardenia resinifera* leaves using appropriate animal models.

By elucidating the anti-inflammatory potential of *Gardenia resinifera* at the in-vivo level, this research aims to contribute valuable insights into its therapeutic applications in managing inflammatory disorders. The findings may provide a scientific basis for the development of novel herbal medicines or therapeutic agents derived from *Gardenia resinifera*.

MATERIALS AND METHODS

Material

Various chemicals were procured from reputable suppliers for the study. Potassium mercuric iodide and picric acid were obtained from Thomas Baker, Mumbai, while Loba chemie Pvt. Ltd., Mumbai supplied iodine, potassium iodide, sodium nitroprusside, sodium hydroxide, lead acetate, copper acetate, and Folin-Ciocalteu reagent. S. D. Fine Chem. Ltd., Mumbai provided potassium bismuth iodide, pyridine, gelatin, nitric acid, and ferric chloride. Additionally, Qualigens Fine Chemicals, Mumbai supplied methanol, ethanol, and chloroform, whereas Fehling's solution was sourced from Central Drug House Ltd., New Delhi.

Methods

Collection of plant material

The plants have been selected on the basis of its availability and folk use of the plant. Leaves of *Gardenia resinifera* were collected from Bhopal in the month of January, 2024. Drying of fresh plant parts was carried out in sun but under the shade. Dried leaves of *Gardenia resinifera* were preserved in plastic bags, closed tightly and powdered as per the requirements.

Figure 1: Collection of leaves of *Gardenia resinifera*

Extraction by maceration process

Leaves of *Gardenia resinifera* were shade dried at room temperature. 50 gram dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place.

Defatted dried powdered leaves of *Gardenia resinifera* has been extracted with ethanol solvent using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC(Mukherjee, 2015; Kokate, 1991).

Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powdered drug}} \text{ x } 100$$

Phytochemical screening

Medicinal plants are traditional pharmaceutical commodities and many of the current medicinal drugs are derived indirectly from plants. Phytochemical materials consist of two main bioactive components (Chlorophyll, vitamins, amino acids, sugar etc.) and secondary bioactive components; (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical analyses were performed according to the normal protocols for extract. Phytochemical examinations were carried out for all the extracts as per the standard methods (Audu *et al.,* 2007).

1. Detection of alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test: Filtrates was treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's Test: Filtrates was treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test: Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Detection of flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extract was treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

Total flavonoids content estimation

Estimating the total flavonoid content in plant materials is a crucial aspect of understanding their potential health benefits, as flavonoids are known for their antioxidant, anti-inflammatory, and anti-carcinogenic properties. To estimate the total flavonoid content (TFC) in plant samples, several analytical techniques are employed. These methods aim to quantify flavonoid compounds using various chemical reactions and measurements. The estimation of total flavonoid content is a fundamental process in the analysis of plant materials for their health-promoting properties. By using methods like spectrophotometry, researchers can quantify flavonoid levels and explore their potential benefits in food science, nutrition, and medicine.

Determination of total flavonoids content was based on Aluminum chloride method (Mishra *et al.,* 2017). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filtered. 3 ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Total phenolic content estimation

The total phenolic content of the extract was determined by the modified folin-ciocalteu method (Mishra *et al.,* 2017). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10-50µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

In vitro antioxidant activity using DPPH assay

Antioxidant activity was performed using 1,1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assay. Sample and standard (10 mg) were dissolved in methanol (10 mL) to obtain a stock concentration of 1000 µg/mL. The stock solution was further diluted to final concentrations of 10, 20, 40, 60, 80 and 100 µg/mL in methanol. The DPPH solution was freshly prepared in MeOH (6mg in 100ml methanol). Then, 1.5 mL of DPPH solution was added to 1.5 mL of sample solution of different concentrations. An equal volume of DPPH and methanol except sample was used as control. Methanol was used as blank in this method. The mixture was allowed to react at room temperature in the dark. After 15 minutes, the absorbance of the reaction mixture was recorded at 517 nm (Mishra *et al.,* 2017). Then, Final decrease in absorbance was noted of DPPH with the sample at different concentration. The percentage inhibition (%) was calculated using the following formula:

$$Calculation\ of\ \%\ Inhibition\ = \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \times 100$$

The concentration of plant extract necessary to scavenge 50% of radicals (IC_{50}) was calculated by plotting inhibition percentages against concentrations of sample.

In vivo anti-inflammatory activity

Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Toxicity study

Preliminary experiments were carried out on rats (n=6). Ethanolic extract of *Gardenia resinifera* were administered orally in different doses to determine the range of doses that cause zero and 100 % mortality of animals.

Acute oral toxicity was conducted according to the method of the Organisation for Economic Co-operation and Development (OECD) (Tan-No *et al.,* 2006). Animals were kept fasting, providing only water; extracts were given p.o. in doses of 500, 1000and 2000 mg/kg/p.o. administered orally for 4 days in different groups of rats (n=6), and the animals were kept under observation for mortality and any behavioural changes to evaluate a possible anti-inflammatory effect.

Table 1: Toxicity study

Observations	Acute toxicity
Skin and Fur	Normal
Eyes	Normal
Respiration	Normal
Sleep	Normal
Coma	Not seen
Mortality	Not seen

Experimental designs

Group –1: Control

Group –2: Indomethacin (30 mg/kg, bw, Standard)

Group –3: Ethanolic extract of *Gardenia resinifera* (100mg/kg, p.o.)

Group –4: Ethanolic extract of *Gardenia resinifera* (200mg/kg, p.o.)

Carrageenan-induced hind paw edema

The carrageenan-induced hind paw edema model is a widely used experimental method for evaluating the anti-inflammatory properties of drugs and natural compounds. This model is favored due to its simplicity, reproducibility, and the relevance of the inflammatory response it induces, which closely mimics human acute inflammation. the carrageenan-induced hind paw edema model remains a cornerstone in the study of inflammation and the development of anti-inflammatory therapeutics. Its well-characterized response and ease of use make it an essential tool in pharmacological and biomedical research. Male Wistar rats were fasted for 16 h and used in groups of 4, each containing 6 individuals. Ethanolic extract of *Gardenia resinifera* (100 mg/kg), Ethanolic extract of *Gardenia resinifera* (200 mg/kg), and indomethacin (30 mg/kg) were suspended in a 5% solution of a vehicle and were administered orally. One hour later, paw edema was produced by injecting 100 µl of 1% carrageenan solution in saline into the left hind paw. Paw volume was measured after up to 5 h carrageenan injection, using a water displacement plethysmometry. The swelling ratio (% swelling) was expressed as the percentage of the increase in paw volume before carrageenan injection (Zarei *et al.,* 2023).

Statistical Analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The ethanolic extract of *Gardenia resinifera* leaves demonstrated promising phytochemical composition and pharmacological activities in this study. The extraction process yielded 8.6% w/w of the ethanolic extract, indicating moderate extraction efficiency.

Phytochemical screening revealed the presence of alkaloids (confirmed by Hager's and Wagner's tests), flavonoids, phenols, proteins, saponins, diterpenes, and tannins, suggesting a diverse range of bioactive compounds. Quantitative analysis showed the ethanolic extract contained 1.26 mg/100 mg of total flavonoids and 1.45 mg/100 mg of total phenols. In the DPPH assay, the extract exhibited an IC50 value of 83.06 µg/mL, indicating moderate antioxidant potential compared to ascorbic acid (IC50 = 34.57 µg/mL). Furthermore, in the carrageenan-induced paw edema model, the extract demonstrated dose-dependent anti-inflammatory effects, significantly reducing paw volume compared to the control group. The observed anti-inflammatory activity supports the traditional use of *Gardenia resinifera* in treating inflammatory conditions. These findings underscore the potential of *Gardenia resinifera* as a source of bioactive compounds with antioxidant and anti-inflammatory properties, warranting further investigation into its therapeutic applications.

Table 2: % Yield of leaves extract of *Gardenia resinifera*

S. No.	Extract	% Yield (w/w)
1.	Ethanolic	8.6 %

Table 3: Phytochemical screening of extract *of* leaves of *Gardenia resinifera*

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	+ve
	Dragendroff's Test	-ve
	Hager's Test	+ve
2.	**Glycosides**	
	Legal's Test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric chloride test	+ve
5.	**Proteins**	
	Xanthoproteic test	+ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's Test	-ve
7.	**Saponins**	
	Froth Test	+ve
	Foam Test	+ve
8.	**Diterpenes**	
	Copper acetate test	+ve
9.	**Tannins**	
	Gelatin Test	+ve

Table 4: Estimation of total flavonoids and phenol content of *Gardenia resinifera*

S. No.	Extract	Total flavonoids content (mg/ 100 mg of dried extract)	Total phenol content (mg/ 100 mg of dried extract)
1.	Ethanolic	1.26	1.45

Table 5: IC_{50} value for DPPH assay of Ascorbic acid and ethanolic extract of *Gardenia resinifera*

S. No	Standard / Plant Extract	IC_{50} (µg/mL)
1.	Ascorbic acid	34.57
2.	Ethanolic extract	83.06

Table 6: Effect of Ethanolic extract of *Gardenia resinifera* on paw oedema induced by carrageenan in rats

Treatment	Dose (mg/kg)	Mean ± SEM (Inhibition of paw volume)			
		0 min.	60 min.	120 min.	180 min.
Control	0.1 ml of 1% (w/v)	0.373±0.052	0.73±0.050	0.86±0.060	0.850±0.062
Indomethacin	10	0.33±0.052***	0.45±0.051***	0.33±0.052***	0.30±0.051***
Ethanolic extract of *G. resinifera*	100	0.300±0.054**	0.60±0.052**	0.57±0.056*	0.49±0.053*
Ethanolic extract of *G. resinifera*	200	0.333±0.052***	0.56±0.053***	0.650±0.052**	0.43±0.052**

*P < 0.05-significant compared to the carrageenan treated group.

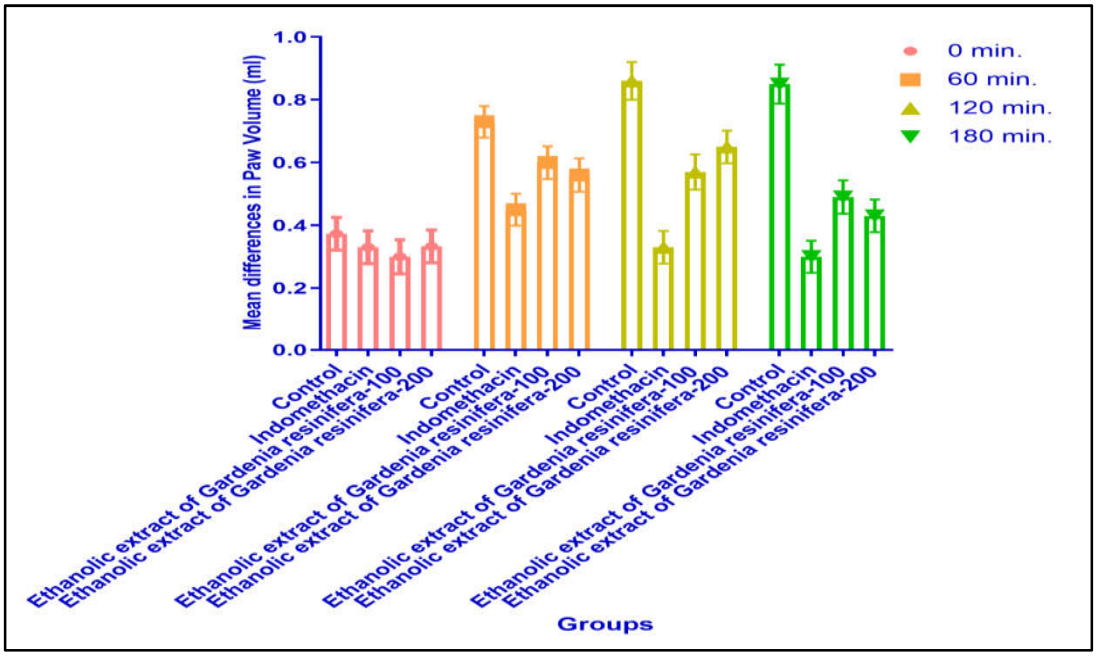

Figure 2: Effect of Ethanolic extract of *Gardenia resinifera* on paw oedema induced by carrageenan in rats

CONCLUSION

In conclusion, the ethanolic extract of *Gardenia resinifera* leaves emerges as a valuable natural resource rich in bioactive compounds. The presence of saponins, flavonoids, phenols, proteins, and diterpenes, along with demonstrated antioxidant and anti-inflammatory activities, underscores its potential therapeutic applications. These findings support its use in pharmaceuticals, particularly in formulations targeting oxidative stress-related conditions and inflammatory disorders. The extract's moderate antioxidant activity, as evidenced by the DPPH assay, and significant reduction in paw edema in the carrageenan-induced inflammation model suggest promising avenues for further research. Additionally, the presence of saponins hints at its potential as a natural surfactant, which could find applications in eco-friendly detergents or cosmetics. Further studies are warranted to explore the extract's mechanisms of action, safety profile, and efficacy in diverse biological systems. This research lays a foundation for harnessing *Gardenia resinifera* as a sustainable source of therapeutic agents, contributing to the development of natural and environmentally friendly healthcare solutions.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Audu, S.A., Mohammed, I. & Kaita, H.A. (2007) Phytochemical screening of the leaves of Lophira lanceolata (Ochanaceae). *Life Science Journal*, 4, 75–79.
- Kokate, C.K. (1991) Practical pharmacognosy, 3^< rd> ed. New Delhi. VPBN, 107–111.
- Mishra, A.G., Singh, R., Meha, P. & Parkhe, G. (2017) Determination of total phenolic, flavonoid content, antioxidant and antimicrobial activity of *Gloriosa superba* seed extract. *Asian Journal of Pharmaceutical Education and Research*, 6, 12–17.
- Mukherjee, P.K., Bahadur, S., Chaudhary, S.K., Kar, A. & Mukherjee, K. (2015) Quality related safety issue-evidence-based validation of herbal medicine farm to pharma. In:. Elsevier, pp. 1–28.
- Sarris, J., McIntyre, E. & Camfield, D.A. (2013) Plant-based medicines for anxiety disorders, Part 2: a review of clinical studies with supporting preclinical evidence. *CNS Drugs*, 27, 301–319.
- Singh, M., Kumar, V., Singh, I., Gauttam, V. & Kalia, A.N. (2010) Anti-inflammatory activity of aqueous extract of Mirabilis jalapa Linn. leaves. *Pharmacognosy Research*, 2, 364–367.
- Tan-No, K., Nakajima, T., Shoji, T., Nakagawasai, O., Niijima, F., Ishikawa, M., Endo, Y., Sato, T., Satoh, S. & Tadano, T. (2006) Anti-inflammatory effect of propolis through inhibition of nitric oxide production on carrageenin-induced mouse paw edema. *Biological and Pharmaceutical Bulletin*, 29, 96–99.
- Waterhouse, D.N. (2009) A novel combination of Chinese medicines to treat advanced cancers and

lymphomas tested in rats. *Chinese Medicine*, 4, 22.

- Zarei, M.H., Lorigooini, Z., Amini Khoei, H.A. & Bijad, E. (2023) Acute oral toxicity assessment of galbanic acid in albino rat according to OECD 425 TG. *Toxicology Reports*, 11, 111–115.