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RESEARCH ARTICLE

In-vitro anti-inflammatory potential of *Nelumbo nucifera* Rhizomes

Archana Shantaram Gadakh*[1], Abhijeet Dattatraya Kulkarni[2]

[1] *Research Scholar, School of Pharmaceutical Sciences, Sandip University, Nashik, Maharashtra, India*
[2] *Associate Professor, School of Pharmaceutical Sciences, Sandip University, Nashik, Maharashtra, India*

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Abstract: *Nelumbo nucifera*, commonly known as sacred lotus, is an aquatic plant with significant medicinal properties utilized in traditional Chinese and Indian medicine for centuries. This study investigated the in-vitro anti-inflammatory potential of aqueous and hydroalcoholic extracts of *N. nucifera* rhizomes through protein denaturation inhibition and red blood cell membrane stabilization assays. The rhizomes were collected, authenticated, and extracted using both aqueous and 80% hydroalcoholic solvents through Soxhlet extraction. Preliminary phytochemical screening revealed the presence of various bioactive compounds including alkaloids, glycosides, triterpenoids, flavonoids, tannins, and steroids in the hydroalcoholic extract, while the aqueous extract contained flavonoids, saponins, tannins, steroids, and triterpenoids. Both extracts demonstrated concentration-dependent anti-inflammatory activity across the tested concentrations (100, 200, and 500 µg/mL). At 500 µg/mL, the hydroalcoholic extract showed maximum protein denaturation inhibition of 54.18%, while the aqueous extract exhibited 49.50% inhibition. In the membrane stabilization assay, the hydroalcoholic and aqueous extracts at 500 µg/mL demonstrated significant protection against red blood cell hemolysis, showing 78.32% and 70.46% protection, respectively, compared to 84.12% protection by the standard drug aspirin at 200 µg/mL. The results suggest that *N. nucifera* rhizome extracts possess promising anti-inflammatory properties, potentially attributed to their ability to stabilize cell membranes and prevent protein denaturation.

Keywords: *Nelumbo nucifera*; Anti-inflammatory activity; Membrane stabilization; Protein denaturation; Rhizome extracts.

1. Introduction

Nelumbo nucifera, commonly known as sacred lotus or Indian lotus, is a perennial aquatic plant that has garnered significant attention in traditional medicine systems worldwide [1]. The plant belongs to the monogeneric family Nymphaeaceae and exists in two species globally: *N. nucifera* Gaertn, predominantly found in Asia and Australia, and N. lutea Willd (American lotus), native to North America [2, 3]. Throughout history, various parts of *N. nucifera*, including leaves, seeds, flowers, and rhizomes, have been utilized for their therapeutic properties. The plant's significance is particularly notable in Chinese medicine, where it has been documented in classical texts for over four centuries [4]. The rhizomes, which are the underground stem modifications, have emerged as a particularly valuable part of the plant, containing a rich array of bioactive compounds [5].

Figure 1. *Nelumbo nucifera* flowers and leaves (left) and rhizomes (right)

* Corresponding author: Archana Shantaram Gadakh

The chemical composition of *N. nucifera* rhizomes is diverse and nutritionally significant. Fresh rhizomes contain approximately 83.80% water, 9.25% starch, 2.70% crude protein, and various essential nutrients including riboflavin (0.6 mg/100g), thiamine (0.22 mg/100g), and niacin (2.10 mg/100g). Additionally, the presence of asparagine-like compounds, amino acids (2%), and ascorbic acid (1.5 mg/100g) contributes to its nutritional value [6]. Inflammation, while being a crucial defensive mechanism in tissue repair and host defense, can also lead to various pathological conditions when uncontrolled [7]. Current therapeutic approaches primarily rely on non-steroidal anti-inflammatory drugs (NSAIDs), which, despite their effectiveness, are associated with numerous adverse effects including gastrointestinal complications, hepatotoxicity, and renal disorders [8]. This scenario has prompted increased interest in plant-based alternatives, which often exhibit better physiological compatibility and fewer side effects [9].

Previous investigations have identified several bioactive compounds in *N. nucifera* rhizomes, including betulinic acid, a steroidal triterpenoid with potential therapeutic properties [10]. The optimal extraction of these constituents has been reported with 50% (v/v) alcohol, suggesting the importance of solvent selection in obtaining beneficial compounds [11]. This study aims to evaluate the in-vitro anti-inflammatory potential of *N. nucifera* rhizomes using both aqueous and hydroalcoholic extracts. The investigation focuses on two crucial mechanisms of inflammation: protein denaturation and membrane stabilization, which are fundamental to understanding the anti-inflammatory properties of natural compounds [12].

2. Materials and methods

2.1. Plant Material Collection and Authentication

N. nucifera rhizomes were sourced from Om Lotus Garden, Pune, India. The botanical identity was authenticated by Dr. A. Benfiamin, Scientist F & Head of Office, Botanical Survey of India, Western Regional Centre, Pune (Voucher specimen No. BSI/WRCTech./2024/JVD-60).

2.2. Preparation of Plant Extract

The collected rhizomes underwent thorough washing to remove extraneous materials, followed by shade drying [13]. The dried material was pulverized into a fine powder and subjected to two different extraction methods. For hydroalcoholic extraction, the powdered rhizomes were extracted using 80% ethanol-water mixture in a Soxhlet apparatus. The extraction process continued until complete extraction was achieved. The resulting extract was concentrated using a water bath, yielding 8.2% w/w of dried extract. Similarly, for aqueous extraction, the powder underwent extraction using distilled water in a Soxhlet apparatus. The extract was concentrated to dryness, yielding 6.9% w/w of the final product [14].

2.3. Phytochemical Screening

Both extracts underwent preliminary phytochemical analysis following standard protocols described by Pavani and Shasthree [15] to identify major chemical constituents including alkaloids, glycosides, tannins, amino acids, proteins, terpenoids, steroids, flavonoids, and saponins. The screening procedures were performed according to established methodologies [16].

2.4. Anti-inflammatory Activity Assessment

The protein denaturation inhibition assay was conducted using fresh hen egg albumin. The assay mixture comprised fresh hen egg albumin (0.2 mL), phosphate buffer saline (2.8 mL, pH 6.4), and extract solution (2.0 mL) at varying concentrations (100, 200, and 500 µg/mL). The mixtures were incubated at 37°C for 15-20 minutes, followed by heating at 70°C for 5 minutes. After cooling, absorbance was measured at 660 nm using a UV spectrophotometer. Aspirin served as the standard reference drug at concentrations of 100 and 200 µg/mL [17].

Protein denaturation inhibition as a percentage was calculated using the following formula:

$$\% \text{ inhibition} = [Vt / Vc - 1] \times 100$$ Where, Vt = absorbance of test sample, Vc = absorbance of control.

For the red blood cell membrane stabilization assay, rat blood was collected from healthy adults and mixed with Alsever's solution containing sodium citrate (0.8%), dextrose (2.0%), NaCl (0.42%), and citric acid (0.5%). The prepared RBC suspension (10% v/v) was mixed with hyposaline solution (2.0 mL, 0.36%), phosphate buffer (1.0 mL, pH 7.4), and test extracts at concentrations of 100, 200, and 500 µg/mL [18, 19]. The mixtures were incubated at 37°C for 30 minutes, followed by centrifugation at 3000 rpm for 20 minutes. The supernatant's hemoglobin content was measured spectrophotometrically at 560 nm.

Percentage protection = [(OD1-OD2)/OD1] x 100 OD1 means Optical density of drug treated sample, OD2 means Optical density of control

2.5. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean ± SEM. The data analysis included calculation of percentage inhibition for protein denaturation and percentage protection for membrane stabilization using standard formulae as described by Ramalingam [20]

3. Results and Discussion

3.1. Phytochemical Analysis

The preliminary phytochemical screening revealed distinct chemical profiles for both extracts. The hydroalcoholic extract demonstrated the presence of glycosides, triterpenoids, alkaloids, tannins, steroids, and flavonoids, while the aqueous extract contained tannins, saponins, flavonoids, steroids, and triterpenoids [21]. These findings align with previous studies that have identified similar bioactive compounds in *N. nucifera* rhizomes.

3.2. Protein Denaturation Inhibition

Both extracts exhibited concentration-dependent inhibition of protein denaturation, a key mechanism in inflammatory processes. The hydroalcoholic extract demonstrated superior activity compared to the aqueous extract, with maximum inhibition of 54.18% at 500 µg/mL, while the aqueous extract showed 49.50% inhibition at the same concentration. The reference drug aspirin exhibited 52.20% inhibition at 200 µg/mL [22].

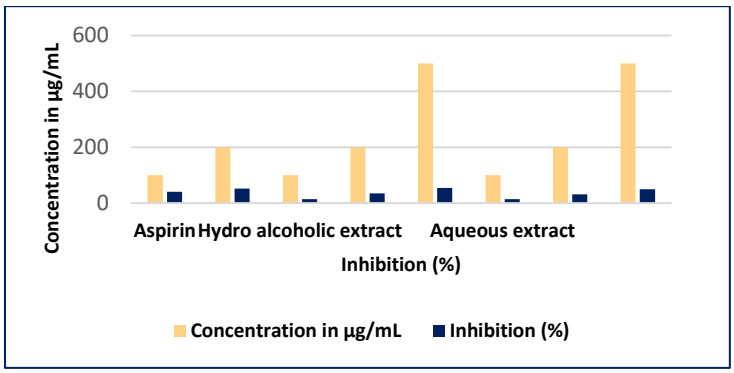

Figure 1. Effects of *Nelumbo nucifera* extracts against denaturation of protein

3.3. Membrane Stabilization Activity

The membrane stabilizing effects of both extracts were evaluated through their ability to prevent hypotonic solution-induced erythrocyte hemolysis. The hydroalcoholic extract demonstrated significant membrane stabilization with 78.32% protection at 500 µg/mL, while the aqueous extract showed 70.46% protection at the same concentration. These results were comparable to the standard drug aspirin, which provided 84.12% protection at 200 µg/mL [23].

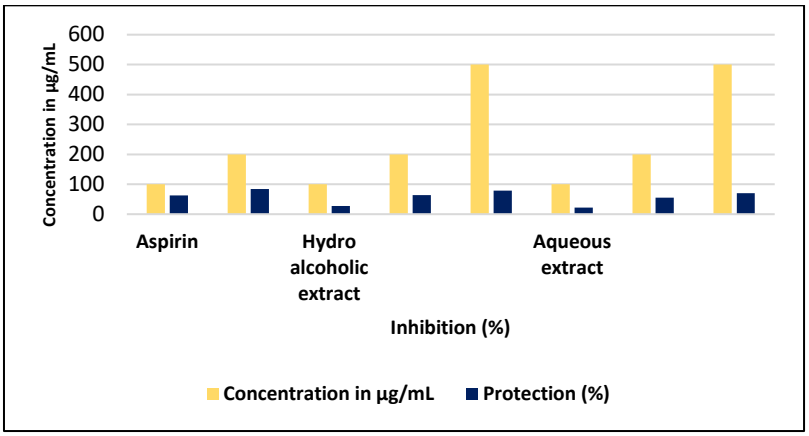

Figure 2. Effect of *Nelumbo nucifera* extracts to stabilize red blood cells

3.4. Discussion

The observed anti-inflammatory activity can be attributed to multiple mechanisms. The protein denaturation inhibition suggests that the extracts may prevent the formation of autoantigens, which are commonly associated with inflammatory conditions such as rheumatoid arthritis [24]. The similarity between lysosomal membranes and erythrocyte membranes implies that the membrane-stabilizing effect of the extracts could potentially prevent the release of lysosomal contents during inflammation [25].

The higher efficacy of the hydroalcoholic extract compared to the aqueous extract might be due to the better extraction of both polar and non-polar bioactive compounds. This observation corresponds with previous studies indicating that hydroalcoholic solutions are more efficient in extracting a broader range of phytochemicals from medicinal plants [26]. The presence of various phytochemicals, particularly flavonoids and triterpenoids, may contribute to the observed anti-inflammatory effects. These compounds are known to interfere with various phases of the inflammatory process, including the inhibition of pro-inflammatory mediators and the stabilization of cellular membranes [27]. The results demonstrate that *N. nucifera* rhizome extracts possess significant anti-inflammatory potential through multiple mechanisms, including protein denaturation inhibition and membrane stabilization. These findings provide scientific validation for the traditional use of *N. nucifera* rhizomes in treating inflammatory conditions [28].

4. Conclusion

The present investigation demonstrates significant in-vitro anti-inflammatory potential of both hydroalcoholic and aqueous extracts derived from *Nelumbo nucifera* rhizomes. The hydroalcoholic extract exhibited superior anti-inflammatory activity compared to the aqueous extract, suggesting that the combination of water and alcohol provides optimal extraction of bioactive compounds. The observed protein denaturation inhibition and membrane stabilization effects provide mechanistic insights into the anti-inflammatory properties of these extracts. These findings support the traditional therapeutic applications of *N. nucifera* rhizomes in inflammatory conditions and suggest their potential development as natural anti-inflammatory agents.

Compliance with ethical standards

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Conflict of interest statement

The authors declare no conflict of interest.

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Author's short biography

Ms. Archana Shantaram Gadakh

Ms. Archana Shantaram Gadakh currently working as Assistant Professor in PES Modern college of Pharmacy (for ladies) Moshi Pune, Maharashtra. She is perusing her Ph.D. from School of Pharmaceutical Sciences in Sandip University, Nashik, Maharashtra. She has 6 years of teaching and research experience. She has published many research and review articles along with 2 patent and 1 book with national and international publishers. Her research expertise lies in the Nano drug delivery especially in Nano fibers

Dr. Abhijeet Dattatraya Kulkarni

Dr. Abhijeet Dattatraya Kulkarni currently working as Associate Professor in School of Pharmaceutical Sciences in Sandip University, Nashik, Maharashtra. He has earned his Ph.D. from North Maharashtra University Jalgaon (M.S.). He has 13 years of teaching and research experience. He has to his credit more than 42 International and national publications in journals of repute with cumulative impact factor of more than 54.45 and having 700+ citations with H-Index of 12 & I10- Index of 14. He has published 1 patent, 3 books and 4 book chapters with national and international publishers. He has received various grants from University Grant Commission (UGC), Department of Biotechnology (DBT), All India Council of Technical Education (AICTE) and recently his proposal is shortlisted with MSME worth around 1.3 cr. At first stage of evaluation. He is recipient of Best Researcher Award (2021), Research Excellence Award (2020) and Young Achiever Award (2019) by the Institute of Scholars. His research expertise lies in the phytochemical drug delivery, nose to brain drug delivery, Nano drug delivery.