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Phytochemical and wound healing potential of ethanolic extract of *Nymphaea alba*

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Abstract

The present study evaluates the effectiveness of the ethanolic extract of Nymphaea alba in wound healing and administers phytochemicals using "an excision and incision wound model on Wistar strain albino rats". Plant extracts have a higher phytoconstituent concentration, according to "phytochemical screening". "Phenolic compounds, alkaloids, carbohydrates, flavonides, glycosides, and tannin" were all detected in the ethanolic extract of Nymphaea alba. "The tensile strength of the healed wounds" was assessed using the incision wound model, and the influence of wound healing was assessed using the excision wound model, which took into consideration the epithelialisation time and the percentage of wound closure. Wistar albino rats' dorsal surfaces were subjected to excision and incision wounds. Four groups of six rats each were randomly selected from among them. Group I served as the control group, receiving no treatment other than a basic ointment. Group II was used with framycetin sulphate cream (Soframycin, Aventis) and was regarded as the standard group. Group III was given EENA 2% (w/w), while Group IV was given EENA 5% (w/w). These were considered test groups. Compared to the usual control group, Nymphaea alba significantly rises the percentage of wound contraction, reduces its duration of epithelialisation, and increases the tensile strength of the skin in all forms. In view of the positive results, it may be feasible to conduct additional research to evaluate the efficacy of various wound models.

Keywords; Nymphaea alba, Wound healing, Incision, Excision epithelialisation, tensile strength.

INTRODUCTION

A skin wound occurs when the epidermal layer's integrity is compromised. Any tissue injury that loses function and disturbs anatomical integrity is called a wound. Skin healing is the primary outcome of wound healing. When the epidermal layer is damaged, the wound begins to heal immediately and might take years to fully heal. This dynamic process includes highly organised humoral, molecular, and cellular systems. Proliferation, inflammation, and remodelling are the three overlapping phases of wound healing. Any disruption causes abnormal wound healing. Wound healing may sometimes be separated into primary and secondary healing. The simple healing of a clearly defined, non-infected wound is known as primary healing. The wounds from surgery are the best example of primary healing. If the wound's natural healing process is interrupted by "infection, dehiscence, hypoxia, or immune dysfunction", the secondary healing stage begins. During secondary healing, granulation tissue develops and is covered by epithelization. With these types of wounds, "infections and poor healing" are more common. The aquatic flowering plant Nymphaea alba, often called "the European white water lily, white water rose, or white nenuphar, belongs to the Nymphaeaceae family". "North Africa, Asia, Europe, and Tropical Asia (India)" are its native regions. "One of the cultivated species" known to exist at Ward's Lake, Shillong, Meghalaya, and other locations in India is Nymphaea alba. The active alkaloids nymphaeine and nupharine, as well as an aphrodisiac or an anaphrodisiac depending on the source, are found in Nymphaea alba. The petals and other flower parts are the most powerful, even if the roots and stalks are also used in traditional herbal therapy. The active alkaloids may be extracted using alcohol, which also intensifies the sedative effects. For hundreds of years, monks and nuns mashed the plant's root and combined it with wine as an aphrodisiac.

The authors of the first written medical textbooks upheld its practice while warning against taking high and frequent dosages. "Hydroxylyzable tannins, alkaloids, sterols, flavonoids, glycosides, and tannic and gallic acids" are abundant in Nymphaea alba. The traditional medical system employs all parts of the plant for therapeutic purposes. "Anodyne, astringent, cardiotonic, demulcent, antioxidant, sedative, antiscrophulatic, aphrodisiac, and anti-inflammatory" are some of its uses. Additionally, it calms and sedatively affects the nervous system, making it helpful for treating anxiety, sleeplessness, and other related conditions. "Renal oxidative stress and hyperproliferative response inhibition", as well as its anticarcinogenic properties. In order to cure vaginal discharges or pain, it may be used externally as a douche. Irritable bowel syndrome-related diarrhea is treated with a root decoction. It treats renal discomfort and bronchial catarrh. As a gargle, it is used to treat sore throats. The decotion is utilised to treat uterine cancer, according to studies. Additionally, diaphoresis is treated using it. Boils and abscesses are treated with it as a poultice when combined with "slippery elm (Ulmus rubra) or flax (Linum usitatissimum)".

METHOD AND MATERIAL

1. Selection of plant and authentication

“Botanists from the Department of Botany at Safia Science College in Bhopal” verified the authenticity of fresh Nymphaea alba flowers that were gathered from the open field and the cultivated farm.

2. Extraction of Plant

Soxhlation with continuous hot percolation was used for extraction. Nymphaea alba flowers that had been dried and ground up were put in a Soxhlet apparatus thimble. Petroleum ether (40–60°C) was first used as a nonpolar solvent for soxhlation, which was carried out at 60°C. Ethanol was used to remove the dried plant material (marc). To make sure that the plant marc had been used up or that the extraction procedure was finished, a colourless solvent was removed from the syphon tube and evaporated for residue. The absence of residue verified that the extraction process was finished. A rotating vacuum evaporator (Bucchi type) was used to evaporate the obtained extracts at 40°C. The dried extract was weighed using the following procedure, which also determined the yield percentage for each extract:

$$\text{Percentage yield} = \frac{Weight\ of\ (extract)}{Weight\ of\ (powdered\ drug\ taken)} \times 100$$

Physical Characteristics- The extract's solubility in "ethanol, water, acetone, chloroform, ethylacetate, and DMSO" was examined.

Phytochemical investigation- To identify the chemical components included in the extracted materials, a number of chemical tests were performed on them.

i. Tests for Carbohydrates

Molish Test

"Two drops of alcoholic α-naphthol solution" were added to two millilitres of ethanol extract in a test tube. Next, a careful injection of "1 millilitre of concentrated sulphuric acid" was made around the walls of the test tube. The formation of a violet ring at the junction indicates the presence of carbohydrates.

Fehling’s Test

"One millilitre of Fehling's A and Fehling's B solutions" were combined with one millilitre of ethanol extract in a test tube, and the combination was heated for ten minutes in a water bath. When decreasing sugar is present, a red precipitate will form.

Benedict’s Test

"Benedict's reagent and ethanol extract" were mixed in equal parts in a test tube, and the mixture was heated for five to ten minutes in a water bath. Whether the test solution appears "green, yellow, or red" depends on how much reducing sugar is added.

ii. Tests for Protein and Amino acids

Biuret’s Test

The extract was boiled in a test tube after "one millilitre of a 10% sodium hydroxide solution was added". "A drop of a 0.7% copper sulphate solution" was added to the mixture above. The development of a violet or pink colour indicates the presence of proteins.

iii. Tests for Glycosides

Borntrager’s Test

3 millilitres of test solution were treated with diluted sulphuric acid, which was then boiled for five minutes and subsequently filtered. The cooled filtrate was well agitated before an equal quantity of either benzene or chloroform was added. After the organic solvent layer was separated, ammonia was added. A pink to red ammonical layer indicates the presence of anthraquinone glycosides.

Legal's Test

The test solution was dissolved in millilitres of pyridine. To make the mixture alkaline, 10% sodium hydroxide solution was added after "1 millilitre of sodium nitropruside solution". "A pink to blood red colouration" indicates the presence of cardiac glycosides.

Keller-Killiani Test

In a test tube, two millilitres of test solution were mixed with "three millilitres of glacial acetic acid and one drop of 5% ferric chloride". "Add 0.5 millilitres of concentrated sulphuric acid" to the side of the test tube. When the acetic acid layer becomes blue, cardiac glycosides are present.

iv. Tests for Alkaloids

The extract was mixed with diluted hydrochloric acid, thoroughly shaken and filtered. The filtrate was used in the subsequent studies.

Mayer's Test

"Two to three millilitres of filtrate" were mixed with a few drops of Mayer's reagent along the tube's walls. When the precipitate becomes white or creamy, alkaloids are present.

Hager's Test

One to two millilitres of filtrate were treated with a few droplets of Hager's reagent in a test tube. Alkaloids are present when a yellow precipitate occurs.

Wagner's Test

"1-2 millilitres of filtrate" were treated with a few droplets of Wagner's reagent in a test tube. When the precipitate becomes reddish-brown, alkaloids are present.

v. Tests for Saponins

Froth Test

Once the extract had been diluted with distilled water, "it was shaken in a graduated cylinder" for fifteen minutes. The formation of a foam layer indicates the presence of saponins.

vi. Tests for Flavonoids

Lead Acetate Test

The extract was mixed with "a few drops of lead acetate solution". "By the formation of a yellow precipitate", the presence of flavonoids may be detected.

Alkaline Reagent Test

A few droplets of sodium hydroxide were applied to the extract in a test tube on an individual basis. Flavonoids are detected by the development of "a bright yellow colour that fades" upon the addition of a few droplets of diluted acid.

vii. Tests for Triterpenoids and Steroids

Salkowski's Test

The extract was treated with chloroform before filtering. The filter was filled with "a few drops of concentrated sulphuric acid", shaken and left to stand. If the lower layers become red, there is sterol. "When a golden yellow layer" appears at the bottom, triterpenes are present.

Libermann-Burchard's Test

The extract was treated with chloroform. Following the addition of a few droplets of acetic anhydride to the solution, it was boiled and allowed to settle. Through the test tube's sides, concentrated sulphuric acid was introduced. Whereas the generation of a deep red colour indicates the presence of triterpenoids, the formation of a brown ring at the intersection of two layers indicates the presence of steroids.

viii. Tests for Tannin and Phenolic compounds

Ferric Chloride Test

A small amount of the extract was dissolved in distilled water. This mixture was mixed with "two millilitres of a 5% ferric chloride solution". When the colour becomes blue, green, or violet, it indicates the presence of phenolic compounds.

Lead Acetate Test

A small amount of the extract was dissolved in distilled water. This combination was mixed with "a few drops of lead acetate solution". A white precipitate indicates the presence of phenolic compounds.

Gelatin Test

A certain amount of extract was dissolved in purified water. "A 1% gelatin solution" containing 10% sodium chloride was incorporated into this mixture in the amount of two millilitres. A white precipitate indicates the presence of phenolic compounds.

ix. Tests for Fats and Oils

Two filter papers were pressed with a little amount of extract. When persistent spot oil appears on the paper, it indicates the existence of fixed oils.

Solubility test

Solubility was assessed by adding a few millilitres of chloroform to two to three millilitres of "the extract's alcoholic solution".

Solubility was seen when "two to three millilitres of the extract's alcoholic solution" were mixed with a few millilitres of 90% ethanol.

Spectrophotometric Quantification of Total Phenolic Content:

Principle

The phenolics are distinguished by a single aromatic ring that contains "one or more hydroxyl groups". Phenolic compounds' strong tendency to chelate metals accounts for their antioxidant action. Phenolics have carboxyl and hydroxyl groups and may bind, especially copper and iron. In an alkaline medium, phenols react with phosphomolybdic acid in the Folin-Cioocalteau reagent to form “a blue-colored complex that can be measured using spectrophotometrically”.

Determination of total phenolics by folin-ciocalteu colorimetic assay

The total amount of phenolic in the extracts was determined using the FolinCiocalteu reagent. “Total phenolics” were reported as milligrammes per gramme of gallic acid equivalent (GAE), with galic acid used as the standard. Gallic acid concentrations of “0.01, 0.02, 0.03, 0.04, and 0.05 mg/ml were made in methanol”. 0.5 ml of each sample was added to the test along "with 2.5 ml of a folin Ciocalteure agent that had been diluted ten fold" and 2 ml of 7.5% sodium carbonate. Plant extract concentrations of 0.1 and 1 mg/ml were also produced in methanol. "The absorbance was measured spectrometerically at 760 nm" after the tubes were covered with parafilm and allowed to sit at room temperature for 30 minutes. Every decision was made in triplicate. Polyphenols are among the reducing substances that “the folin-Ciocalteu reagent is sensitive to”. When they react, they generate a blue hue. Spectrophotometric measurements were made of this blue colour. The calculation of the unknown phenol concentration was done using “the line of regression from gallic acid”. The line of regression from the gallic acid standard curve was determined “to be y = 0.005x + 2.569 and R2 = 0.991”. Consequently, it was determined that the chosen standard curve had a satisfactory goodness of fit. By aligning the absorbance of the test sample (y = absorbance) with the previously mentioned GA's line of regression.

Spectrophotometric Quantification of Total Flavonoid Content

Principle

According to their chemical structure, flavonoidsn polyphenolic compounds found in nature are classified "as flavonols, flavones, flavanoes, isoflavones, catechins, anthocyanidins, and chalcones". When flavonoids and $AlCl_3$ combine, a coloured product is created that may be detected using spectrophotometrically.

Procedure

A colorimetric test was used to determine the total flavonoids. 0.15 ml of $AlCl_3$ (100 g/L) was added after 6 minutes of combining "a 75 µl $NaNO_2$ solution with an aliquot of diluted Rutin sample or reference solution". After 5 minutes, "0.5 cc of NaOH was added". After adding distilled water to get the final amount down to 2.5 ml, everything was well combined. The absorbance of the mixture was measured at 510 nm and compared to "a blank sample of the same mixture". The total flavonoid content was determined "as mg rutin/g dry weight (mg rutine/g DW)" using the Rutin calibration curve. Three replications of each sample's analysis were conducted. The unknown flavonoid content was estimated using the line of regression from rutin. Based on the rutin standard curve, the regression line was found to "bey = 0.001x - 0.020 and R2 = 0.994". The selected standard curve was found to have a sufficient goodness of fit as a result. By aligning the absorbance of the test sample (y = absorbance) with the previously defined rutin line of regression.

Pharmacological Activity

Animals

"Wistar Strain Albino rats" of both sexes, weighing 120–150 g, were purchased and kept in 6-rat groups. The animals were kept in polypropylene cages "with 12:12 hour light/dark cycles in a well-ventilated room". There was an abundance of "standard pelleted feed and drinking water" throughout the trial. The animals were acclimated to the laboratory conditions one week prior to the trial's start. “With a 12-hour light-dark cycle and proper ventilation”, the animal house's temperatures ranged from 28 to 30 °C and its relative humidity was between 56 and 60%. Strict adherence to the ethical guidelines regulating the use of live animals in research was maintained. Both the CPCSEA and the Ethics Committee approved the procedure.

Acute oral toxicity

Acute oral toxicity was evaluated in "accordance with OECD 423 recommendations". Standards for oral acute toxicity studies are governed by the "Organisation for Economic Co-operation and Development (OECD)". It is a global organisation that wants to reduce the amount of suffering and "the quantity of animals used in acute toxicity testing". Animals were given single doses of Nymphaea alba extract in an acute toxicity investigation. Groups of albino

wistar rats were created. Every animal that was given a typical “rat pelleted diet had unlimited access to tap water”. For the trial, the following dosages were chosen: “2000 mg/kg, 300 mg/kg, 50 mg/kg, and 5 mg/kg”. Within 72 hours of the sample being administered, “the animals were monitored for mortality”. Since no lethality was seen up to 2000 mg/kg, two tests were selected "for pharmacological screening based on maximum tolerated dose limit (MTD)" based on the acute toxicity study. In the end, certain doses (2000 mg/kg) were chosen for further pharmacological investigation.

Wound healing activity

Excision wound model

The animals' dorsal thoracic central region hairs were removed after they were anaesthetised with a little intake of diethyl ether vapour. Skin fragments from the shaved area were taken out to create excision incisions that were 500 mm2 in size and 2 mm deep. The wound was left exposed all the way. Animals that showed any indication of illness were isolated, removed from the trial, and reinstalled after being carefully monitored for infections. Then, for 16 days, the normal medications were administered to the designated groups daily. All groups' wound areas were measured using a permanent marker and a transparency sheet on days 4, 8, 12, and 16. On graph paper, the wound areas that were noted on “those days were then measured”. The day "when the scar disappeared after wounding" and there were no more raw wounds was known as the day of epithelization. This model was used to monitor "the rate of wound contraction". Wound contraction was calculated as the percentage reduction in wound area "(% of wound contraction = healed area/total area × 100)". The end of full epithelization was determined by the scab sliding off the wound, and the number of days needed to achieve this was determined as the epithelization period.

Statistical Analysis

“Mean ± standard error of mean (SEM)” was used to show the results. Using “Stats 3.5 software, a p-value of $p < 0.05$” was deemed statistically significant.

Incision wounds

Four groups of six rats each were randomly selected from among the animals. Simple ointment was used to treat Group I, which served as the control group and was left untreated. Framycetin sulphate cream (Soframycin, Aventis) was administered to Group II, which was regarded as the standard group. The test groups were group IV, which received EENA 5% (w/w), and group III, which received EENA 2%(w/w). Prior to the injury, a depilator cream was used to remove “the animal's dorsal fur”. A longitudinal paravertebral incision was formed "through the cutaneous muscle and skin on the dorsal surface using a sterile scalpel". The incision was 6 cm long and 2 mm deep. The wounds were closed with surgical sutures placed one centimetre apart. Starting on the first day, the extracts were applied topically once daily for 10 days. The sutures were removed on the eighth day, and using the "Continuous Water Pouring Technique," the tensile strength of the healed incision was assessed on the tenth day (Lee et al., 1970).

Measurement of tensile strength

After being put to sleep, the animals were transferred to the surgical table. On the other side of the cut damage, two forceps were firmly used, facing each other and 3 mm from the edge of the incision. One forceps was set up on supports, while the other was attached to a 1000 millilitre lightweight plastic container that was suspended in the open and had a string connecting it to a pulley. Water from the repository was permitted to flow into the holder gradually, slowly, and relentlessly. The water stream was recorded as soon as the wound opened, and the amount of water collected in the holder roughly equal to its weightmwas recorded as the tensile strength (Ilango and Chitra, 2010).

$$\%\ Tensile\ strength\ (TS) of\ control = \frac{TS\ (Control) - TS\ (Untrated)}{TS\ (Untreated)} \times 100$$

$$\%\ Tensile\ strength\ (TS) of\ Standard = \frac{TS\ (Standard) - TS\ (Control)}{TS\ (Control)} \times 100$$

$$\%\ Tensile\ strength\ (TS) of\ Test\ sample = \frac{TS\ (extract) - TS\ (Control)}{TS\ (Control)} \times 100$$

Where TS is tensile strength.

RESULTS & DISCUSSION

Plant Extraction

Hot percolation, or "Soxhlation," was used to remove the plant material, and the yield percentages were “0.45% for petroleum ether and 8.43% for ethanol”.

Solubility Determination

Table 1 Solubility determination of extract

S. No.	Solvent	Solubility of Petroleum ether extract	Solubility of Ethanolic extract
1.	Water	Soluble	Soluble
2.	Ethanol	Soluble	Soluble
3.	Petroleum ether	Soluble	Partial soluble
4.	DMSO	Insoluble	insoluble

Phytochemical Investigation:

Table 2 Phytochemical Investigation of extract

S. No.	Experiment	Presence or absence of Phytochemical test	
		Pet. Ether extract	Ethanolic extract
1.	Alkaloids		
1.1	Mayer's reagent test	Absent	Present
1.2	Wagner's reagent test	Absent	Present
1.3	Hager's reagent test	Absent	Present
2.	Carbohydrates		
2.1	Molish's test	Absent	Present
2.2	Fehling's test	Absent	Present
2.3	Benedict's test	Absent	Present
2.4	Barfoed's test	Absent	Present
3	Proteins and Amino Acids		
3.1	Biuret test	Absent	Absent
4.	Flavonoids		
4.1	Alkaline reagent test	Absent	Present
4.2	Lead Acetate test	Absent	Present
5.	Glycoside		
5.1	Borntrager test	Absent	Present
5.2	Legal's test	Absent	Present
5.3	Killer-Killiani test	Absent	Present
6.	Tannin and Phenolic Compounds		
6.1	Ferric Chloride test	Absent	Present
6.2	Lead Acetate test	Present	Present
6.3	Gelatin test	Absent	Present
7.	Saponin		
7.1	Foam test	Absent	Absent
8.	Test for Triterpenoids and Steroids		
8.1	Salkowski's test	Absent	Absent
8.2	Libbermann-Burchard's test	Absent	Absent

Quantitative analysis

Total Phenolic Content Estimation:

Table 3 Standard Table of Gallic acid

S.no	Concentration	Absorbance
1	10 µg/ml	0.1098
2	20 µg/ml	0.1763
3	30 µg/ml	0.2468
4	40 µg/ml	0.2981
5	50 µg/ml	0.3258

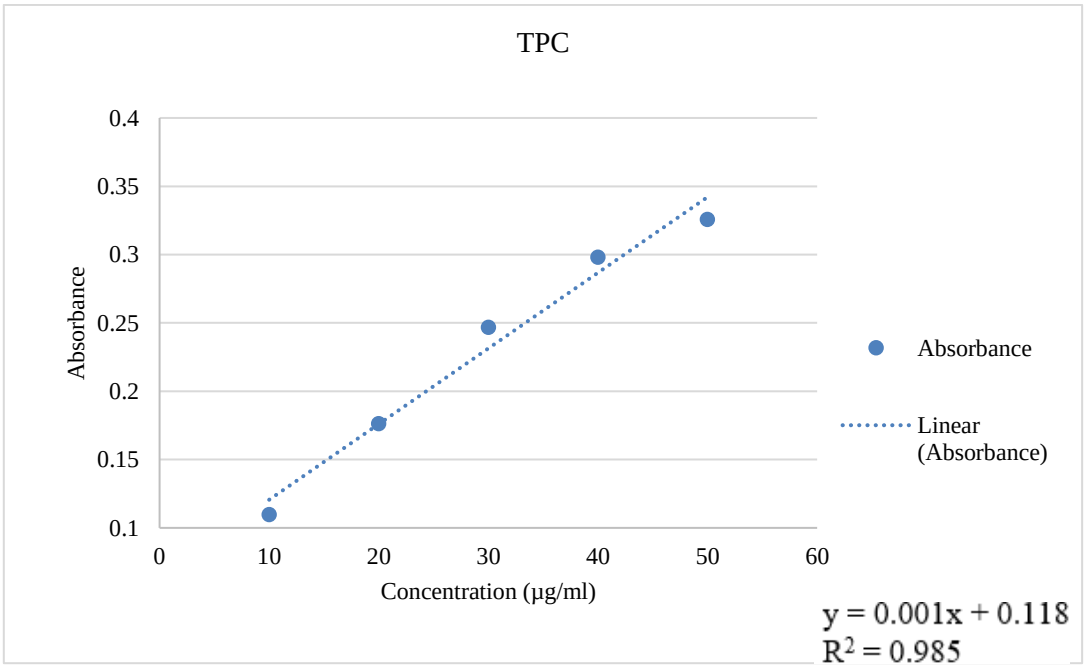

Figure 1 Standard graph of TPC

Total Phenolic Content in Plant Extract Nymphaea alba

Table 4 Total Phenolic Content in Nymphaea alba

S.no	Conc(mg/ml)	Absorbance	TPC in mg/g equivalent of gallic acid
1	1 mg/ml	0.093	5.054
2	1 mg/ml	0.095	5.418
3	1 mg/ml	0.093	5.054
		Mean± Sd	5.175±0.2101

Total Flavonoid Content

Table 5 Standard Reading of Rutin

S.no	Concentration	Absorbance
1	10 µg/ml	0.135
2	20 µg/ml	0.151
3	30 µg/ml	0.165
4	40 µg/ml	0.177
5	50 µg/ml	0.201

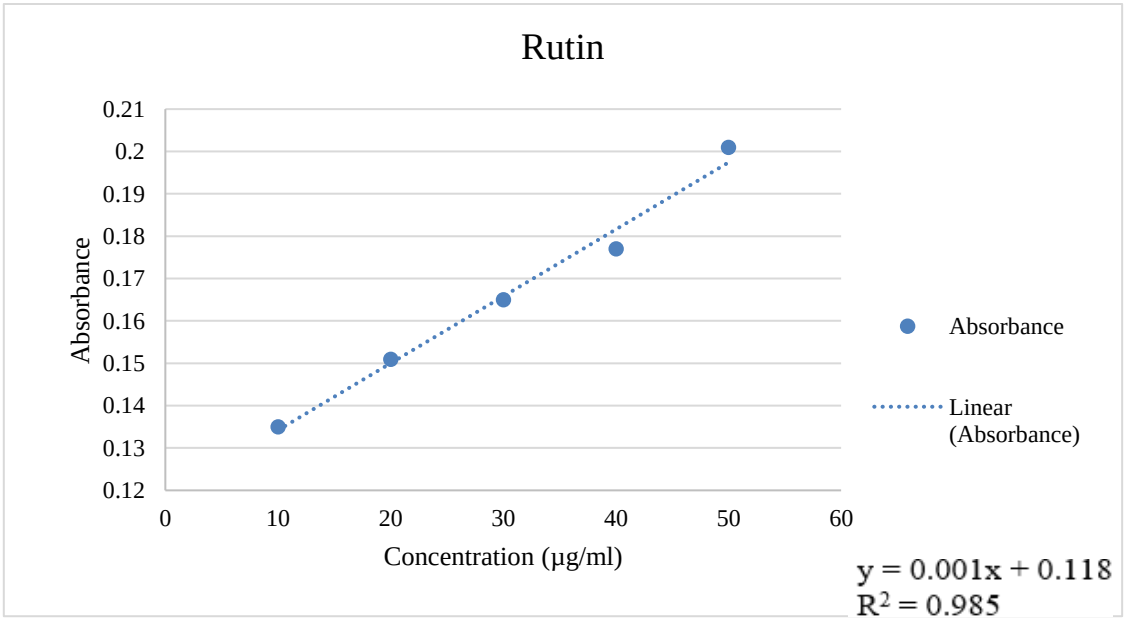

Figure 2 Standard graph of Rutin

Table 6 Total flavonoid Content in Nymphaea alba

S.no	Concentration (mg/ml)	Absorbance	TFC in (mg/g) equivalent of rutin
1	1mg/ml	0.122	2.25
2	1mg/ml	0.124	3.50
3	1mg/ml	0.124	3.50
		Mean±Sd	3.0833±0.7216

Acute oral toxicity:

"The OECD 423 guidelines" were followed for conducting the acute oral toxicity research. For toxicity investigations, four dosage ranges "5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg were used".

"For the next four hours after dose" and for seventy-two hours following sample administration, each animal was monitored separately for the occurrence of death.

Table 7 Results of toxicity studies

S. No.	Groups	Observations/ Mortality
1.	5 mg/kg Bodyweight	0/3
2.	50 mg/kg Bodyweight	0/3
3.	300 mg/kg Bodyweight	0/3
4.	2000 mg/kg Bodyweight	0/3

Wound healing activity

Excision wound model

Slight vapour inhalation of diethyl ether was employed to anaesthetise the rats. To make a single excision incision, 500 mm 2 of skin was removed from the depilated area. The wound was then left open to the weather. From the animals, "four unique groups of six creatures" were generated. The test groups were group I (untreated) animals, group II (standard) animals treated with 5%(w/w) framycetin sulphate cream (Soframycin, Aventis), "group III (treated with EENA 2%(w/w), and group IV (treated with EENA 5%(w/w)". The extract was applied topically once daily from the operating day until complete epithelization. The contraction of the wound was seen using this model. The % decrease in wound area was used to determine wound contraction. Every other day, the wound boundary was traced on graph paper to enable "planimetric monitoring of the wound region's progressive alterations".

Table 8 Effect of EENA on % wound contraction in excision wound model.

Group	4th day	8thday	12th day	16th day
	% wound contraction			
Group I (untreated)	4.19±0.023	15.42±1.497	40.24±1.428	63.51±2.476
Group II (standard)	13.78±0.395	34.58±2.841	61.03±1.508	90.63±1.46
Group III (2%w/w)	10.42±0.284	30.05±2.541	53.04±1.450	77.73±1.416
Group IV (5%w/w)	11.1±0.3	31.44±1.537	54.2±1.277	84.46±1.427

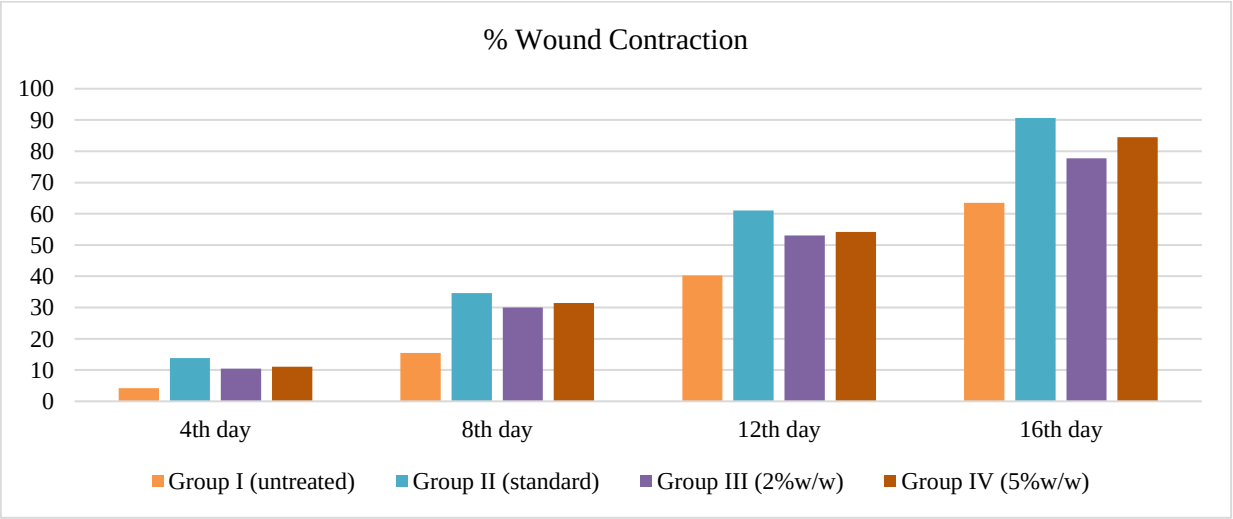

Figure 3 Effect of EENA on % wound contraction in excision wound model

Incision wounds

In comparison to the control group, the formulations containing 10% ointment of the crude extract exhibited "a remarkable increase ($p<0.01$) in wound tensile strength". Starting on the first day and continuing for up to ten days, "the extracts were applied topically once per day". The sutures were removed on the eighth day, and using the "Continuous Water Pouring Technique," the tensile strength of the healed incision was assessed on the tenth day (Lee et al., 1970). The Nymphaea alba group treated with 5% (w/w) had a higher breaking strength (479.85 ± 12.92** g) in accordance with the incision wound healing model results. Its potency was comparable to that of "the standard drug framycetin sulphate cream (Soframycin, Aventis), which had 494.42 ± 15.82 g (Table 9)". "In the incision wound model", each test sample exhibited a notable level of wound healing activity.

Table 9 Effect of ethanol extract of Nymphaea alba on Tensil strength (Incision wound model)

Group	Treatment	Breaking Strength
I	Control	328.21 ± 16.20
II	Standard (Framycetin Sulphate)	494.42 ± 15.82**
III	2% w/w of EENA	377.97 ± 17.92*
IV	5%w/w of EENA	479.85 ± 12.92**

CONCLUSION

The research report concentrated on the resource's capacity to heal wounds since plants have long been used to cure ailments like burns and wounds and are known to be reasonably resistant to most microorganisms. As a result, wound healing activities received particular attention. Phytochemical screening demonstrated that the plant extracts had a higher concentration of phytoconstituents. The existence of alkaloids, carbohydrates, flavonides, glycosides, tannin, and phenolic compounds was shown by the ethanolic extract of Nymphaea alba. Based on the calibration curve ($R2 = 0.991$), the ethanolic extract's total phenolic content was 5.175 ± 0.2102 "gallic acid equivalents/g, and its total flavonoid content ($R2 = 0.094$) was 3.0833 ± 0.7216 rutin equivalents/g". All of the extracts and powerful extract fractions were used in the wound-healing activities. The Nymphaea alba fraction's 5% w/w ethanol extract had the strongest efficacy in both the incision and excision models. The excision wound model's epithelization duration was 84.46±1.427**, the highest of the two fractions and on par with the standard medication. It showed almost the same level of activity as the conventional medication, with "a value of 492.59± 11.32** in the incision wound model".

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