WOUND HEALING ACTIVITY OF HERBAL FORMULATION OF *EUPHORBIA CYATHOPHORA* (LEAVES) EXTRACTS

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Abstract

The present study was designed to investigate the phytochemical profile, antioxidant potential, antimicrobial activity, and wound healing efficacy of *Euphorbia cyathophora* leaf extract formulated into a topical herbal gel. Methanolic extraction of shade-dried leaves yielded 2.95% semisolid crude extract, which on preliminary phytochemical screening revealed the presence of flavonoids, phenolic compounds, alkaloids, carbohydrates, and steroids/triterpenoids. Quantitative estimation showed appreciable total phenolic content (59.5 mg GAE/g) and total flavonoid content (57.5 mg rutin equivalent/g), indicating strong antioxidant constituents. The extract demonstrated concentration-dependent DPPH free radical scavenging activity with an IC_{50} value of 50.35 µg/mL. The formulated Carbopol-based gels exhibited acceptable physicochemical properties including suitable pH (5.8–6.6), good viscosity, satisfactory spreadability, homogeneity, and absence of skin irritation. In vitro antimicrobial studies showed significant activity with a maximum zone of inhibition of 14 mm. Furthermore, in vivo evaluation using the excision wound model in Wistar rats revealed enhanced wound contraction and faster epithelialization in treated groups compared to control. Overall, the findings suggest that *Euphorbia cyathophora* possesses notable antioxidant, antimicrobial, and wound healing properties, supporting its potential use as an effective herbal topical formulation for wound management.

Keywords: *Euphorbia cyathophora, Herbal gel formulation, Phytochemical screening, Antioxidant activity, DPPH assay, Antimicrobial activity, Wound healing, Excision wound model.*

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1. Introduction

Wound healing is a complex and well-orchestrated biological process involving hemostasis, inflammation, proliferation, and remodeling phases that restore tissue integrity after injury (Gurtner et al., 2008). Any disruption in these phases, particularly due to infection, oxidative stress, or prolonged inflammation, can delay healing and lead to chronic wounds (Guo & DiPietro, 2010). Excessive generation of reactive oxygen species (ROS) at the wound site damages cellular lipids, proteins, and DNA, thereby impairing fibroblast proliferation, collagen synthesis, and angiogenesis (Sen et al., 2009). Antioxidants play a crucial role in neutralizing ROS and promoting tissue repair, making them important therapeutic agents in wound management (Pham-Huy et al., 2008).

Medicinal plants have long been recognized as valuable sources of bioactive compounds for the treatment of wounds and skin disorders (Kumar et al., 2007). Phytoconstituents such as flavonoids, phenolic acids, tannins, alkaloids, and terpenoids possess significant antioxidant, anti-inflammatory, and antimicrobial properties that contribute synergistically to enhanced wound contraction and epithelialization (Agyare et al., 2009; Lodhi et al., 2010). Flavonoids, in particular, are known to promote collagen stabilization, improve vascularization, and accelerate tissue regeneration (Middleton et al., 2000). Phenolic compounds exhibit strong free radical scavenging activity and protect tissues from oxidative damage (Rice-Evans et al., 1997).

Microbial infection is another major factor responsible for delayed wound healing, as pathogens such as Staphylococcus aureus and Escherichia coli prolong inflammation and tissue destruction (Bowler et al., 2001). Therefore, herbal formulations with combined antioxidant and antimicrobial properties are considered promising alternatives to conventional synthetic drugs, which may cause adverse effects and antibiotic resistance (Ventola, 2015). Topical gel formulations are particularly advantageous because they ensure localized drug delivery, improved patient compliance, enhanced permeability, and minimal systemic toxicity (Bhowmik et al., 2012).

Euphorbia cyathophora, belonging to the family Euphorbiaceae, is widely distributed in tropical and subtropical regions. Various species of the genus Euphorbia have been traditionally used for the treatment of wounds, inflammation, and microbial infections (Ernst et al., 2015). Members of this genus are reported to contain diverse phytochemicals including flavonoids, diterpenes, triterpenoids, and phenolic compounds that exhibit antioxidant, antimicrobial, and anti-inflammatory activities (Vasas & Hohmann, 2014; Salehi et al., 2019). Although several Euphorbia species have demonstrated significant pharmacological

potential, systematic scientific evaluation of Euphorbia cyathophora leaves, particularly in a topical gel formulation for wound healing, remains limited.

Considering the therapeutic importance of plant-derived antioxidants and antimicrobials in wound management, the present study was undertaken to investigate the phytochemical profile, antioxidant potential, antimicrobial activity, and wound healing efficacy of methanolic leaf extract of Euphorbia cyathophora, and to formulate it into a stable and effective herbal gel for topical application.

2. Selection of plant material

Leaves of *Euphorbia cyathophora* were collected from the medicinal plant garden of Pinnacle Biomedical Research Institute, Bhopal, Madhya Pradesh, and authenticated by Dr. Jagrati Tripathi, Department of Botany, Government College Khimlasa, Sagar. The leaves were cleaned, shade-dried at room temperature for 2–3 weeks, powdered using a mechanical grinder, and stored in an airtight container for further study.

3. Extraction by Soxhlation Process

Shade-dried leaves of *Euphorbia cyathophora* were coarsely powdered and stored in an airtight container. About 480 g of the powder was extracted using methanol (45–55 °C) in a Soxhlet apparatus for 72 hours. The extract was concentrated using a rotary vacuum evaporator to obtain a semisolid crude extract, which was stored at 4 °C for further formulation and phytochemical analysis. The percentage yield was calculated using the standard formula, and the dried extract was evaluated for organoleptic properties such as color and odor before being preserved at low temperature for further studies.

Figure 1: Soxhlation Process

4. Phytochemical Test of *Euphorbia cyathophora*

4.1 Test for Saponins

- **Froth Test:** The extract was mixed with 1 mL of distilled water and shaken vigorously. Formation of stable, persistent foam indicated the presence of saponins.

4.2 Test for Tannin and Phenolic Compounds

- **Ferric Chloride Test:** The extract was heated with water, filtered, and treated with ferric chloride solution. Appearance of a blue color indicated the presence of tannins.
- **Gelatin Test:** The extract was dissolved in distilled water and mixed with 1% gelatin solution containing 10% sodium chloride. Formation of a white precipitate confirmed tannins.
- **Lead Acetate Test:** The extract was treated with a few drops of lead acetate solution. A white precipitate indicated the presence of phenolic compounds.

4.3 Test for Triterpenoids and Steroids

- **Libermann-Burchard Test:** The extract was dissolved in chloroform and treated with acetic acid, acetic anhydride, and concentrated sulfuric acid. A blue-green coloration indicated the presence of steroids.
- **Salkowski Test:** The extract in chloroform was mixed with concentrated sulfuric acid. A bluish-red to cherry-red color in the chloroform layer with green fluorescence in the acid layer confirmed steroids/triterpenoids.

4.4 Test for Carbohydrates

- **Molisch's Test:** The extract was treated with Molisch's reagent and concentrated sulfuric acid. Formation of a purple ring at the interface indicated carbohydrates.
- **Fehling's Test:** The extract was boiled with Fehling's A and B solutions. A brick-red precipitate confirmed reducing sugars.
- **Benedict's test:** The extract was heated with Benedict's reagent. Appearance of green, yellow, or red precipitate indicated reducing sugars.
- **Barfoed's Test:** The extract was heated with Barfoed's reagent. A red precipitate indicated the presence of monosaccharides.

4.5 Test for Glycosides

- **Borntragers Test:** The extract was boiled with dilute sulfuric acid, filtered, and the filtrate was extracted with benzene or chloroform. The organic layer was treated with ammonia. A pink to scarlet color indicated anthraquinone glycosides.

- **Keller Killiani Test:** The extract was mixed with glacial acetic acid and ferric chloride, followed by careful addition of concentrated sulfuric acid. A blue coloration in the acetic acid layer confirmed cardiac glycosides.

4.6 Test for protein and amino acids

- **Biuret's test:** The extract was treated with 10% sodium hydroxide and a few drops of copper sulfate solution. A violet or pink color indicated the presence of proteins.
- **Ninhydrin test:** The extract was heated with 5% ninhydrin solution for 10 minutes. Development of a blue color confirmed the presence of amino acids.

4.7 Test for Flavonoids

- **Lead Acetate test:** A small amount of lead acetate solution was added to the extract. Flavonoids may be present if a yellow precipitate forms.
- **Alkaline reagent test:** In a test tube, a few drops of sodium hydroxide were added to the extract separately. Intense yellow coloration that turns colorless when a few drops of diluted acid are added is a sign that flavonoids are present.

4.8 Tests for Alkaloids

- **Dragendorff's Test:** The extract was treated with acetic acid and Dragendorff's reagent. An orange-red precipitate indicated alkaloids.
- **Wagner's Test:** The extract was mixed with acetic acid and Wagner's reagent. A reddish-brown precipitate confirmed alkaloids.
- **Mayer's Test:** The extract was treated with Mayer's reagent. A dull white precipitate suggested alkaloids.
- **Hager's Test:** The extract was mixed with Hager's reagent. Formation of a yellow precipitate indicated the presence of alkaloids.

5. Quantitative Estimation of Phytoconstituents

The plant material was taken for quantitative estimation after initial phytochemical tests confirmed the presence of phenols, alkaloids, flavonoids, saponins, and tannins.

5.1 Total phenolic content:

TPC was determined by the Folin–Ciocalteu method. The methanolic extract was reacted with Folin–Ciocalteu reagent followed by sodium carbonate and incubated for 30–60 minutes. Absorbance was measured at 760 nm using a UV spectrophotometer. Gallic acid (30–130 µg/mL) was used to prepare the calibration curve. Results were expressed as mg gallic acid equivalents (GAE)/g of dry extract (mean ± SEM, n=3).

5.2 Total flavonoid content

TFC was estimated using the aluminium chloride colorimetric method. The extract was

mixed with aluminium chloride and sodium acetate, and the volume was adjusted with distilled water. After 30 minutes of incubation at room temperature, absorbance was recorded at 510 nm. Rutin (30–130 µg/mL) was used as the standard. Results were expressed as mg rutin equivalents/g of dry extract (mean ± SEM, n=3).

6. DPPH (2,2-Diphenyl-1-Picrylhydrazyl)

Antioxidant activity was evaluated using the DPPH assay. Extract solutions (20–100 µg/mL) were mixed with 0.1 mM DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 515 nm using methanol as blank.

Percentage antioxidant activity of sample/standard was calculated by using formula:

% Inhibition = [(Ab of control- Ab of sample)/ Ab of control x 100]

The decrease in absorbance indicated the free radical scavenging activity of the extract.

7. Preparation of herbal formulation

Herbal gel was prepared by dispersing Carbopol 934 in 50 mL warm water (Solution A) and allowing it to swell for 2 hours, followed by stirring at 600 rpm for uniformity. Solution B was prepared by dissolving carboxymethyl cellulose and methyl paraben in 50 mL warm water with continuous stirring until a gel-like consistency formed.

Both solutions were mixed under constant stirring, and triethanolamine was added dropwise to adjust pH and stabilize the gel. The herbal extract (1% and 2% for Formulations I–III) was then incorporated into the base. Propylene glycol was added as a permeation enhancer. The mixture was stirred until a smooth, homogeneous gel suitable for topical application was obtained.

Table 1: Composition of prepared herbal gel

Name of Ingredient	Formulation I	Formulation II	Formulation III
Carbopol 940	1.0 gm	1 .0 gm	1.0 gm
Carboxymethyl cellulose	1 gm	1 gm	1 gm
Propylene glycol	0.6 ml	0.6 ml	0.6 ml
Methyl paraben	0.2 ml	0.2 ml	0.2 ml
1% Euphorbia cyathophora	----	1gm	--
2% Euphorbia cyathophora	----	---	2gm
Triethanolamine	q. s	q. s	q. s
Water	100 ml	100 ml	100 ml

8. Quality control parameters of formulation

Quality control tests were performed on formulations of different concentrations to evaluate pH, spreadability, and extrudability. Acute dermal toxicity of the methanolic extract of *Euphorbia cyathophora* was also assessed.

8.1 pH

The pH of the herbal gel was measured using a digital pH meter. One gram of gel was dissolved in 100 mL of distilled water and allowed to stand for 2 hours. The pH was recorded in triplicate, and the average value was calculated.

Figure 2: pH meter

8.2 Spreadability

Spreadability of the herbal gel was evaluated by the slide and drag method, in which a known quantity of gel was placed between two glass slides and a specific weight was applied to the upper slide. The time required for the upper slide to move a certain distance and separate from the lower slide was recorded. Spreadability (S) was calculated using the formula $S = (M \times L) / T$, where M is the weight applied to the upper slide, L is the length moved by the slide, and T is the time taken (in seconds) for separation.

8.3 Viscosity

A Brookfield viscometer was used to measure the produced gel's viscosity. At 100 rpm, the reading was obtained.

Figure 3: Viscometer

9. Skin irritation studies

Skin irritation was evaluated using Wistar rats (200–300 g). The dorsal fur was shaved three days prior to application. The test group received herbal gel containing the extract, while the control group received gel base only. Formulations were applied once daily for seven days. After treatment, the skin was examined for redness, swelling, or other signs of irritation to assess safety and tolerability.

10. Antimicrobial Activity (Well Diffusion Assay)

- **Preparing Diluting the Samples**

Extract concentrations of 100, 150, 200, and 250 µg/mL were prepared and adjusted to 1 mL with purified water.

- **Preparation of Nutrient Agar Media**

Twenty-eight grams of nutrient agar were dissolved in 1 L distilled water, pH checked, and sterilized at 121°C (15 lbs) for 15 minutes. The sterile medium was poured into Petri plates and allowed to solidify.

11. Well Diffusion Assay

Bacterial *E. coli* strains were cultured on nutrient agar medium. A standardized bacterial suspension (10^8 CFU/mL) was spread evenly on sterile agar plates. Wells (6 mm diameter) were made using a sterile cork borer, and 100 µL of each formulation was introduced into respective wells. Plates were incubated at 37°C for 18–24 hours. Antibacterial activity was determined by measuring the zone of inhibition (mm) around each well.

12. Pharmacological study

12.1 Animal

Healthy Wistar albino rats (150 ± 60 g) were used. The study protocol was approved by the Institutional Animal Ethics Committee and conducted at the animal facility of Pinnacle Biomedical Research Institute (PBRI), Bhopal. Animals were acclimatized under standard laboratory conditions (25 ± 2°C, 44–56% humidity, 12-hour light/dark cycle) with free access to standard diet and water throughout the study.

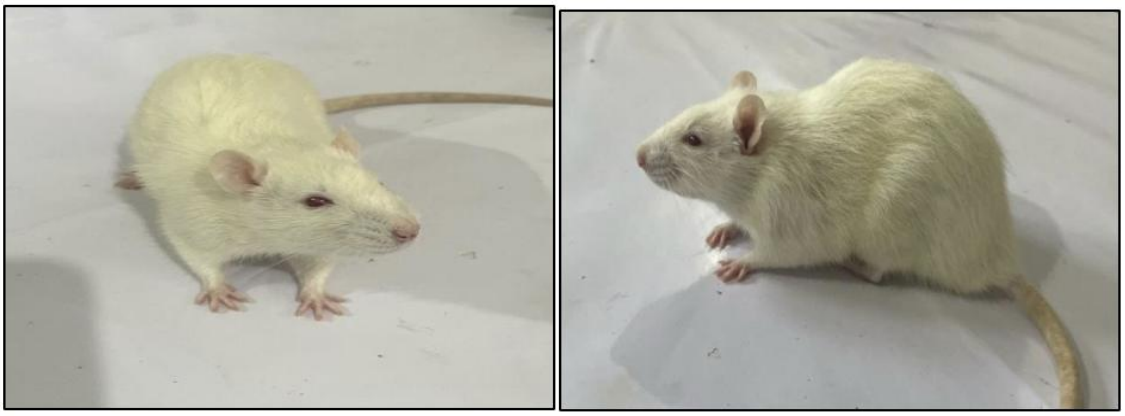


Figure 4: Wistar albino rats

12.2 Acute dermal toxicity

Acute dermal toxicity of the *Euphorbia cyathophora* extract was evaluated according to OECD guideline 404. Three rats (n=3) were acclimatized for one week and housed individually. Approximately 10% of the dorsal skin was shaved 24 hours prior to the study. The gel was applied to the exposed area at regular intervals, and animals were observed daily for 14 days for signs of erythema, edema, or other adverse reactions.

12.3 Experimental group and treatment

For the excision wound model, the dorsal surface of rats was shaved and sterilized with 70% ethanol. A full-thickness excision wound was created using sterile surgical instruments, leaving the wound untreated except for the test formulations. The wound area was traced every four days, and percentage wound contraction was calculated considering the initial wound size as 100%.

Animals were randomly divided into five groups (n=6 per group), and treatments were applied topically once daily:

- **Group I:** Negative control (Gel base)
- **Group II:** *Euphorbia cyathophora* 1% gel

- **Group III:** *Euphorbia cyathophora* 2% gel
- **Group IV:** Standard drug (Gentamicin 1% gel)

Healing progress and wound contraction were compared among groups.

12.4 Excision wound model

The excision wound model was performed with slight modifications to a standard method. The dorsal region of anesthetized rats (ketamine 100 mg/kg) was shaved, and a full-thickness circular wound was created using sterile instruments. From Day 0, 500 mg of gel (base, extract formulation, or standard drug) was applied topically once daily until complete healing. Wound contraction and epithelialization time were recorded to evaluate healing efficacy.

13. RESULTS

13.1 Selection of plant material

Methanolic extraction of *Euphorbia cyathophora* leaves was performed using 480 g of dried powder, yielding 14.16 g of crude extract with a percentage yield of 2.95%. This reflects the efficiency of methanol in extracting bioactive constituents from the plant material. Figure represents the percentage yield obtained.

Table 2: Percentage Yield of plant material

S. No	Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
1	*Euphorbia cyathophora*	Methanol	480	14.16	2.95

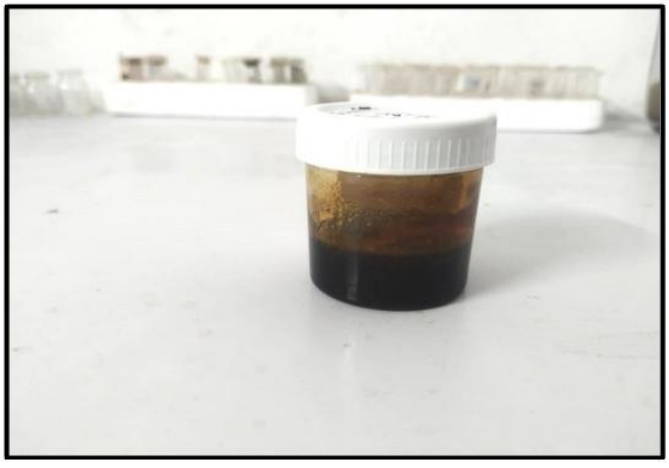


Figure 5: Yield of plant material *Euphorbia cyathophora by* methanol

13.2 Phytochemical Test

Preliminary phytochemical screening of the methanolic extract of *Euphorbia cyathophora* revealed the presence of several important bioactive constituents. Alkaloids were confirmed by Dragendorff's, Mayer's, Wagner's, and Hager's tests. Carbohydrates showed positive results in Molisch's, Fehling's, Benedict's, and Barfoed's tests. Flavonoids and phenolic compounds were also present, as indicated by alkaline reagent, lead acetate, and ferric chloride tests. Steroids and triterpenoids were confirmed by Salkowski's and Liebermann–Burchard's tests.

However, glycosides, proteins, amino acids, and saponins were absent in the methanolic extract. These findings suggest that the plant is rich in alkaloids, flavonoids, phenolics, and steroidal compounds, which may contribute to its pharmacological potential. The results are illustrated in the corresponding figure.

Table 3: Phytochemical analysis of fruit extracts of *Euphorbia cyathophora*

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	**Absent (- ve)**
8.	Test for Triterpenoids and Steroids	
8.1	Salkowski's test	**Present (+ ve)**
8.2	**Libbermann-Burchard's test**	**Present (+ ve)**

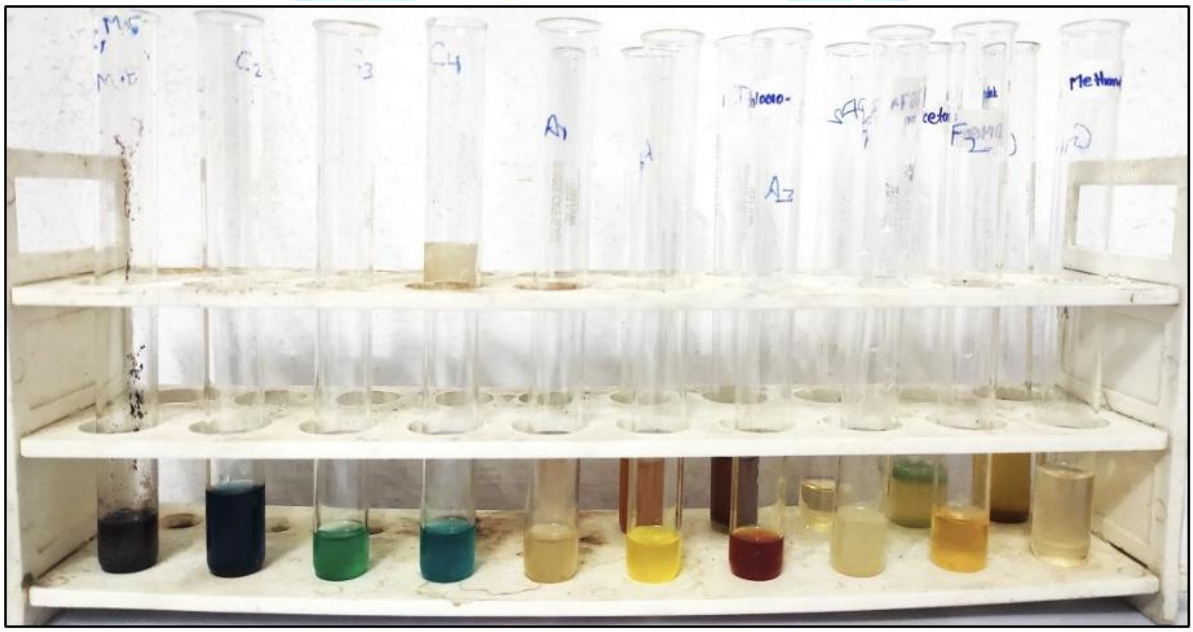


Figure 6: Phytochemical Testing of *Euphorbia cyathophora by* Methanol

13.3 Quantitative Estimation of Phytoconstituents

13.3.1 Total Phenolic content (TPC) estimation

The total phenolic content of the extract was determined using the Folin–Ciocalteu method, and a calibration curve was constructed using gallic acid as the standard. Different concentrations of gallic acid (20–100 µg/mL) were prepared, and their absorbance was measured at 760 nm. The absorbance values increased proportionally with concentration,

indicating good linearity of the standard curve. This calibration curve was used to calculate the total phenolic content of the extract, which was expressed as mg of gallic acid equivalents (GAE) per gram of dry extract. The graphical representation of the gallic acid standard curve is shown in the figure.

Table 4: Standard table for Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.172
2.	40	0.198
3.	60	0.215
4.	80	0.284
5.	100	0.345

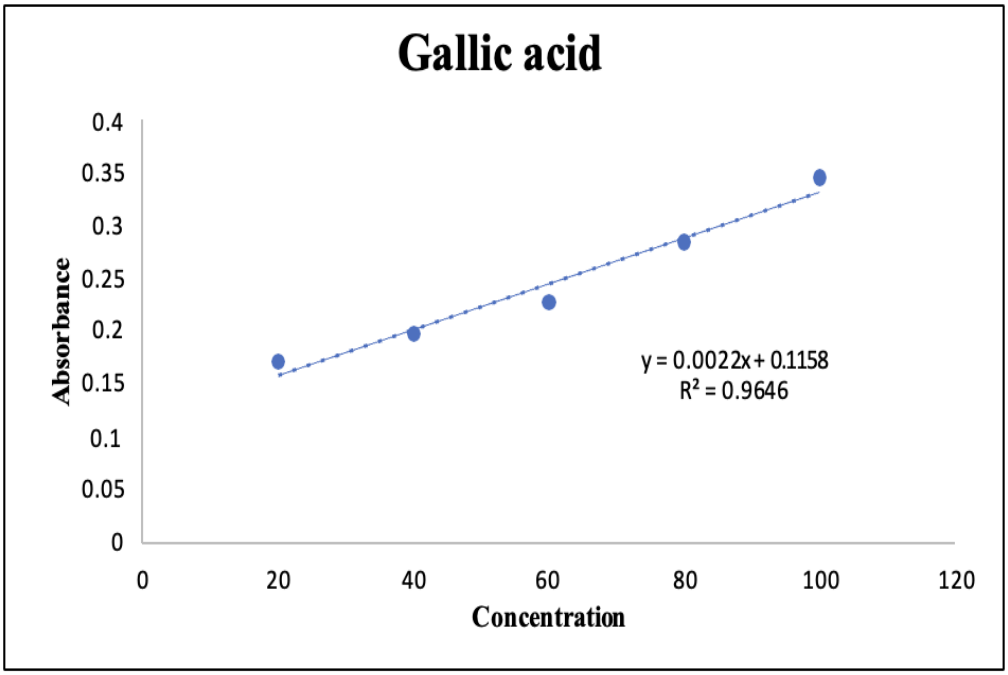


Figure 7: Represent standard curve of Gallic acid

13.3.2 Total Phenolic Content

The total phenolic content (TPC) of the methanolic extract of *Euphorbia cyathophora* was determined using the Folin–Ciocalteu method and calculated from the gallic acid standard calibration curve. The extract showed absorbance values of 0.178, 0.226, and 0.300, corresponding to a total phenolic content of 59.5 mg/g expressed as gallic acid equivalents (GAE). These results indicate that the extract possesses a considerable amount of phenolic compounds, which may contribute to its antioxidant potential.

Table 5: Total Phenolic Content in *Euphorbia cyathophora* extract

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.178	59.5 mg/gm
2	0.226	
3	0.300	

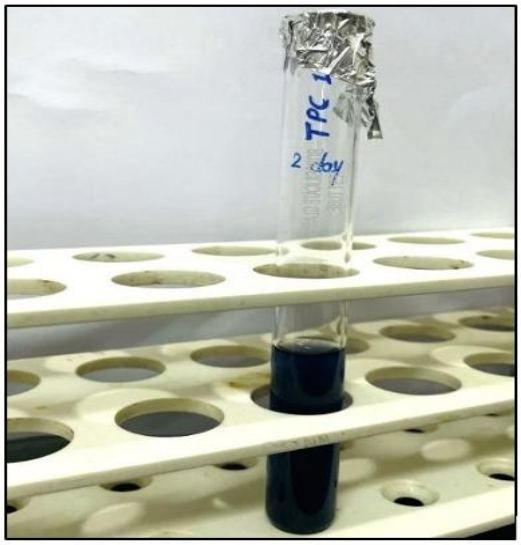


Figure 8: Total Phenolic content (TPC) *chinensis* estimation of *Euphorbia cyathophora*

13.3.4 Total Flavonoids content (TFC) estimation

The total flavonoid content was determined using the aluminium chloride colorimetric method, and a calibration curve was prepared using rutin as the standard. Standard solutions of rutin at concentrations ranging from 20–100 µg/mL showed a gradual increase in absorbance (0.168–0.378) at 510 nm, indicating good linearity. This standard curve was used to calculate the total flavonoid content of the extract, which was expressed as mg of rutin equivalents per gram of dry extract.

Table 6: Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.168
2.	40	0.204
3.	60	0.275
4.	80	0.312
5.	100	0.378

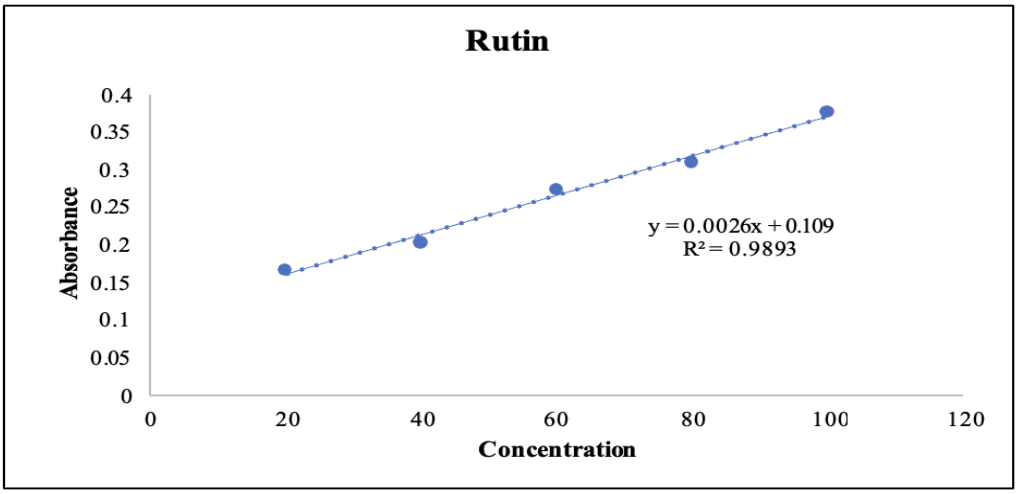


Figure 9: Represent standard curve of Rutin

13.3.5 Total Flavonoid Content

The total flavonoid content (TFC) of the methanolic extract of Euphorbia cyathophora was determined using the aluminium chloride colorimetric method and calculated from the rutin standard calibration curve. The extract exhibited absorbance values of 0.170, 0.215, and 0.289, corresponding to a total flavonoid content of 57.5 mg/g expressed as rutin equivalents. These findings indicate that the extract contains a considerable amount of flavonoids, which may contribute to its antioxidant and pharmacological activities.

Table 7: Total Flavonoid Content in Euphorbia cyathophora extract

S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.170	57.5 mg/gm
2	0.215	
3	0.289	

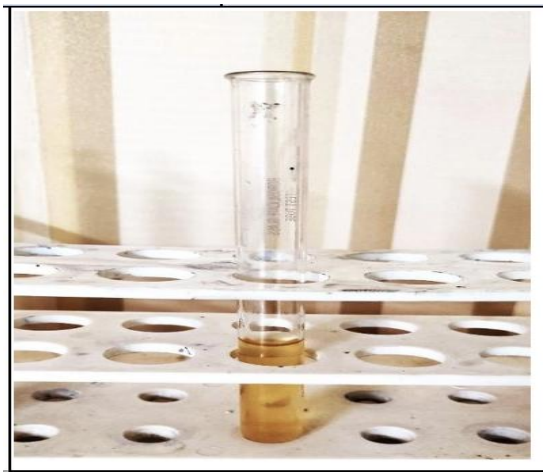


Figure 10: Total Flavonoids content (TFC) estimation of *Euphorbia cyathophora*

13.4 In vitro Antioxidant Assays

The antioxidant potential of the methanolic extract of *Euphorbia cyathophora* was evaluated using the DPPH radical scavenging assay and compared with the standard ascorbic acid.

For ascorbic acid, the percentage inhibition increased in a concentration-dependent manner from 51.06% at 20 µg/mL to 82.94% at 100 µg/mL, with a control absorbance of 0.985. The IC_{50} value was found to be 21.497 µg/mL, indicating strong antioxidant activity (Figure 11).

Similarly, the methanolic extract of Euphorbia cyathophora showed increasing radical scavenging activity from 43.10% at 20 µg/mL to 66.63% at 100 µg/mL, with a control absorbance of 0.935. The IC_{50} value of the extract was calculated as 50.35 µg/mL, demonstrating moderate antioxidant potential compared to the standard (Figures 12 and 13).

Overall, the results confirm that the extract possesses significant free radical scavenging activity, likely due to its phenolic and flavonoid constituents.

Table 8: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.482	51.065
40	0.425	56.852
60	0.362	63.248
80	0.295	70.050
100	0.168	82.944
Control = 0.985		
IC50 = 21.497		

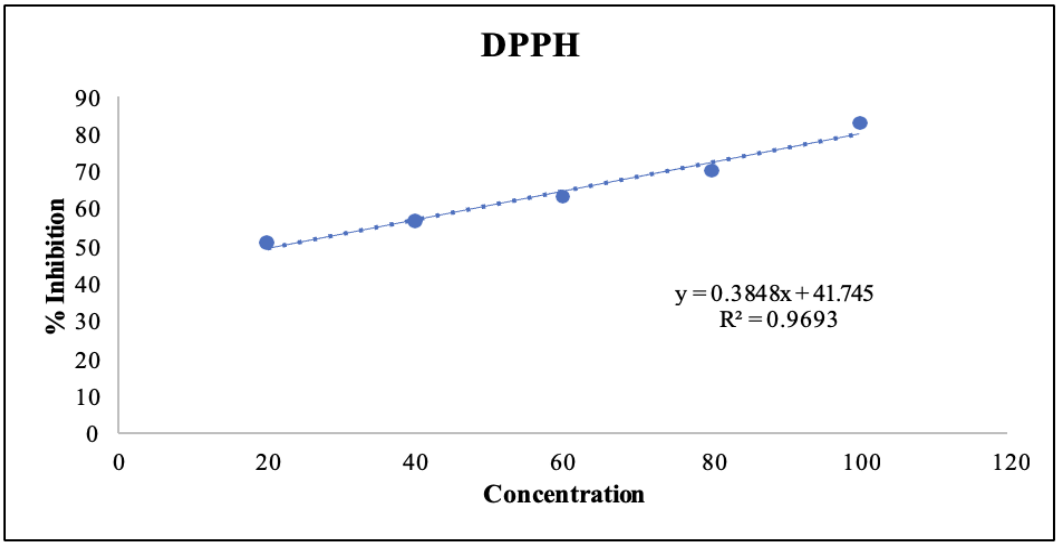


Figure 11: DPPH radical scavenging activity of Std. Ascorbic acid

Table 9: DPPH radical scavenging activity of methanol extract of *Euphorbia cyathophora*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.532	43.101
40	0.487	47.914
60	0.452	49.518
80	0.408	56.363
100	0.312	66.631
Control=0.935		
IC50=50.35		

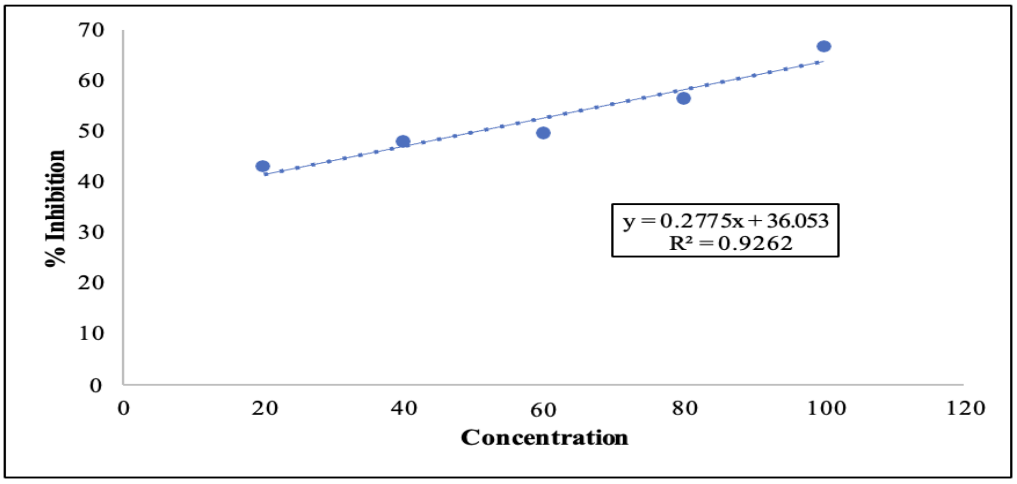


Figure 12: Represents the Percentage Inhibition *Vs* Concentration of Extract of *Euphorbia cyathophora*

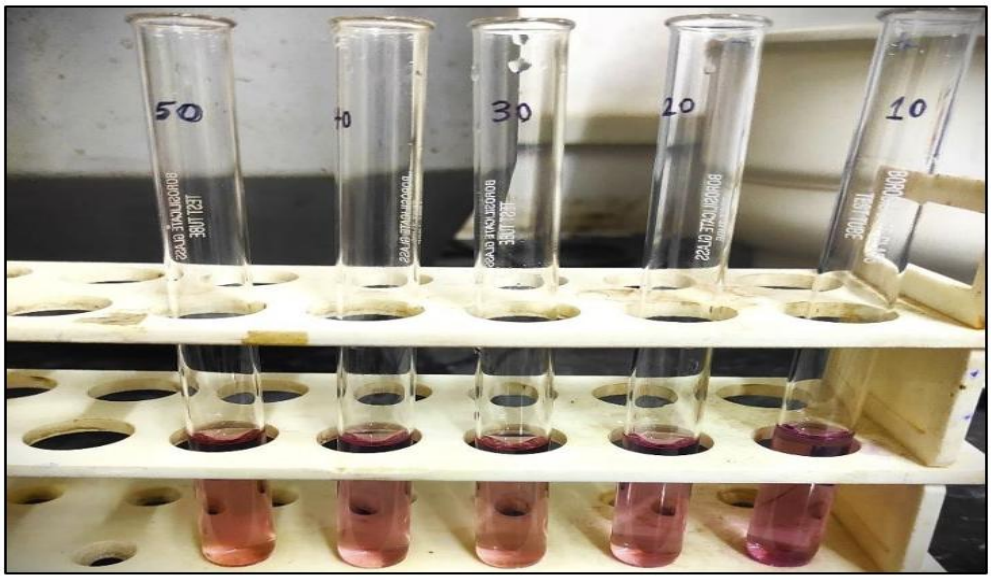


Figure 13: DPPH radical scavenging activity of extract of *Euphorbia cyathophora*

13.5 Evaluation parameter of herbal gel formulation

13.5.1 Organoleptic properties

The organoleptic evaluation of the formulated herbal gel revealed satisfactory physical characteristics. The gel was found to be homogeneous in nature, indicating uniform distribution of ingredients. It exhibited a yellowish-brown color and a smooth appearance, confirming good formulation consistency and aesthetic acceptability.

Table 10: Organoleptic properties

S. No	Parameters	Results
1.	Homogeneity	Homogeneous
2.	Color	Yellowish Brown
3.	Appearance	Smooth

Figure 14: Herbal Gel Formulation

13.5.2 Measurement of pH, Viscosity and Spreadability test

The formulated herbal gels were evaluated for pH, viscosity, spreadability, and skin irritation. The pH of all formulations ranged from 5.8 to 6.6, which is within the acceptable range for topical application and compatible with skin pH. Viscosity values increased slightly from Formulation 1 (3984 ± 0.25 cps) to Formulation 3 (4237 ± 0.11 cps), indicating good consistency and stability of the gel system. Spreadability also improved progressively, with values ranging from 11.94 to 14.83 gm·cm/sec, suggesting ease of application. No signs of skin irritation were observed in any formulation, confirming their safety for topical use.

Table 11: pH, Viscosity and Spreadability test

S. No	Formulation	pH	Viscosity determination (cps)	Spreadability test (gm.cm/sec)	skin irritation study
1.	Formulation1	5.8	3984±0.25	11.94	Not irritation observed
2.	Formulation 2	6.2	4124±0.43	13.09	Not irritation observed
3.	Formulation 3	6.6	4237±0.11	14.83	Not irritation observed

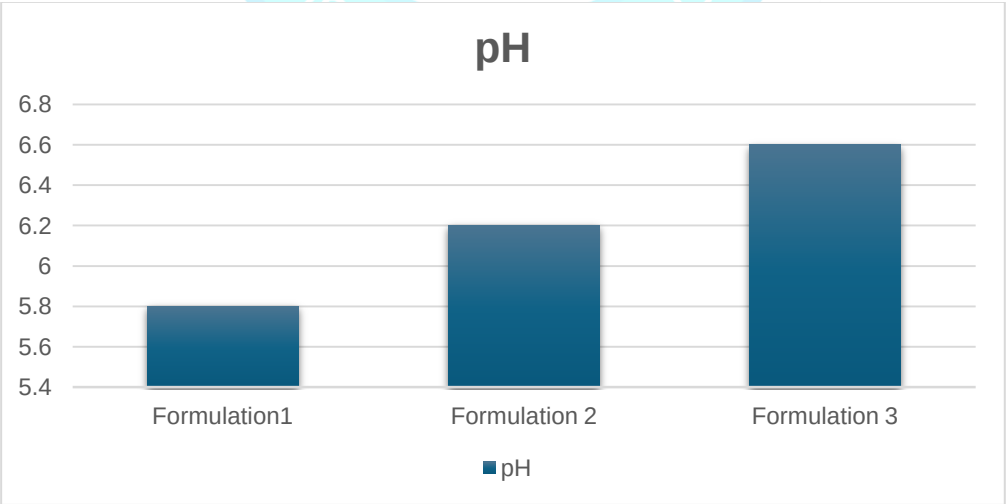


Figure 15: pH

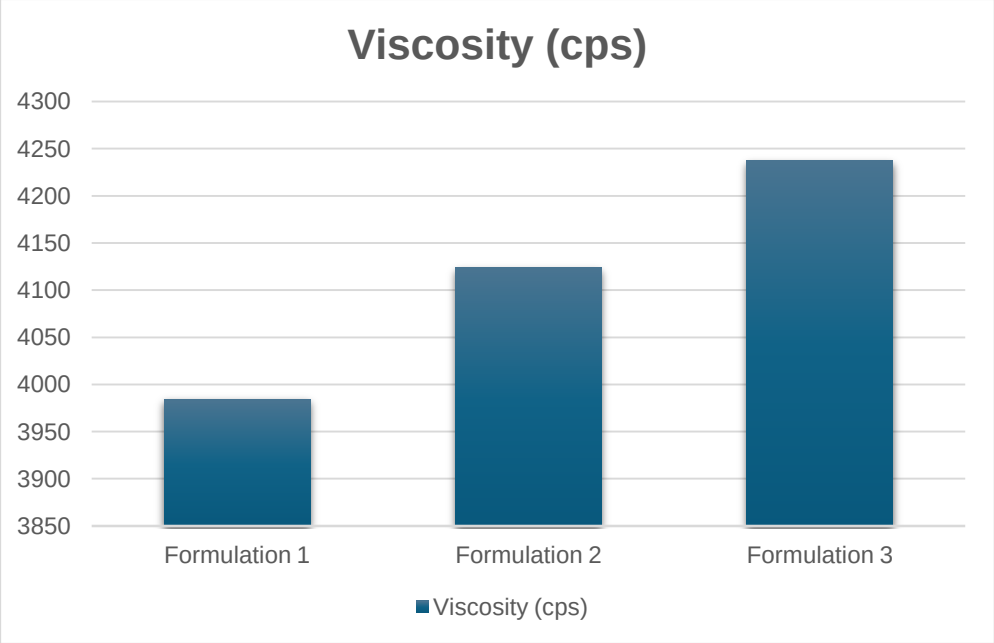


Figure 16: Viscosity

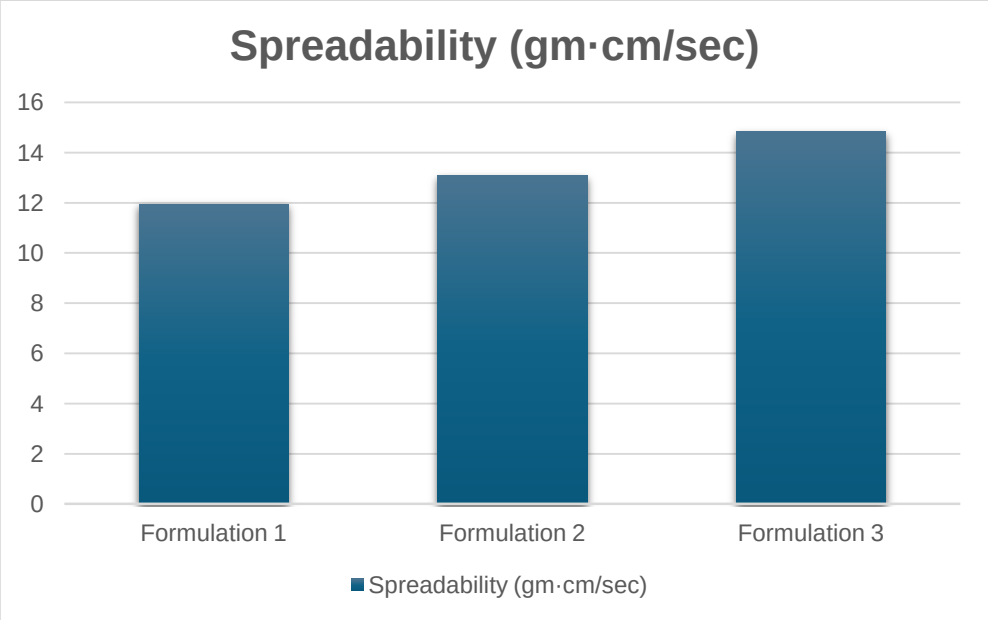


Figure 17: Spreadability

13.6 In-vitro antimicrobial activity

The antimicrobial activity of the formulated herbal gels was evaluated using the well diffusion method, and the results are presented in Figure 18 and Table 12. The zone of inhibition increased with higher concentrations of the extract in the formulation. Formulation I showed a zone of inhibition of 9 mm, Formulation II exhibited 11 mm, and Formulation III demonstrated the highest activity with a 14 mm zone of inhibition. These results indicate that the antimicrobial efficacy of the herbal gel is concentration-dependent, with Formulation III showing the most potent antibacterial activity.

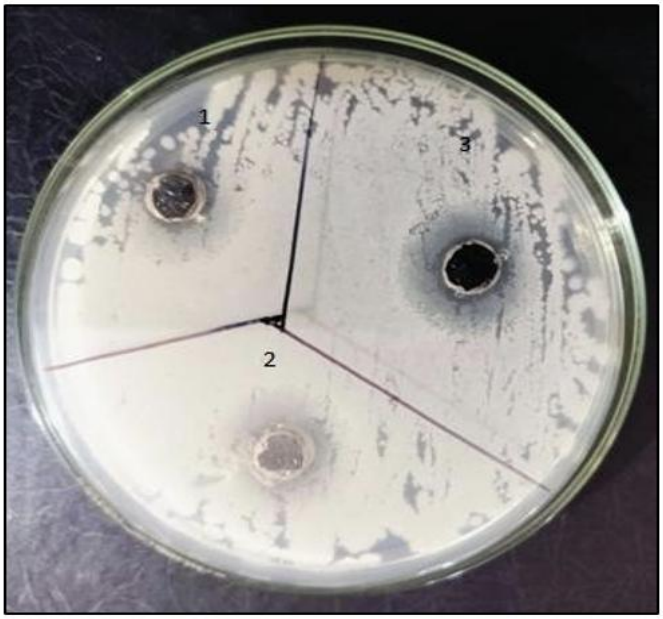


Figure 18: Antimicrobial Activity of Herbal gel

Table 12: Zone of Inhibition of Antimicrobial Activity

S. No	Sample Name	Zone of Inhibition (mm)
1.	Formulation I	9mm
2.	Formulation II	11mm
3.	Formulation III	14mm

13.7 Wound Healing Excision Model

The wound healing potential of the formulated gels was evaluated using the excision wound model, and representative images are shown above for different groups at 4th, 8th, 12th, 16th, and 21st days.

In the **control group**, wound contraction was slow, and complete healing was delayed compared to treated groups.

Formulation I (Euphorbia cyathophora 1% gel) showed moderate improvement in wound contraction with gradual reduction in wound size over time.

Formulation II (Euphorbia cyathophora 2% gel) demonstrated enhanced wound healing activity, with faster contraction and improved epithelialization compared to Formulation I.

Formulation III (Polyherbal gel) exhibited better healing progression than individual extract formulations, indicating synergistic effects of combined constituents.

The **standard group (Gentamicin gel)** showed rapid wound contraction and near-complete epithelialization by the 21st day, serving as a positive control.

Overall, the results indicate that the herbal formulations, particularly the higher concentration and polyherbal gel, significantly promoted wound contraction and tissue regeneration compared to the control

Wound Healing Excision Model

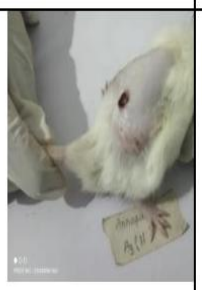
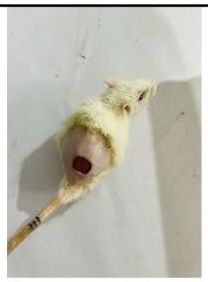
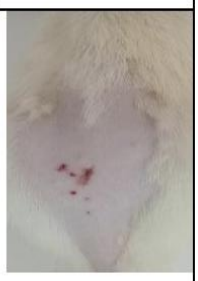
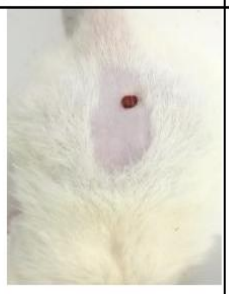
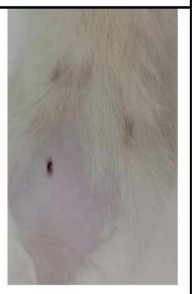
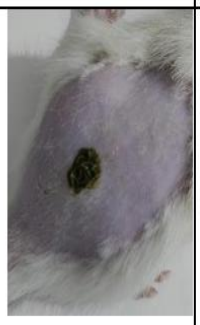
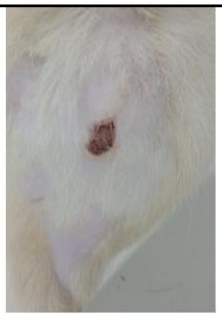
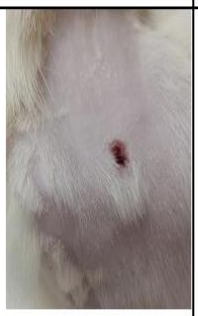
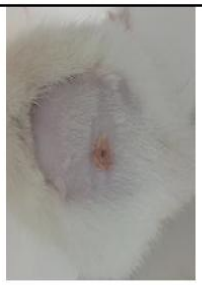
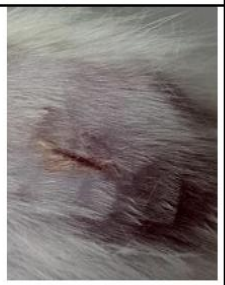
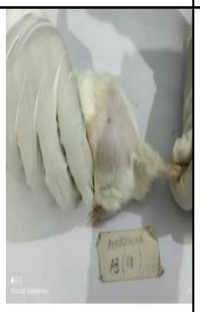
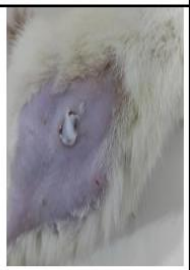
Group	4 Day	8 Day	12 Day	16 Day	21 Day
Control					
Formulation I *Euphorbia cyathophora 1%*					
Formulation II *Euphorbia cyathophora 2%*					
Formulation III Polyherbal gel					
Reference Standard (Gentamicin gel)					

Table 13: Percentage wound closure in various treatment groups

Area of wound during different days of observation (%)						
Sr. No.	**Formulation**	**4 days**	**8 days**	**12 days**	**16 days**	**21 days**
1	**Control**	6.25 ± 0.32	8.10 ± 0.41	10.45 0.48	12.22 0.50	14.08 ± 0.54
2	**Formulation I**	15.80 0.52	28.65 ± 0.63	46.25 0.57	52.40 0.61	62.30 ± 0.54
3	**Formulation II**	9.45 ± 0.61	25.32 ± 0.51	49.15 0.59	58.27 0.53	62.64 ± 0.53
4	**Polyherbal formulation**	7.25 ± 0.52	20.48 ± 0.72	42.30 0.55	50.15± 0.51	60.85 ± 0.46
5	**Reference Standard (Gentamicin gel)**	6.35 ± 0.62	20.22 ± 0.65	39.40 0.58	49.75 0.48	57.90 ± 0.51

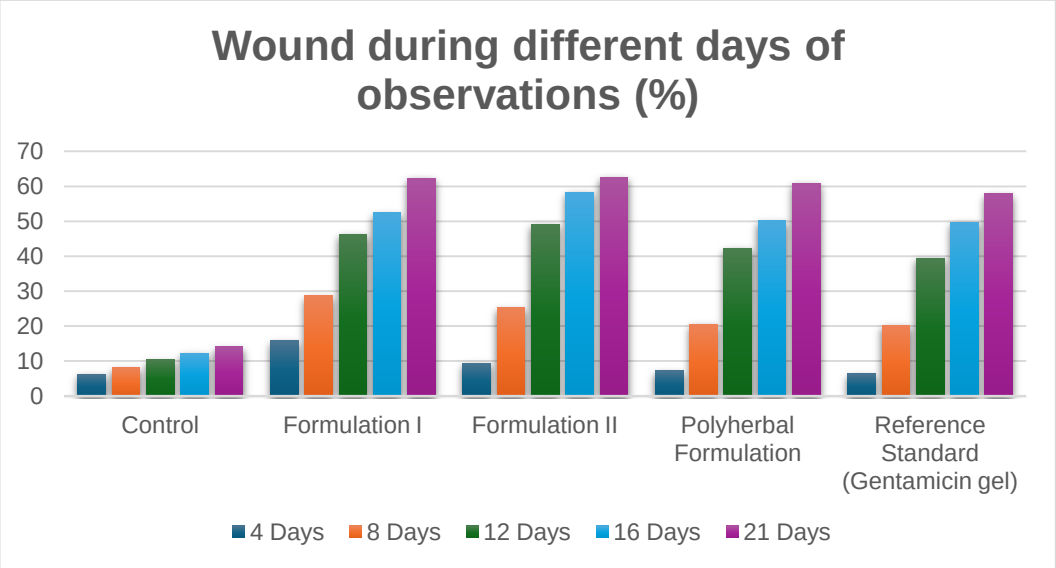


Figure 19: Evaluation of wound healing activity

14. Acknowledgements

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15. Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this review.

16. Conclusion

The present investigation demonstrated that *Euphorbia cyathophora* leaf extract possesses significant phytochemical constituents, particularly flavonoids and phenolic compounds, which contribute to its notable antioxidant activity. The formulated herbal gel exhibited satisfactory physicochemical characteristics, including suitable pH, good viscosity, spreadability, homogeneity, and absence of skin irritation, indicating its stability and safety for topical application. The extract-based formulations showed appreciable antimicrobial activity and significantly enhanced wound contraction and epithelialization in the excision wound model compared to the control group. Overall, the findings suggest that *Euphorbia cyathophora* has promising therapeutic potential as a natural, effective, and safe topical agent for wound management, supporting its further development and clinical exploration.

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