

Pharmacological assessment of wound healing activity of *Terminalia arjuna* and *Neolamarckia cadamba* (Bark) extract on wistar albino rats

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ABSTRACT

This study focuses on the pharmacological assessment of methanolic bark extracts of *Terminalia arjuna* and *Neolamarckia cadamba* for their wound healing potential in Wistar albino rats. The extracts demonstrated considerable yields of 10.28% and 10.68% respectively. Phytochemical screening revealed a rich presence of bioactive compounds such as flavonoids, tannins, triterpenoids, and carbohydrates, which are known contributors to tissue regeneration. Quantitative analysis showed that *N. cadamba* contained higher total phenolic content (88 mg/gm gallic acid equivalent) compared to *T. arjuna* (50 mg/gm), with both extracts exhibiting comparable flavonoid levels (~46 mg/gm rutin equivalent). Herbal gel formulations were developed and evaluated for organoleptic properties, pH, viscosity, and spreadability, all of which fell within acceptable dermal application parameters. The polyherbal gel formulation exhibited superior antimicrobial activity (zone of inhibition: 14 mm) and significantly enhanced wound contraction (75.50% by day 21), approaching the efficacy of the standard Gentamicin gel (90.79%). These findings suggest a synergistic effect between the two plant extracts and support their traditional use as effective wound healing agents.

Keywords: *Terminalia arjuna*, *Neolamarckia cadamba*, wound healing, herbal gel, phytochemical screening, antioxidant activity, flavonoids, phenolics, Wistar rats, excision model.

1. INTRODUCTION

Herbal medicines are the study or use of medicinal herbs to prevent and treat diseases and ailments or to promote health and healing. It is a drug or preparation made from a plant or plants and used for any of such purposes (**Moreira et al., 2014**). Many herbal remedies have originated via traditional use in a particular cultural environment. For certain societies, traditional usage is documented in written books, while for others, traditional knowledge and use are passed down orally from one generation to another (**Mahuika, 2019**).

Wound healing is a complicated and long-term process, which includes interactions with immunological and biological systems. Wounds are mostly considered acute wounds and chronic wounds (**Hurlow and Bowler 2022**). Wound healing is mainly a process of a repair mechanism that recovers injury to the skin and other soft tissues. In acute wounds, the process of healing includes the initial inflammation and immunological reactions, accumulation of collagen and fibroblast, angiogenesis, contraction of the wound

area, and scar remodelling. In chronic wounds, more clinical challenges occur, and the healing rate is slow. This is because of some underlying conditions such as microbial infections and several disease states (**Sorg et al., 2017**).

Terminalia arjuna, with its anti-inflammatory and wound-healing properties, offers immense potential in this area. Its bioactive compounds, such as flavonoids and tannins, have demonstrated efficacy in controlling bacterial colonization and reducing inflammation in periodontal pockets, making it a promising candidate for inclusion in herbal-based local drug delivery systems (**Rajaram et al., 2024**). *Neolamarckia cadamba* (Rubiaceae) is a plant that is well-known for its wide range of pharmacological properties. The plant is well known as anti-hyperglycemic, wound healing, membrane stabilizing, anthelmintic, antioxidant and, anti-Alzheimer agent (**Yadav et al., 2022**).

The main purpose of this study was to assessment of the wound healing potential of bark extracts of *Terminalia arjuna* and *Neolamarckia cadamba* on wistar albino rats.

2. METHODS AND MATERIALS

2.1 Chemical

Glacial acetic acid, Nitroprusside, Ammonia and Sodium hydroxide was procured from Merk. Magnesium was received from Himedia. Chloroform, Conc. Hcl and 95% alcohol was acquired from Clorofiltind. Conc. H₂SO₄ was received from Fizmerck.

2.2 Collection of Plant

The therapeutic plant *Neolamarckia cadamba* and *Terminalia arjuna* were gathered. Following cleaning, the sections of plants, dried about 3 days at ambient temperature in shade and for a final 3 days at 45°C in oven to ensure complete dryness. Dried plant components were sorted in dry, cool environment in airtight glass containers to prevent degradation and contamination. Verification of specific traditional plant's authenticity—a medicinal herb to verify its identification and purity, *Neolamarckia cadamba* and *Terminalia arjuna* was verified by a plant taxonomist.

2.3 Extraction

The material of plant was extracted for this experiment utilizing both a continuous hot percolation process and Soxhlet device. A Soxhlet device was filled with a thimble containing *Neolamarckia cadamba* and *Terminalia arjuna* powder. Methanol solvent was employed to do soxhlation at 60°C. The used-up plant material (marc) was again extracted using an ethanol solvent once it had dried. Every solvent was soxhlation treated until there wasn't any discernible color shift in the siphon tube. The procedure of extraction was deemed complete when the solvent evaporated and disappeared without a trace. 40°C accustomed to evaporate the extracted extracts in Buchi type revolving vacuum evaporator (**Bennour et al., 2020**). The % yield for every extract in the application formula was determined by weighing the dried extract:

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Weight of Plant Material used}} \times 100$$

When the extracted materials were ready, they were tagged, kept in sealed container until needed, and evaluated for organoleptic traits (color, aroma, and percentage yield).

2.5 Quantitative Phytochemical Estimation

2.5.1 TPC

After extracting *Neolamarckia cadamba* and *Terminalia arjuna*. The total quantity of phenol was quantified using the Folin-Ciocalteu Assay. Along with 0.2 mL of extracts, 2.5 mL of Folin-Ciocalteu Reagent, and 2.5% sodium carbonate were added. The solution was diluted up to maximum of 7 ml of pure water. After two hours at normal temperature, spectrophotometric measurements of solutions were taken at 760 nanometers. The concentration of 20, 40, 60, 80, and 100 µg/mL were used to make Gallic Acid. A blue color is produced as a result of their reaction (Islam *et al.*, 2015).

2.5.2 TFC

The amount of flavonoid determined using the aluminum chloride method. 0.5 ml solution of the *Neolamarckia cadamba* and *Terminalia arjuna* extract and two milliliters of distilled water was made. Sodium nitrite (5%) in 0.15 ml was added and mixed. After that, add 0.15 ml of 10% aluminum chloride and wait six minutes. Next, give it 6 min. Next, 2 ml of the sodium hydroxide (4%), were added. Sufficient mixing was achieved by shaking the mixture. Use of UV spectrophotometer, the amount that absorbs mixture at the 510 nm was determined. Rutin concentrations ranged from 20 to 100 µg/mL. The calibration curve was used to quantify total flavonoid content, that was then stated equivalent of milligrams of Rutin per gram of dry extract weight (Bhagat *et al.*, 2021).

2.6 DPPH

To investigate the antioxidant capacity of *Neolamarckia cadamba* and *Terminalia arjuna* extracts, free radical scavenging by the DPPH test was used. A methanol solution containing 1 mg/ml of extracts was prepared alongside the usual. The absorbance of the combined mixture was measured using a UV spectrophotometer after vortexing and allowing it to stand for 30 minutes at room temperature in a dark environment. Standard *Neolamarckia cadamba* and *Terminalia arjuna* extracts (20-100 µg/ml) were made by dilution 1 mg/mL stock solution with 2 ml of 0.1 mM DPPH solution (Shimadzu 1700). To serve as the control, 3 ml of a 0.1 ml DPPH solution should be used (Engida *et al.*, 2015).

The following approach was used to calculate the % antioxidant activity of the standard sample:

$$\% \text{inhibition} = \frac{(\text{Ab control} - \text{Ab of sample})}{\text{Ab control}} \times 100$$

2.7 Acute Toxicity of Dermal Analysis

The crude extract was exposed to dermal toxicity test of *Neolamarckia cadamba* and *Terminalia arjuna* in compliance with OECD draft guideline number 404. Prior to test began, three rats (n=3) with similar skin surfaces were selected randomly, housed in separate cages, and given a week to adjust to their new circumstances. Approximately 10% of the trunk was cut off 24 hours before the research by shaving the animal's dorsal side. Rats were housed in different enclosures to control the timing of exposure. After the exposure ended, the test chemical was taken out, and over the course of 14 days, rat were watched every day to have the ability to detect any negative skin reactions (Myers, 2017).

2.8 Preparation of polyherbal formulation

Initially, 50 mL of warm water (A) was dissolved in carbopol-934 for 2 hours, and then, with the help of a 600 rpm magnetic stirrer, the mixture was mixed uniformly. A firm gel was produced by mixing 50 ml of warm water (B), methyl paraben, and carboxymethyl cellulose in a separate container while stirring continuously. Constant stirring was used for both A and B mixes. To neutralize the pH, triethanolamine was next added drop by drop. While each extract was concentrated to 1% in the first two formulations, the third formulation—used to generate the gel—included 2% concentration, or 1% of each extract. The next step was to add propylene glycol, which increases permeability. After the final dispersion was stirred, a smooth gel was produced without lumps.(Sharma *et al.*, 2022).

Table 1: Composition of prepared herbal gel

Name of Ingredient	Formulation I	Formulation II	Polyherbal Formulation
Carbopol 940	1.0 gm	1.0 gm	1.0 gm
Carboxymethyl Cellulose	1.5 gm	1.5 gm	1.5 gm
Propylene Glycol	0.7 ml	0.7 ml	0.7 ml
Methyl Paraben	0.3ml	0.3ml	0.3ml
<i>Neolamarckia cadamba</i>	1.0 gm	—	1.0 gm
<i>Terminalia arjuna</i>	—	1.0 gm	1.0 gm
Triethanolamine	q.s (pH 6.5–7.0)	q. s	q. s
Distilled Water	up to 100 ml	up to 100 ml	up to 100 ml

2.9 Evaluation parameters

2.9.1 Organoleptic parameters:

Measurements were made of every organoleptic trait among the formulations, including odor, color, and taste (Patil *et al.*, 2018).

2.9.2 Determination of pH:

The solution of pH was ascertained using a pH meter. The manufacturer's instructions for making use of the meter for pH were followed. First, the apparatus was calibrated using a pH 4–7–9 buffer (Springer, 2014).

2.9.3 Homogeneity

Visual inspection was accustomed to confirm homogeneity of each created ointment. Their appearance was put to the test.

2.10 In-vivo evaluation of the wound healing process

Animals Protocol

- **IAEC Approval:** The IAEC or Institutional Animal Ethics Committee gave their approval to all animal research. (CPCSEA No.- PBRI/IAEC/07-04-25/019)
- **Animal utilized Weight :** 200±50gm
- **Strain:** Wistar rat
- **Sex:** Either
- **Condition of Housing:** - Animals were stored individually in cages with temperature controls set at $22 \pm 2^{\circ}\text{C}$, in groups of six. Each animal was fed a conventional diet (golden feed) and given frequent watering.

2.10.1 Wound healing activity

After the animals' backs were cleaned and sterilized with a surgical blade and 70% ethanol was utilized to make excision wound from a pre-shaven area. The injury was left undressed, and neither local nor systemic antibiotic treatment was administered (**Garg *et al.*, 2011; Sara *et al.*, 2024**). The pace at which a contraction of wounds is tracked using this model. Periodically, at every fourth day interval, the wound zone was noted to be gradually decreasing. Using a tracing paper on wounded margin, the wound contractions are measured and the % reduction in zone of wound is computed. 5 groups of 6 animals each were divided by randomly dividing the animals. The polyherbal gel treatments were put on skin every day for 21 days.

Table 2: Experimental procedure

Group of animals	Treatment
Group I	Animals received distilled water
Group II	Test group treated with <i>Neolamarckia cadamba</i> 1% gel. (Formulation I)
Group III	Test group treated with <i>Terminallia arjuna</i> 1% gel. (Formulation II)
Group IV	Test group treated with (<i>Neolamarckia cadamba</i> and <i>Terminallia arjuna</i>) (Formulation III (1:1))
Group V	Standard Gentamicin gel (1%)

2.11 Wound contraction rate

The proportion of wounds that have healed, which shows how new epithelial cells have grown to fix the damage, is evaluated through measurement the shrinkage of wounds with a transparent paper sheet and an appropriate marker at regular intervals (**Masson-Meyers *et al.*, 2020**). The term "contraction of wound" refers to reduction in percentage of initial damage size.

$$\text{Wound Contraction (\%)} = \left(\frac{\text{Initial Wound Area} - \text{Specific Day Wound Area}}{\text{Initial Wound Area}} \right) \times 100$$

2.12 Antimicrobial Activity (Well Diffusion Assay)

• Anti-bacterial Activity Preparing Diluting the Samples

The concentrations of specifically 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml were included into the distilled samples in that order. Next, using purified water, the sample's volume was increased to 1 milliliter (**Syed Najmuddin *et al.*, 2017**).

• Nutrient Agar Media Preparation

Nutrient Media (28 grams) was mixed into one liter of filtered water. Before sterilization, the pH of the medium was measured. For fifteen minutes, the medium was autoclaved at 121 degrees Celsius and fifteen pounds of pressure. After the agar had solidified, plates containing nutritional media were placed in a laminar flow.

• Well Diffusion method

On nutritional agar medium, or NAM, bacterial strains (*E. coli*) were cultured. The next step involved forming wells to collect gel samples at different concentrations during ovulation. The volume was adjusted with a diluent until it reached 1 ml. A 100 µl sample was used. To obtain optimal results, the plates were incubated at 37°C for 48 to 72 hours. The bacterial suspension was standardized to 10⁸ CFU/mL and then placed in the shaker. Afterwards, 100µl of inoculum (10⁸ CFU/ml) was transferred from the broth to a new sealed Agar Media, Sterile using a micropipette. Using a sterilized cork borer, four 6 mm wells were bored into the sick materials. Each person received a varied amount of the polyherbal gel mixture. After allowing it to permeate for roughly half an hour was incubated for three days at room temperature for 18–24 hours at a temperature of 37 degrees Celsius. When measured using a ruler attached to the reverse side of a Petri dish, the zone of inhibition (ZOI) was determined to be exact to within 1 ml. The Petri dish is held just inches above a non-reflective black background. The well's diameter and the region of complete inhibition were measured (**Erhonyota *et al.*, 2023**).

3. RESULT

3.1 Procurement of plant material

Table 3: Percentage Yield of plant material

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
<i>Neolamarckia cadamba</i>	Methanol	400	31.63	10.68
<i>Terminallia arjuna</i>	Methanol	300	30.02	10.28



Figure 1: Yield of plant material of *Neolamarckia cadamba* and *Terminalia arjuna* by methanol

3.2 Phytochemical Test

Table 4: Phytochemical analysis of extracts of *Neolamarckia cadamba*

S. No.	Experiment	Presence or absence of phytochemical test
		Methanolic extract
1.	Alkaloids	
1.1	Dragendroff's test	Absent (- ve)
1.2	Mayer's reagent test	Absent (- ve)
1.3	Wagner's reagent test	Absent (- ve)
1.3	Hager's reagent test	Absent (- ve)
2.	Glycoside	
2.1	Borntrager test	Present (+ ve)
2.2	Legal's test	Present (+ ve)
2.3	Killer-Killiani test	Present (+ ve)
3.	Carbohydrates	
3.1	Molish's test	Present (+ ve)
3.2	Fehling's test	Present (+ ve)
3.3	Benedict's test	Present (+ ve)
3.4	Barfoed's test	Present (+ ve)
4.	Proteins and Amino Acids	
4.1	Biuret test	Present (+ ve)
4.2	Ninhydrin test	Present (+ ve)
5.	Flavonoids	
5.1	Alkaline reagent test	Present (+ ve)
5.2	Lead Acetate test	Present (+ ve)
6.	Tannin and Phenolic Compounds	
6.1	Ferric Chloride test	Present (+ ve)
7.	Saponin	
7.1	Foam test	Absent (- ve)
8.	Test for Triterpenoids and Steroids	
8.1	Salkowski's test	Present (+ ve)
8.2	Libbermann-Burchard's test	Present (+ ve)

Table 5: Phytochemical analysis of extracts of *Terminallia arjuna*

S. No.	Experiment	Presence or absence of phytochemical test
		Methanolic extract
1.	Alkaloids	
1.1	Dragendroff's test	Present (+ ve)
1.2	Mayer's reagent test	Present (+ ve)
1.3	Wagner's reagent test	Present (+ ve)
1.3	Hager's reagent test	Present (+ ve)
2.	Glycoside	
2.1	Borntrager test	Absent (- ve)
2.2	Legal's test	Absent (- ve)
2.3	Killer-Killiani test	Absent (- ve)
3.	Carbohydrates	
3.1	Molish's test	Present (+ ve)
3.2	Fehling's test	Present (+ ve)
3.3	Benedict's test	Present (+ ve)
3.4	Barfoed's test	Present (+ ve)
4.	Proteins and Amino Acids	
4.1	Biuret test	Absent (- ve)
4.2	Ninhydrin test	Absent (- ve)
5.	Flavonoids	
5.1	Alkaline reagent test	Present (+ ve)
5.2	Lead Acetate test	Present (+ ve)
6.	Tannin and Phenolic Compounds	
6.1	Ferric Chloride test	Present (+ ve)
7.	Saponin	
7.1	Foam test	Present (+ ve)
8.	Test for Triterpenoids and Steroids	
8.1	Salkowski's test	Present (+ ve)
8.2	Libermann-Burchard's test	Present (+ ve)

**Figure 2: Phytochemical Testing of *Neolamarckia cadamba* by Methanol****Figure 3: Phytochemical Testing of *Terminallia arjuna* by Methanol**

3.3 Quantitative Estimation of Phytoconstituents

3.3.1 Total Phenolic content (TPC) estimation

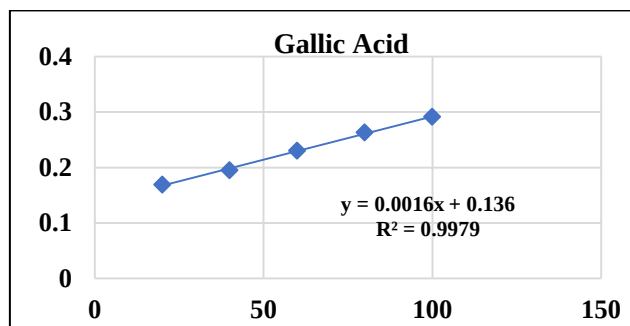


Figure 4: Represent standard curve of Gallic acid

3.3.1.1 Total Phenolic Content

Table 6: Total Phenolic Content in *Neolamarckia cadamba* extract

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.170	88 mg/gm
0.237	
0.266	

Table 7: Total Phenolic Content in *Terminallia arjuna* extract

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.160	50 mg/gm
0.195	
0.205	

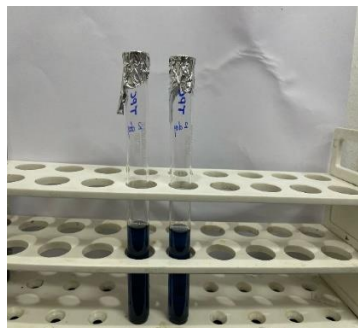


Figure 5: Total Phenolic Content of *Neolamarckia cadamba* and *Terminallia arjuna*

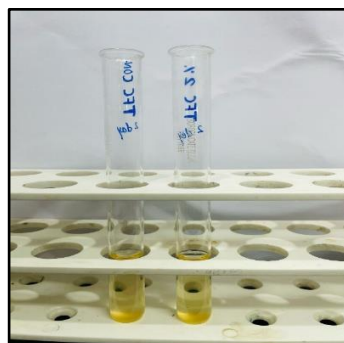


Figure 6: Total Flavonoid Content of *Neolamarckia cadamba* and *Terminallia arjuna*

3.3.2 Total Flavonoids content (TFC) estimation

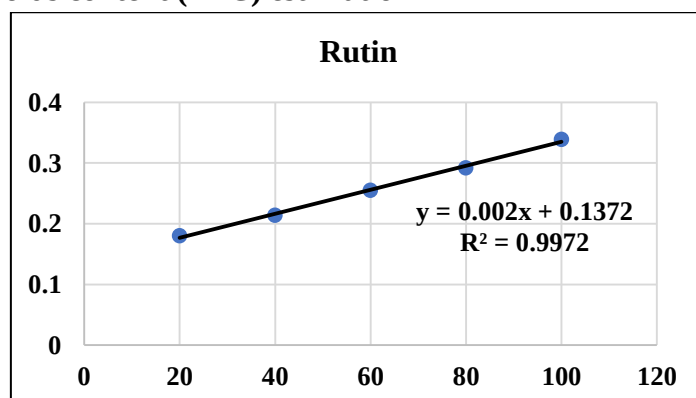


Figure 7: Represent standard curve of Rutin**3.3.2.1 Total Flavonoid Content****Table 8: Total Flavonoid Content in *Neolamarckia cadamba* extract**

Absorbance	TFC in mg/gm equivalent of Rutin
0.173	46.9 mg/gm
0.220	
0.300	

Table 9: Total Flavonoid Content in *Terminallia arjuna* extract

Absorbance	TFC in mg/gm equivalent of Rutin
0.169	45.4 mg/gm
0.205	
0.310	

3.4 Evaluation parameter of herbal gel formulation**3.4.1 Organoleptic properties****Table 10: Organoleptic properties**

Parameters	Results
Appearance	Semisolid gel
Color	Slightly yellowish
Homogeneity	Absence of aggregates

The gel was assessed based on its appearance, color, and uniformity. When tested, the gel appeared yellowish.

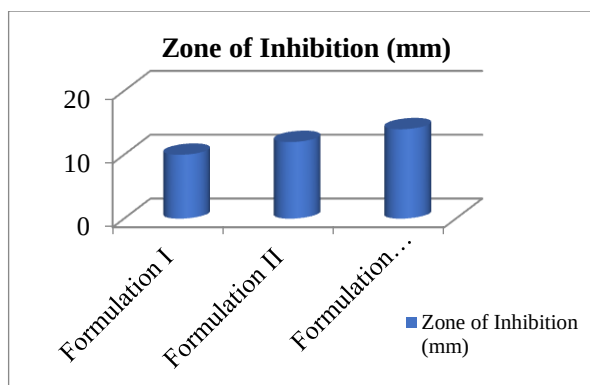
3.4.2 Measurement of pH, Viscosity and Spreadability test**Table 11: pH, Viscosity and Spreadability test**

Formulation	pH	Viscosity determination(cps)	Spreadability test(gm.cm/sec)	skin irritation study
Formulation1	6.3	5455±0.71	11.99	Not irritation observed
Formulation 2	6.5	5509±0.56	11.19	Not irritation observed
Formulation 3 Polyherbal formulation	6.7	5691±0.82	13.20	Not irritation observed

3.5 *In-vitro* antimicrobial activity**Figure 8: Antimicrobial Activity of Polyherbal gel**

Table 12: Zone of Inhibition of Antimicrobial Activity

Sample Name	Zone of Inhibition (mm)
Formulation I	10mm
Formulation II	12mm
Formulation III Polyherbal formulation	14mm



3.6 Wound Healing

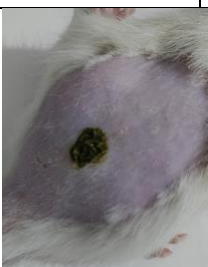
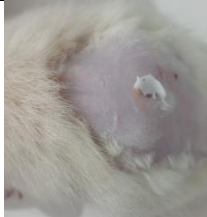
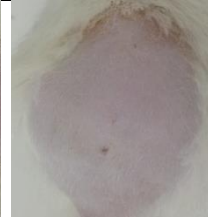
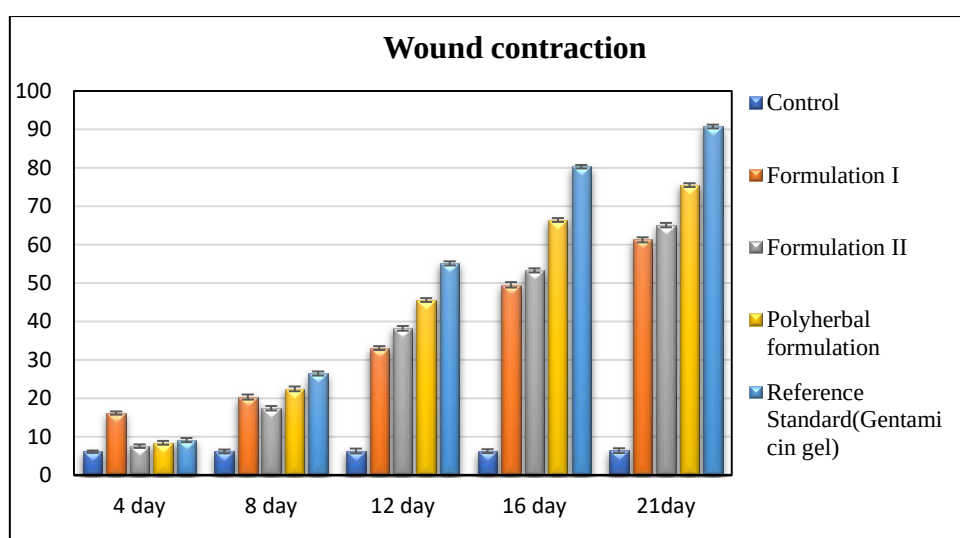
Group	4 Day	8 Day	12 Day	16 Day	21 Day
Control					
Formulation I <i>Neolamarckia cadamba</i>					
Formulation II <i>Terminallia arjuna</i>					
Formulation III Polyherbal gel					
Reference Standard (Gentamicin gel)					

Table 13: Percentage wound closure in various treatment groups

Sr. No.	Formulation	Area of wound during different days of observation (%)				
		4 days	8 days	12 days	16 days	21days
1	Control	6.11±0.3144	6.20±0.4213	6.29±0.4563	6.27±0.4892	6.36±0.5125
2	Formulation I	16.13±0.5011	20.35±0.6632	33.10±0.5779	49.54±0.6129	61.27±0.5408
3	Formulation II	7.50±0.6123	17.45±0.5132	38.22±0.5982	53.32±0.5275	65.13±0.5367
4	Polyherbal formulation	8.39±0.5167	22.48±0.7212	45.60±0.5526	66.40±0.5131	75.50±0.4565
5	Reference Standard (Gentamicin gel)	9.12±0.6235	26.42±0.6499	55.11±0.5854	80.30±0.4772	90.79±0.5123

**Graph 1: Evaluation of wound healing activity**

4. CONCLUSION

The findings clearly show that *Neolamarckia cadamba* bark and *Terminalia arjuna* have wound-healing and antibacterial capabilities. However, the combination of polyherbal ingredients that contains both extracts had the best outcomes, probably because synergistic interactions between the bioactive components. This supports the barks' medicinal potential in natural wound care treatments. Further research, including molecular mechanisms and clinical research, are necessary to validate and optimize these discoveries for human application.

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