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In-Vitro Anti-Inflammatory Activity Of Nerium Oleander Flowers Extract (Pink Flower)

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Abstract

Nerium oleander, a widely cultivated ornamental plant, is known to contain a diverse range of secondary metabolites, including flavonoids, phenolic compounds, tannins, saponins, terpenoids, and cardiac glycosides, several of which are associated with anti-inflammatory activity. The present study evaluated the in-vitro anti-inflammatory activity of *Nerium oleander* (pink flower) extract using the egg albumin denaturation method, with diclofenac sodium employed as the standard reference drug. Egg albumin solution was treated with varying concentrations (100–500 µg/mL) of the flower extract and the standard drug, subjected to heat-induced denaturation at 70°C, and the resulting turbidity was measured spectrophotometrically at 660 nm to determine the percentage inhibition of protein denaturation. The extract exhibited a clear concentration-dependent inhibition of protein denaturation, increasing from 7.14% at 100 µg/mL to 85.7% at 500 µg/mL, compared with 35.7% to 92.8% inhibition shown by diclofenac sodium over the same concentration range. These findings indicate that *Nerium oleander* flower extract possesses notable in-vitro anti-inflammatory activity, likely attributable to its phenolic, flavonoid, tannin, and terpenoid constituents, which may act by stabilizing protein structure and modulating inflammatory mediators. The results support the potential of *Nerium oleander* flowers as a natural source of anti-inflammatory agents, warranting further in-vivo evaluation and isolation of the constituents responsible for this activity.

Keywords: Nerium oleander; anti-inflammatory activity; egg albumin denaturation; flower extract; diclofenac sodium

Introduction

The extraction of bioactive compounds from medicinal and ornamental plants has emerged as a significant area of research, driven by the growing demand for natural products across pharmaceutical, agricultural, and industrial sectors. The flowers of *Nerium oleander* are known to contain a diverse array of secondary metabolites—including flavonoids, phenolic compounds, alkaloids, tannins, saponins, and cardiac glycosides—that underlie many of the plant's biological activities [1-4].

Flower extraction refers to the process of isolating these phytochemicals from plant tissue using appropriate solvents, such as methanol, ethanol, acetone, water, or hydroalcoholic mixtures. The yield and chemical composition of the resulting extract are strongly influenced by the extraction technique and solvent selected. Commonly employed methods include maceration, Soxhlet extraction, ultrasonic-assisted extraction, and general solvent extraction, each of which yields concentrated extracts suitable for subsequent chemical and biological analysis [5].

Research has shown that extracts derived from *Nerium oleander* flowers exhibit antioxidant, antimicrobial, anti-inflammatory, insecticidal, and potentially anticancer properties, attributable to their diverse bioactive constituents. Phytochemical screening and extraction of the flowers thus offer valuable insight into the plant's medicinal potential and support the identification of compounds relevant to drug development and other therapeutic applications. Nonetheless, given that *Nerium oleander* contains toxic cardiac glycosides such as oleandrin, rigorous safety precautions must be observed throughout the extraction process and in all subsequent experimental procedures [5-8].

Overall, the extraction of *Nerium oleander* flowers represents a critical step in characterizing their phytochemical profile, assessing their biological activities, and exploring their applications in pharmacology, biotechnology, and natural product research. This work lays the foundation for a deeper understanding of the relationship between the plant's chemical constituents and its therapeutic and toxicological properties [8-12].

Inflammation arises from a range of triggers, including physical injury, ultraviolet exposure, microbial infection, and immune responses. Its hallmark signs—redness, heat, swelling, and pain—are well established in classical pathology. When left unchecked, these inflammatory cascades can progress into chronic conditions such as asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis. These disorders are increasingly prevalent and often debilitating, with rheumatoid arthritis and osteoarthritis representing major contributors to disability in aging populations.

Treatment options—corticosteroids, NSAIDs, and biologic agents—each carry significant drawbacks. Long-term corticosteroid use is associated with a constellation of adverse effects resembling Cushing's syndrome, including facial rounding, a “moon face” appearance, weight gain concentrated in the trunk, elevated blood pressure, high blood sugar, muscle wasting, weakened immunity, bone density loss, cataracts, and psychiatric disturbances. NSAIDs, meanwhile, act by inhibiting COX-1-derived compounds necessary for protective functions, which explains their tendency to cause stomach irritation, bleeding, and platelet dysfunction [13]. Since the anti-inflammatory, pain-relieving, and fever-reducing benefits of NSAIDs stem specifically from COX-2 inhibition, selective COX-2 inhibitors (coxibs) were developed in recent years to preserve therapeutic effect while reducing gastrointestinal risk.

Inflammatory diseases

Immune cells involved in inflammation release a complex array of growth factors, cytokines, and bioactive metabolites. They also generate reactive oxygen species (ROS), reactive nitrogen species (RNS), and free radicals during oxidative processes. Neutrophils in particular produce ROS and RNS—superoxide, hydrogen peroxide, and nitric oxide—that can damage nucleic acids, proteins, carbohydrates, and lipids, worsening tissue injury and intensifying inflammation. Consequently, compounds capable of neutralizing these radicals or inhibiting lipid peroxidation hold promise as therapeutic candidates across multiple inflammatory conditions [14,15].

Anti-inflammatory activity of plant extract is commonly performed by protein denaturation assay such as Egg Albumin denaturation method. Inflammation is protective biological response of body against infection, injury or tissue damage. *Nerium oleander* flowers contains various bioactive phytochemicals such as phenolics, tannins, flavonoids, terpenoids that exhibits anti-inflammatory properties. These compounds help in reducing the inflammation which inhibits release of inflammatory mediators and stabilize proteins involved in inflammatory process [16,17].

Inflammation is the body's protective response to harmful stimuli pathogens, damaged cells, or irritants and involves a cascade of vascular and cellular events: vasodilation, increased vascular permeability, migration of leukocytes, and release of chemical mediators (histamine, prostaglandins, bradykinin, cytokines like TNF-α and IL-6). While protective, excessive or chronic inflammation contributes to diseases like arthritis, asthma, and autoimmune disorders [17,18].

One well-established in vitro marker of inflammation is protein denaturation — the alteration of a protein's secondary and tertiary structure due to heat, pH changes, or chemical stress. Denaturation of tissue proteins is a documented cause of inflammatory and autoimmune reactions, as denatured proteins can act as

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autoantigens, triggering conditions such as rheumatoid arthritis. Because of this well-documented link, inhibition of protein denaturation is used as a simple, reproducible, and cost-effective screening method for anti-inflammatory activity [19,20].

Materials And Methods

Uv- analytica, Egg albumin, Phosphate buffer (PH:6.4), 0.2m Potassium dihydrogen phosphate, Di sodium hydrogen phosphate, Diclofenac sodium, Incubator

Anti-inflammatory activity

Anti-inflammatory activity of *Nerium oleander* flower extract carried out by Egg Albumin Denaturation Method. The principle involved in this method is the Egg albumin (mainly ovalbumin) serves as a model protein. When exposed to heat (typically 70°C or higher) or extreme pH, it denatures and precipitates visible as turbidity. If a test substance (drug or plant extract) can prevent or reduce this denaturation, it is considered to possess anti-inflammatory potential, analogous to how NSAIDs stabilize proteins and reduce inflammatory tissue damage.

Core principle: Protection of protein structure ∝ anti-inflammatory activity. Denaturation of proteins (egg albumin) when heat is induced and thus the substances which prevent this denaturation are considered to have anti-inflammatory activity.

In-vitro anti-inflammatory activity of Nerium oleander flowers extract

The ability to inhibit heat-induced protein denaturation is measured by inhibiting the release of inflammatory mediators and stabilizing the proteins involved in this process, higher inhibition of protein denaturation indicates higher anti-inflammatory potential. To prepare 1% of egg albumin solution, weigh accurately 1 gm egg albumin powder and dissolve in 100 mL water and carefully mix, avoiding foam, then allow to stand for 10–15 minutes and filter it. 6.4 pH Phosphate buffer preparation was done by 0.2 M potassium dihydrogen phosphate (6.8 g) and 0.2 M disodium hydrogen phosphate (7.1 g) mixed in 250 mL distilled water respectively; this is considered as stock solution, and the buffer solution is prepared by mixing 77 mL of potassium dihydrogen phosphate and 23 mL of sodium hydrogen phosphate, which gives the pH of 6.4 approximately; the pH is then adjusted using addition of potassium dihydrogen phosphate to decrease the pH and sodium hydrogen phosphate to increase the pH of the solution. The solution of flower extract and standard drug (diclofenac sodium) with concentrations 100–500 µg/mL was prepared by following standard procedure; then from the above solutions, 2 mL from each concentration of extract and standard drug were collected in different test tubes, to which 2.8 mL phosphate buffer and 0.2 mL egg albumin solution were added. The test tubes were then incubated at 37°C for 15 minutes, heated at 70°C for 5 minutes in a heating mantle, and allowed to cool at room temperature. Absorbance was then measured at 660 nm using a UV–Visible spectrophotometer.

Percentage inhibition (%) = $[(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$

Results

The absorbance values and percentage inhibition of heat-induced egg albumin denaturation obtained for *Nerium oleander* flower extract and diclofenac sodium (standard) at concentrations of 100–500 µg/mL are summarized in Table 1. The extract produced a concentration-dependent increase in percentage inhibition, from 7.14% at 100 µg/mL to 85.7% at 500 µg/mL, while diclofenac sodium showed inhibition ranging from 35.7% to 92.8% over the same concentration range. The control absorbance (without extract or standard) remained constant at 0.14 for both sets. This concentration-dependent trend is illustrated graphically in Figure 1.

The IC_{50} comparison of the flower extract against ascorbic acid (standard) in the same assay system is presented in Table 2, showing a percentage absorbance of 85.7 for the extract against 92.8 for ascorbic acid.

Discussion And Conclusion

The present study evaluated the in-vitro anti-inflammatory activity of *Nerium oleander* flower extract using the egg albumin denaturation method, with diclofenac sodium employed as the standard reference drug. Protein denaturation is recognized as one of the principal mechanisms underlying inflammatory processes; therefore, inhibition of heat-induced protein denaturation was used as a preliminary in-vitro screening approach for anti-inflammatory activity.

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As presented in Table 1 and illustrated in Figure 1, the *Nerium oleander* flower extract exhibited a clear concentration-dependent inhibition of protein denaturation. At concentrations of 100, 200, 300, 400, and 500 µg/mL, the extract produced 7.14%, 28.6%, 42.9%, 64.2%, and 85.7% inhibition, respectively, compared with 35.7%, 50%, 57.1%, 78.5%, and 92.8% inhibition recorded for diclofenac sodium at the same concentrations. This progressive increase in inhibition with rising concentration confirms a dose-dependent protective effect of the extract against heat-induced albumin denaturation.

Although the maximum inhibition achieved by the extract (85.7% at 500 µg/mL) remained slightly lower than that of the standard drug (92.8%), the gap between the two narrowed at higher concentrations, indicating that the extract's anti-inflammatory efficacy approaches that of diclofenac sodium within this range. This suggests that *Nerium oleander* flower extract exerts a genuine, concentration-responsive protective effect on protein structure rather than a marginal or non-specific one.

The anti-inflammatory potential of the extract may be attributed to the bioactive phytoconstituents reported in *Nerium oleander* flowers, including phenolics, tannins, flavonoids, and terpenoids. These constituents are known to stabilize protein structure and modulate the release of inflammatory mediators and may therefore act synergistically to protect albumin from heat-induced denaturation. However, the present assay alone cannot establish the precise mechanism of action or identify which individual constituents are principally responsible for the observed activity.

Overall, the findings indicate that *Nerium oleander* flower extract possesses promising in-vitro anti-inflammatory activity, particularly at higher concentrations. Further studies employing additional in-vitro and in-vivo models, together with isolation and structural characterization of the active constituents, are required to confirm and more fully understand its anti-inflammatory potential.

The present study demonstrates that *Nerium oleander* flower extract possesses significant in-vitro anti-inflammatory activity, as evidenced by its ability to inhibit heat-induced egg albumin denaturation (Table 1, Figure 1). The extract exhibited a concentration-dependent increase in percentage inhibition, reaching a maximum of 85.7% at 500 µg/mL, compared with 92.8% recorded for diclofenac sodium at the same concentration.

This activity may be attributed to the bioactive phytoconstituents present in the flower extract, particularly phenolics, flavonoids, tannins, and terpenoids, which are known to stabilize protein structure and modulate inflammatory processes. These findings suggest that *Nerium oleander* flower extract may serve as a promising natural source of anti-inflammatory compounds. Nevertheless, further investigation is necessary to isolate the active constituents, elucidate their mechanisms of action, and confirm their efficacy and safety through appropriate in-vivo and clinical studies.

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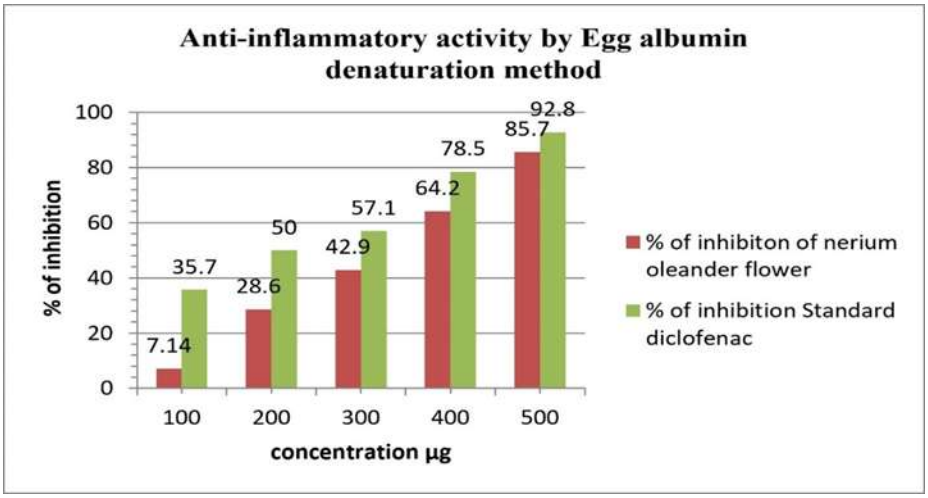
Figure Legends

Figure 1 : Concentration-dependent percentage inhibition of egg albumin denaturation by Nerium oleander flower extract compared with diclofenac sodium (standard), demonstrating dose-dependent anti-inflammatory activity of the extract.



TABLE/S

Table 1 : Absorbance values and percentage inhibition of egg albumin denaturation by *Nerium oleander* flower extract and diclofenac sodium (standard) at 660 nm, showing concentration-dependent anti-inflammatory activity of the extract

Concentration (µg/mL)	Absorbance of Extract at 660 nm	Absorbance of Standard Drug at 660 nm	% Inhibition (Diclofenac)	% Inhibition (Plant Extract)
100	0.13	0.09	35.7%	7.14%
200	0.10	0.07	50%	28.6%
300	0.08	0.06	57.1%	42.9%
400	0.05	0.03	78.5%	64.2%
500	0.02	0.01	92.8%	85.7%
Control	0.14	0.14	–	–

Table 2 : IC_{50} comparison of *Nerium oleander* flower extract and ascorbic acid (standard) in the egg albumin denaturation assay, indicating the relative antioxidant potential of the extract.

Method Used	% Absorbance of Extract	% Absorbance of Ascorbic Acid
Egg Albumin Denaturation Method	85.7	92.8

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