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Original Article

Phytochemical Analysis and Tissue Restoration Activity of *Nelumbo nucifera* Extract

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ABSTRACT

Background: Flavonoids, alkaloids, tannins, saponins, and phenolic substances are among the several secondary metabolites that have been identified by phytochemical analysis of *Nelumbo nucifera*. The hepatoprotective, anti-inflammatory, wound-healing, and antioxidant qualities of these bioactive components are well-known. Since oxidative stress is a major contributor to tissue and cellular damage, these substances antioxidant activity is especially important in reducing it, especially when it comes to organ repairs, wound healing, tissue restoration. **Materials and Methods:** The purpose of the study was to assess the phytochemical components and potential for tissue restoration of *Nelumbo nucifera* extract. *N. nucifera* plants were collected, cleaned, dried in the shade, and ground into powder. Soxhlet extraction was used to extract the powdered material using a hydro-alcoholic solvent. Preliminary phytochemical analysis was undertaken using standard qualitative methods to determine the presence of bioactive components such as alkaloids, flavonoids, phenols, saponins, tannins, glycosides, and terpenoids. The tissue restoration activity was conducted on Wistar albino rats. **Results:** The qualitative and quantitative analysis of *Nelumbo nucifera* extract revealed the presence of various bioactive compounds. Total tannin Content of *Nelumbo nucifera* Extract was found 16.93, 37.34, and 42.44 (µg/mL) for hydro alcoholic, Ethanolic and Aqueous extract. *Nelumbo nucifera* extract significantly enhanced tissue restoration. **Conclusion:** *Nelumbo nucifera* extract is abundant in bioactive phytochemicals, such as flavonoids, alkaloids, tannins, and phenolic compounds, which are recognized for their restorative and antioxidant qualities, as the current study has effectively shown. As demonstrated by the improved healing and regeneration in the treated tissues, the extract demonstrated strong healing and repair activity. According to these results, *Nelumbo nucifera* may be used therapeutically for wound healing and tissue restoration.

Keywords: *Nelumbo nucifera*, Antioxidant, Tissue restoration, Extraction.

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INTRODUCTION

Modern pharmacology and traditional healing systems have long relied on medicinal plants because of their wide range of bioactive chemicals.[1] Among these, the sacred lotus, or *Nelumbo nucifera* Gaertn., is widely used in Southeast Asian, Chinese, and Ayurvedic medicine.[2] All parts of the plant, including the seeds, leaves, flowers, and rhizomes, have been used to treat a variety of illnesses, from skin conditions and diarrhoea to fever and inflammation.

It is well known that *Nelumbo nucifera* contains a diverse range of phytochemicals, including phenolic compounds, alkaloids, tannins, terpenoids, and flavonoids. These secondary metabolites are linked to a wide range of pharmacological characteristics, such as antibacterial, anti-inflammatory, wound-healing, and antioxidant effects.[3,4] *N. nucifera* extract's traditional use in wound and skin care has been supported by recent scientific studies that have shown its potential to modulate oxidative stress and enhance tissue restoration processes. For organ function to be maintained, tissue damage from disease, burns, or injuries must be effectively and promptly restored. With the rise in antibiotic resistance and the negative effects of synthetic medications, there is a great demand for natural solutions that promote tissue regeneration while reducing inflammation and microbial infection. In this regard, plant-based remedies offer a more secure and frequently cost-effective substitute. Analysing the phytochemical components of *Nelumbo nucifera* extract and assessing its capacity for tissue healing are the objectives of this study.[5,6]

MATERIALS AND METHODS

The *Nelumbo nucifera* plant was chosen for investigation. The Plant *Nelumbo nucifera* were obtained from Shreenath Agro Private Ltd., located in Pune, Maharashtra, India. The plant was

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verified as *Nelumbo nucifera* (Family: Nelumbonaceae) by the Botanical Survey of India, Western Regional Centre.

Drying and Grinding

After acquiring the *Nelumbo nucifera* plant, they were left to dry in the shade for two weeks. Continuous monitoring was conducted to avoid microbial contamination. The dried plant materials were extracted and processed to produce a fine powder. To achieve even finer particles, the powder was additionally gone through a 2 mm mesh. The samples were kept in a clean glass jar at a reduced temperature until they were needed for the study.[6,7]

Extraction of Plant

Aqueous extract

The *Nelumbo nucifera* plant was dried and pulverised before being placed into container. The sample was soaked in water for seven days. After this period, the solid material was separated by filtration, and the residue was subsequently pressed. The solvent was eliminated using a rotary evaporator. Finally, the extract was weighed, and its ratio to the weight of the plant material after air drying was determined.

Hydro alcoholic extract

The plant of *Nelumbo nucifera* Plant. was dehydrated, crushed, and then placed in a container. The sample was soaked in a mixture of water and ethanol (in a ratio of 6:4) for a week. Afterward, the solid residue was isolated by means of filtering, and the residue was then pressed. The solvent was removed by rotary evaporator. Finally, the weight of the extract was measured, and ratio to the weight of the plant material after air drying was determined.[8-10]

Chloroform extract

The plant of *Nelumbo nucifera* were desiccated, pulverized, and stored in a glass container. The sample was immersed in Chloroform for a duration of seven days. Afterwards, the marc was separated through filtration and the remaining marc were pressed. The solvent was then evaporated by rotary evaporator. Finally, the sample was measured, and the ratio of the weight of the plant material after air drying was determined.

Ethanol extract

The plants of *Nelumbo nucifera* were desiccated, powdered, and kept in a vessel. The specimen was immersed in ethanol for a period of seven days. Afterwards, the solid material was separated by filtering, and the remaining marc was then pressed. The solvent was removed by rotary evaporator. Finally, the extract was weighed, and the weight of the plant material after air drying was determined.[7-10]

Phytochemical Analysis

The crude extracts were examined for different phytoconstituent by conducting the following phytochemical tests.

Tests for detection of Carbohydrates

Molish's test: To conduct the test, add two to three drops of α-naphthol along with 1 mL of concentrated H_2SO_4 to 2 mL test solution, ensuring to pour from the edges of the test tube to create distinct layers. The formation of a purple color band at the interface of the liquids in the test tube showed the existence of carbohydrates.

Test for detection of Reducing Sugars

Benedict's test: 1 mL test solution mixed with 2 mL of Benedict's reagent, then the mixture was boiled and allowed to sit. The appearance of a red colour precipitate signifies the existence of sugars.

Test for detection of Monosaccharides

Barfoed's test: 2 mL of Barford's reagent were introduced into 1 mL test solution, then boiled for 2 min and allowed to sit. The emergence of a red precipitate indicates the presence of sugar.

Tests for detection of Proteins

Biuret test: 2 mL of 4% sodium hydroxide solution and several drops of 1% $CuSO_4$ were mixed with 3 mL test solution. The appearance of a violet or pink color indicated the existence of proteins.

Tests for detection of Amino Acids

Ninhydrin test: Mix 2 mL test solution with 2 drops of 5% Ninhydrin solution and place it in a boiling water bath for 10 min. The development of a violet or bluish hue signifies the existence of amino acids.

Tests for detection of Glycosides

Keller Killani test

The Glacial acetic acid, along with one drop of 5% $FeCl_3$ and conc. Sulphuric acid, was mixed into extract. The emergence of a rusty brown tint at the boundary of the liquid layers, with a bluish-green tint observed in the upper layer, indicated the existence of cardiac glycosides.

Test for detection of Flavonoids

Shinoda test

95% ethanol (5 mL), three drops of HCl, along with 0.5 g of magnesium turnings were mixed with dry extract of tin. The presence of a pink color signified the existence of flavonoids.

Test for detection of Alkaloids

The extract was allowed to evaporate. Dilute hydrochloric acid was introduced to the remaining residue; it was thoroughly shaken and then filtered. The tests were conducted using the filtrate obtained.

Mayer's test

Several drops of Mayer's reagent are mixed with the filtrate. The existence of alkaloids is signalled by the development of a precipitate.

Test for detection of Terpenoids

Knoller's test

Extract was treated with 0.1% anhydrous stannic chloride in pure thionyl chloride, resulting in a purple hue that shifted to reddish, suggesting the existence of terpenoids.

Test for detection of Tannins and Phenolic Compounds

To 2-3 mL of the aqueous extract, several drops of the subsequent reagents were introduced.

A 5% solution of $FeCl_3$ resulted in a deep blue-black coloration. The Lead Acetate Solution produced a white precipitate.[11-14]

Determination of Tannin Contents

The tannins were measured through Folin-Ciocalteu technique. 0.5 mL of Folin-Ciocalteu phenol reagent, 1 mL of a 35% sodium carbonate solution, and approximately 0.1 mL of each sample extract were mixed. The mixture was then diluted to a final volume of 10 mL using distilled water. After thoroughly mixing the contents, the solution was allowed to stand at room temperature for 30 min. A series of standard solutions of tannic acid (20, 40, 60, 80, and 100 µg/mL) was prepared using the same procedure. The absorbance of both the sample and standard solutions was then recorded at 700 nm with a UV/visible spectrophotometer, using a blank for reference. To assess the tannin concentration, three individual measurements were performed.[15]

Determination of Total Phenolic Contents in the plant extracts

The Folin-Ciocalteu's reagent spectrophotometric technique was used to determine the total phenolic contents. For analysis, the extract methanolic solution with concentrations of 1 mg/mL and 0.5 mg/mL was utilized. A reaction mixture was created by combining 2.5 mL of 10% Folin-Ciocalteu's reagent, 2.5 mL of 7.5% $NaHCO_3$, and 0.5 mL of methanolic extract solution. Blank included 2.5 mL of 7.5% $NaHCO_3$, 0.5 mL of methanol, and 2.5 mL of 10% Folin-Ciocalteu's reagent. After that, the samples were incubated for 45 min at 45°C. A UV spectrophotometer set at λ_{max} 765 nm was used to measure the absorption. The calibration line and the standard gallic acid solution followed the same process. The amount of phenolics in extracts was then expressed as gallic acid equivalent after the concentration of phenolics (mg/mL) was determined from the calibration line based on the observed absorbance.[16]

Determination of Total Flavonoids Contents in the plant extracts

The aluminium chloride colorimetric method was used to determine the extract's total flavonoid concentration. For the analysis, an ethanolic solution containing 1 mg/mL of the extracts was utilized. After 5 min, 0.3 mL of a 10% $AlCl_3$ solution was added, and the combination was let to stand for 6 min. 50 µL of a crude extract built up to 1 mL with methanol was combined with 4 mL of distilled water and then 0.3 mL of 5% NaNO2 solution. After that, 2 mL of 1M NaOH solution was added, then double-distilled water was added to bring the mixture's final volume to 10 mL. After 15 min of standing, the mixture's absorbance was measured at 510 nm. A calibration curve was used to determine the total flavonoid concentration, which was then represented as mg of quercetin equivalent per gram of dry weight.[17]

In vivo Tissue Restoration Activity

Adult Wistar albino rats were split into six groups at random, each containing six rats, and housed individually (one rat per cage) with weights ranging from 170 to 200 g. The rats given a standard diet consisting of four types of pellet feed along with tap water. Their fur was shaved using an electric razor, then disinfected with 70% alcohol before receiving an injection of 1 mL Lignocaine HCl (2%, 100 mg/5 mL). A wound of same size, 2 cm in diameter, was removed from the dorsal region of the neck region at the nape of all rats using a round seal. Care was taken to avoid slicing into the muscle layer, ensuring that skin tension is evenly distributed throughout the process. The control group receive treatment with 0.5 mg of povidone-iodine ointment applied to the wound with cotton once daily for a total of 18 days, beginning on the day of injury. The six groups each receive specific medications applied externally at a dose of 0.5 mg to the wound with cotton once a day for 18 days. Observations of the wound area and the percentage of wound closure were documented on the 3rd, 6th, 9th, 12th, 15th, and 18th days following the injury.[18]

RESULTS

Extraction of Plant Material

The harvesting of plant components is a procedure designed to isolate specific compounds found in plants. The plant of *Nelumbo nucifera* were extracted through cold maceration utilizing different solvents including water, ethanol, and a water-ethanol mixture in a 6:4 ratio. Based on solubility of the chemical components, the ethanol and the water-ethanol mixture were chosen and enhanced for additional investigation.

% Yield / Colour / Nature

An analysis was conducted on the crude extracts for the existence of specific parameters including Color, Percentage Yield, and Nature of Extract. Table 1 shows the Color, % Yield, and Nature of extracts obtained from different solvents. The highest % Yield was observed in the hydro alcoholic solvent, which was 18.13%. The extract was noted to have a brownish-black color. All extracts were characterized as semi-solid in nature.

Preliminary Phytochemical Analysis

The presence of the crude extracts was monitored for various phytoconstituent by performing the following Phytochemical Tests shown in Table 2.

Determination of Tannin Contents

The plant kingdom encompasses a vast array of phytochemicals that can help lower the likelihood of various diseases. According to the curve of calibration the total phenolic content found in plant has been determined. The extract derived from *Nelumbo nucifera* has undergone a different chemical test to identify the presence of several constituents, including flavonoids, carbohydrates, alkaloids, tannins glycosides, and proteins. The results, presented in the table, indicate the existence of necessary terpenoids and phenolics/tannins that contribute to the activity in the optimized extract sample (1 mg/mL). Figure 1 showing the calibration curve for Tannic acid. It was observed that the concentration amounted to 37.34 µg/mL shows in Table 3.

Determination of Total Phenolic Contents in the plant extracts

Bioactive non-nutrient substances called phytochemicals are widely distributed in plants and have the ability to reduce the risk of several illnesses. Significantly, phenolic can be mono- or polyhydroxylated and possess one or more aromatic benzene rings. In human cancers, phenolics are thought to be the most abundant antioxidants. Phenolic acids, stilbenes, tannins, and coumarins are examples of non-flavonoids. Figure 2 shows Gallic Acid Calibration Curve for determination of Total Phenolic Content.

The total phenolic content of the Hydro alcoholic and Aqueous extract (1mg/mL), calculated from the calibration curve ($R^2 = 0.998$), was found to be, 29.3± 2.354 µg/mL and 32.7± 0.643 µg/mL Gallic acid equivalents/g respectively. Table 4 shows the concentration of Total Phenolic Content in extract.

Determination of Total Flavonoids Contents in the plant extracts

Flavonoids are secondary plant metabolites responsible for the colour and aroma, they possess tissue repair activity. In the process of carcinogenesis, flavonoids obstruct with multiple signal transduction pathways and thus limit proliferation and angiogenesis. Figure 3 shows Quercetin Calibration Curve for detection of Total Flavonoid Content.

Table 5 shows the total flavonoid content of the Hydro alcoholic and Aqueous extract sample (1mg/mL), calculated from the

Table 1: Percentage Yield / Colour / Nature of *Nelumbo nucifera* Extract.

Solvent	Initial wt. of powder	Final wt. of powder	Wt. of Crude Extract	Colour of Extract	Consistency of Extract
Ethanol hydroxide	100 gm	90.11 gm	10.87 gm	Green	Semisolid
Water	100 gm	86.71 gm	13.19 gm	Green	Semisolid
Water: Ethanol hydroxide (6:4)	100 gm	83 gm	14.00 gm	Brown black	Semisolid

Table 2: Qualitative Analysis of Phytochemicals.

Sr. No	Phytochemicals	Inference		
		Water	Water: Ethanol (6:4)	Ethanol
1.	Carbohydrates	+	-	+
2.	Proteins	+	-	-
3.	Amino Acids	-	+	+
4.	Flavonoids	+	+	-
5.	Glycoside	+	+	+
6.	Alkaloids	+	+	-
7.	Terpenoids	+	-	-
8.	Tannins	+	+	-

Whereas, +: Present and-: Absent

calibration curve (R^2 = 0.9965), was 391.7±16.493 µg/mL, and 408.3±17.176 µg/mL respectively. Aqueous extract sample was found to contain the highest and significant flavonoids concentration. Figure 4 shows the wound healing in animal.

In vivo Tissue Restoration activity

For the conduction of the *In vivo* Tissue Restoration activity, acceptance was secured by the Institutional Animal Ethics Committee. The study on the tissue Restoration activity of extract in rat physiology indicated a notable improvement in the healing process as compared to the control groups. The

Table 3: **Total tannin Content of different extracts of *Nelumbo nucifera* Extract.**

Sample	Absorbance	Calculated Concentration (µg/mL)
Hydro alcoholic Extract	0.11	16.93
Ethanolic Extract	0.21	37.34

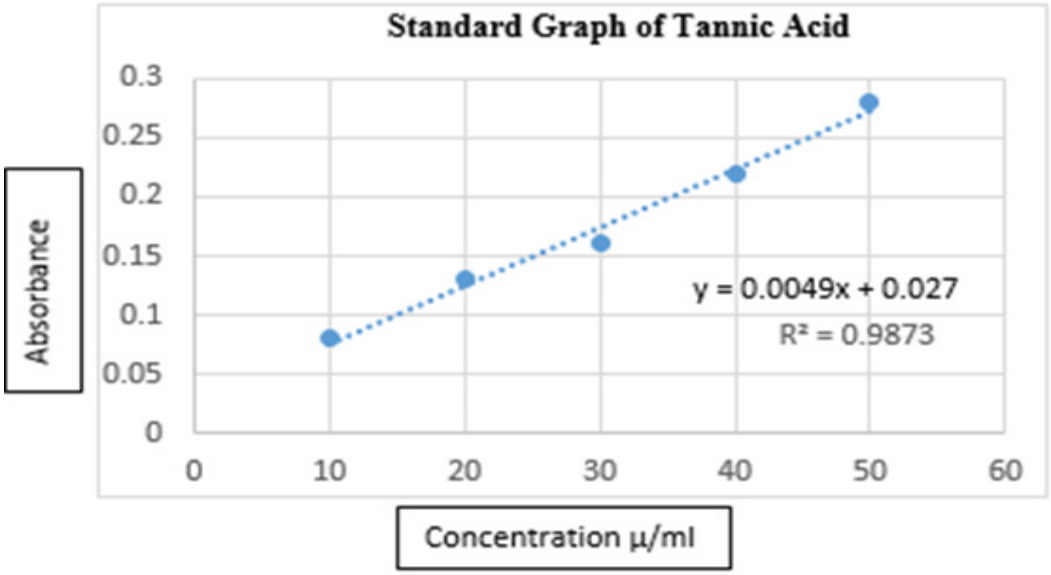

Figure 1: Standard Graph of Tannic Acid.

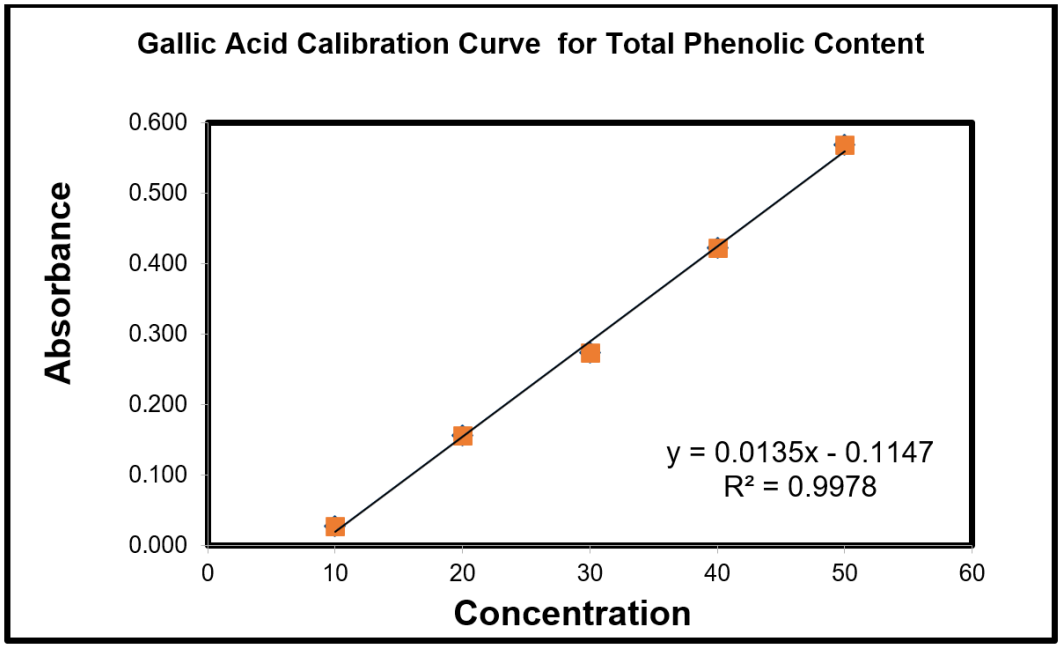

Figure 2: Gallic Acid Calibration Curve for Total Phenolic Content.

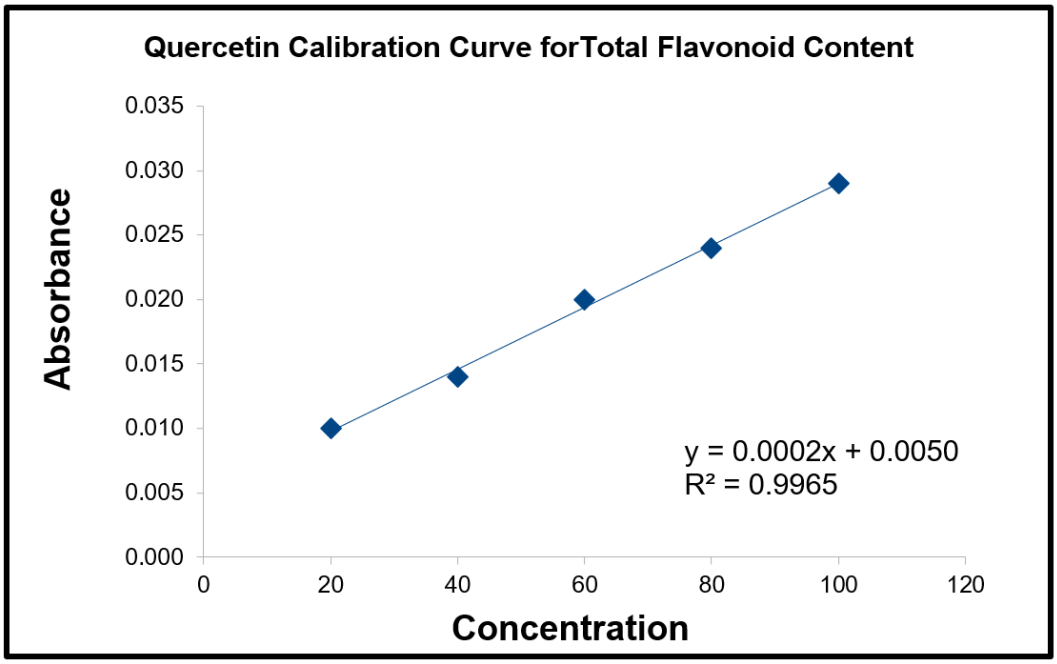

Figure 3: Quercetin Calibration Curve for Total Flavonoid Content.

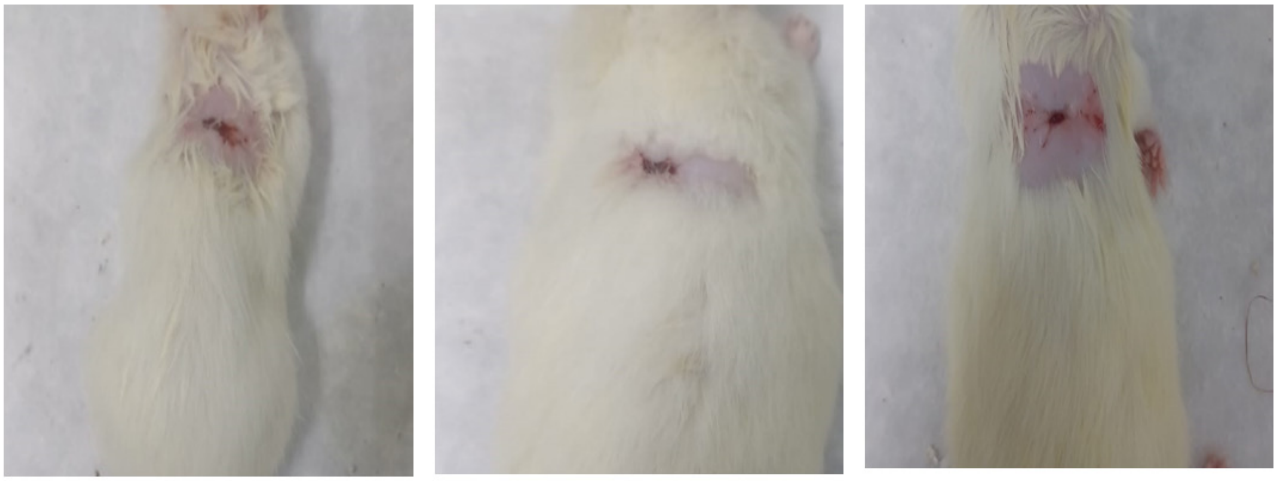

Figure 4: Healing of Wound of animal in each group.

Table 4: **Absorbance of Total Phenolic Content in Plant Extract.**

Sl. No	Sample	Absorbance (nm)	Calculated concentration (µg/mL)
1	Hydro alcoholic Extract	0.281	29.3± 2.354
2	Aqueous extract	0.327	32.7± 0.643

Table 5: **Absorbance of total Flavonoid Content in Plant Extract.**

Sl. No.	Sample	Absorbance (nm)	Calculated concentration (µg/mL)
1	Hydro alcoholic Extract	0.099	391.7±16.493
2	Aqueous extract	0.103	408.3±17.176

Table 6: Effect of *Nelumbo nucifera* Extract on wound area.

Groups	Day 0	Day 5	Day 8	Day 11	Day 14
Vehicle group	10 mm	13.3 mm	13.8 mm	13.9 mm	14.9 mm
Standard group	10 mm	10.8 mm	7 mm	6.5 mm	5 mm
Extract	10 mm	8.5 mm	7mm	5.6 mm	3.1 mm
Marketed Formulation	10 mm	9.8 mm	8 mm	7.3 mm	6.6 mm

analysis of repeated measurements illustrated that the wound area continually diminished over time. This indicates that the formulation effectively supports tissue healing. The investigation into the tissue Restoration activity of *Nelumbo nucifera* rhizome extract in Wistar Rats shows substantial promises for improving wound healing in these animals. Table 6 shows the effect of plant extract on wound.

DISCUSSION

The current investigation sought to assess the phytochemical profile and healing capabilities of *Nelumbo nucifera* extract. The results support the theory that *Nelumbo nucifera* serves as a valuable source of bioactive compounds with notable regenerative properties, affirming its historical medicinal applications. Phytochemical analysis indicated the existence of various bioactive substances, including flavonoids, alkaloids, tannins, and phenolic compounds. These findings align with earlier research that reported similar components in various parts of *Nelumbo nucifera*, particularly in its rhizomes. The notably high levels of flavonoids and phenolics are especially significant because of their well-established antioxidant and anti-inflammatory effects, which are crucial for tissue healing. The observed tissue restoration capabilities exhibited marked regenerative effects in damaged tissues treated with *Nelumbo nucifera* extract. The groups that received the extract displayed improved epithelial regeneration and a decrease in inflammatory cell infiltration. These results can be attributed to the combined effects of the phytochemicals found in the extract. Additionally, the presence of alkaloids and tannins may provide further antimicrobial and angiogenic benefits, which are vital for efficient tissue repair.

CONCLUSION

Nelumbo nucifera Extract enhances Restoration due to their *in vivo* Restoration and *in vitro* anti-inflammatory properties. The *in vitro* anti-inflammatory assay indicates a reduction in inflammation. This combination has potential benefits for tissue repair *in vivo.* The *in vivo* study suggests an enhancement in the speed of Restoration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

UV: Ultra-violate.

ETHICAL APPROVAL

All experiments were performed in accordance with the guidelines for care and use of laboratory animals. Ethical approval was granted by the committee with approval number SCI/IAEC/2024-25/89.

SUMMARY

Nelumbo nucifera, also referred to as the sacred lotus, is a plant characterized by a wide array of phytochemicals and potential abilities for tissue restoration. Research has indicated that extracts derived from rhizomes contain a multitude of bioactive compounds such as alkaloids, flavonoids, and terpenoids. These compounds have been associated with antioxidant, anti-inflammatory, and other advantageous properties, implying the plant's capability to support tissue health and regeneration.

This research examines the phytochemical profile and tissue healing capabilities of *Nelumbo nucifera* extract. Both qualitative and quantitative analyses of the phytochemicals indicated the presence of important bioactive compounds, including flavonoids, alkaloids, tannins, and phenols, known for their properties in antioxidants and anti-inflammatory actions. *In vivo* studies showed that the extract had notable effects on tissue regeneration, such as improved wound healing, decreased inflammation, and increased cellular proliferation. These findings imply that *Nelumbo nucifera* extract could serve as a beneficial natural therapeutic option for tissue repair and regenerative medicine.

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