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"Phytochemical Estimation, Antioxidant and Wound Healing Activity of Polyherbal Gelof Asystasia Gangetica and Euphorbia Cyathophoraextract on Wistar Rats"

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ABSTRACT
This study focuses on the pharmacological assessment of methanolic bark extracts of Asystasiagangetica and Euphorbia cyathophorafor their wound healing potential in Wistar albino rats. The extracts demonstrated considerable yields of 4.90% and 4.25% 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 AsystasiaGangeticacontained higher total phenolic content (92 mg/gm gallic acid equivalent) compared to Euphorbia cyathophora(60 mg/gm), with both extracts exhibiting comparable flavonoid levels (~29 mg/gmrutin 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: 3 mm) and significantly enhanced wound contraction (80.99 % by day 21), approaching the efficacy of the standard Gentamicin gel (94.74%). These findings suggest a synergistic effect between the two plant extracts and support their traditional use as effective wound healing agents.

Keywords: AsystasiaGangetica, Euphorbia cyathophora, Wound healing, Polyherbal gel, Phytochemical screening, Antioxidant activity Wistar rats, Excision model.

I. INTRODUCTION

Wounds are the physical injuries that result in an opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. In other words wound is a break in the epithelial integrity of the skin and may be accompanied by disruption of the structure and the function of underlying normal tissue and may also result from a contusion, haematoma, laceration or an abrasion **(Kirwan et al., 2015).**Wound healing is a complicated and long-term process, which includes interactions with immunological and biological systems. Wound healing is the process of repair that follows injury to the skin and proper healing of wound is essential for the restoration of disrupted anatomical continuity and disrupted functional status of the skin. Several, medicinal plants have been used since time immemorial for treatments of cut wounds, and burns and showed promising effects **(Sorget al., 2017).**

Medicinal plants play a major role in health care system and are exclusive source of life saving drugs for majority of world's population. Anti-cancer activity of medicinal plants is mainly due to presence of phenolic compounds, flavanoids and phenolic diterpenes. Natural products have long been a rich source of cure for cancer. Some of them used in treatment of cancer include taxol, etoposide, topotecan, irinotecan, vincristine, vinblastine, colchicines and ellipticine. Asystasiagangetica belonging to the family Acanthaceae commonly known as Chinese violet.A.gangetica found to contain numerous important phytochemicals like phenols, alkaloids, flavonoids, glycosides, coumarins, tannins, steroids, terpenoids and saponins**(Jose et al., 2018).**It has also been found to contain four important flavonoids, luteolin, quercetin, kaemferol and isorhamnetin. A.gangetica is mainly used for mild hypoglycaemia. It has also been claimed to have anti-asthmatic, antihelminthic, anti-diabetic, anti-oxidant and anti-cancer properties.

Euphorbia species have an increasingly prevalent due to chemical compounds which have different skeletal structure and their therapeutic importance. Euphorbia cyathophora is a perennial and unarmed herb. It has a lot of medicinal uses**(Kemboiet al., 2020).**This has emetic and cathartic effects. A decoction of roots and barks are used to treat ague. Latex is applied to cure erysipelas and corns. Previous works pertaining to

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E. cyathophora showed the antimicrobial and wound healing activities. Linear incision, circular excision wound models and the hydroxyproline assay method was used to assess the wound-healing activity **(Shrivastavet al., 2018).**

Therefore the present study was undertaken to investigate the antioxidant and wound healing activity of polyherbal gel of Asystasiagangetica and Euphorbia cyathophora extract on wistar rats.

II. METHODS AND MATERIALS

2.1 Chemical

Glacial acetic acid, Nitroprusside, Ascorbic Acid and Sodium hydroxide was produced from Merk. Petroleum ether was received from Researchlab. Chloroform, Conc. Hcl and 95% alcohol was acquired from Clorofiltind. Conc. H_2SO_4 was received from Fizmerck.All other solvents, Chemicals and reagents used were of analytical (AR) grade and purchased from Molychem**,** Fizmerck, Rankem and Himedia.

2.2 Collection of Plant

The leaves parts of Euphorbia cyathophora and Asystasiagangetica(400 and 350 gm) were collected from the garden of medicinal plants in Pinnacle Biomedical Research Institute, Bhopal (M.P) authenticated by Dr.Jagrati Tripathi, Department of Botany / Government College Khimlasa, Sagar. The desired parts of the plant were shade dried at room temperature for about two weeks, grind to a fine powder with the aid of electrical grinder.

2.3 Extraction

Extracts were obtained using soxhlet apparatus. 400 gm of leaves powder of Asystasiagangetica and Euphorbia cyathophora were placed in a thimble. The thimble is then placed in distillation flask which contains the methanol solvent (450ml) at 60-80°C. The process runs repeatedly until the extraction was completed. The filtrate was concentrated in rotary vacuum evaporator, dried in desiccators and calculated the percentage yield **(Parliment, 2020).**

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

2.4 Quantitative Estimation of Phytoconstituents

After the confirmation of presence of phenols, alkaloids, flavonoids, saponins and tannins by preliminary phytochemical tests, the plant material was taken for quantitative estimation.

- **Total phenols content:**

The total phenolic content (TPC) of Asystasiagangetica and Euphorbia cyathophora extracts was determined using the Folin-Ciocalteu reagent, with gallic acid (GA) serving as the reference standard. In summary, 0.2 mL of extract solution was poured in a test tube, followed by 5 mL of Folin-Ciocalteu reagent (diluted ten times). The mixture was thoroughly agitated for 4 minutes before adding 4 mL of sodium carbonate solution (7.5% w/v). The final volume was set to 25 mL using deionized distilled water to ensure thorough mixing. The solution was allowed to stand for 90 minutes before its absorbance was measured at 760 nm with a UV-spectrophotometer. The calibration curve was created using standard gallic acid solutions at concentrations of 20, 40, 60, 80, and 100 µg/mL.**(Dilkalalet al., 2024).**

- **Total flavonoid content**

The total flavonoid content (TFC) of Asystasiagangetica and Euphorbia cyathophora extracts was determined using a colorimetric technique with minor modifications. In this step, 0.3 mL of extract solution (made in 45% ethanol) was combined with 8 mL of 10% aluminum chloride solution and 4 mL of 0.2 M sodium acetate. The mixture was immediately diluted to a final volume of 25 mL with deionized distilled water to ensure thorough mixing. The solution was allowed to react at room temperature for 30 minutes before being measured at 350 nm with a UV spectrophotometer. A calibration curve was generated using rutin as the reference standard. Standard solutions were made at 20, 40, 60, 80, and 100 µg/mL. Total flavonoid content was calculated according to this curve, the rutin equivalent is expressed as milligrams per gram of dry extract weight **(Keya, 2020).**

2.5 DPPH (2, 2-Diphenyl-1-Picrylhydrazyl)

Asystasiagangetica and Euphorbia cyathophora extract's antioxidant activity was assessed utilizing the DPPH free radical scavenging test. A methanol solution containing 1 mg/ml extracts/standard was produced. Asystasiagangetica and Euphorbia cyathophora extracts/standards (20-100µg/ml) were produced from a 1mg/mL stock solution with 2mL of 0.1mM DPPH solution added. The resulting mixture was vortexed, incubated for 30 minutes at room temperature in a relatively dark environment, and measured at 517

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494

nm with a UV spectrophotometer. For the control, add 3 ml of 0.1mM DPPH solution and incubate for 30 minutes at room temperature in the dark. The absorbance of the control was measured against methanol (as a blank) at 517 nm **(Dilkalalet al., 2024).**

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

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

2.6 Preparation of Polyherbal gel

Initially carbopol-934 was immersed in 50 mL of warm water (A) for 2 hr and was homogeneously dispersed using magnetic stirrer at 600 rpm. In separate container carboxymethyl cellulose and methyl paraben was added into 50 ml warm water (B) and stirred continuously to make stiff gel. Both the mixtures A and B were mixed with the continuous stirring. Then triethanolamine (Drop wise) was added to neutralize the pH and Formulations I, II, were 1% of each concentration of extract and formulation III was 2% concentration (i.e. 1% of each extract) were incorporated into the dispersion to obtained gel. At this stage, permeation enhancer (Propylene glycol) was added. The final dispersion was agitated until smooth gel was formed without lumps **(Indriastutiet al., 2023).**

Table 1: Composition of prepared herbal gel

Name of Ingredient	Formulation I	Formulation II	Polyherbal formulation III
Carbopol 940	1 gm	1 gm	1 gm
Carboxymethyl cellulose	1 gm	1 gm	1 gm
Propylene glycol	0.5 ml	0.5 ml	0.5 ml
Methyl paraben	0.2 ml	0.2 ml	0.2 ml
Asystasiagangetica	1 gm	----	1 gm
Euphorbia cyathophora	----	1 gm	1 gm
Triethanolamine	q.s	q.s	q.s
Water	100 ml	100 ml	100 ml

2.7 Quality control parameters of polyherbal gel

Quality control of the formulation at different concentrations was carried out to evaluate the pH, Viscosity, Skin irritation test and spread ability.

- **pH determination**

The pH of gel was determined by using digital pH meter.

- **Spreadability**

The polyherbal gel was placed in between the slides under the direction of certain load. The spreadability was expressed in terms of time in seconds taken by two slides to slip off from gel.

- **Viscosity**

The measurement of viscosity of the prepared polyherbal gel was done with Brookfield viscometer.

- **Skin irritation studies:**

The wistar rats of either sex weighing 180-200 gm were used for skin irritation studies. The intact skin was used. The hairs were removed from the rat 3 days before the experiment. The gels containing extracts were used on test animal. Gel base was applied on the back of animal taken as control. The animals were treated daily upto seven days and finally the treated skin was examined.

2.8 Antimicrobial Activity (Well Diffusion Assay)

- **Preparation of Dilutions of the Samples**

The dilutions of the samples were made for the concentration as 100µg/ml, 150µg/ml, 200µg/ml, and 250µg/ml respectively of the sample, after that volume makeup wasdone with distilled water till 1ml.

- **Preparation of Nutrient Agar Media**

28 g of Nutrient Media was dissolved in 1 liter of distilled water. pH of media waschecked before sterilization. Media was sterilized in autoclave at 121 o C at 15 lbspressure for 15 minutes. Nutrient media was poured into plates and placed in thelaminar air flow until the agar was get solidified.

- **Well Diffusion Assay**

Culture of bacterial strains (s. aureus) was spread on the Nutrient agar media(NAM). The wells were then formed for the inoculation of the

International Journal of Pharmaceutical Research and Applications
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samples (extract gel) given in the different concentrations, volume make-up was donetill 1 ml. 100 µl of the sample was loaded. The plates were allowed to incubate at 37^0 C for 48-72 hours for the best results. The bacterial suspension was standardized to10^8 CFU/ml of bacteria and kept into the shaker. Then, 100µl of the inoculum fromthe broth (containing 10^8 CFU/ml) was taken with a micropipette and then transferredto fresh and sterile solidified Agar Media Plate. Four wells of 6 mm were bored in the inoculated mediawith the help of sterile cork-borer. Each well was filled with different concentrationof formulations (1, 2 and 3). It was allowed to diffuse for about 30 minutes at room temperature andincubated for 18-24 hours at 37^o C. The zone of inhibition (ZOI) was observed andmeasured in mm. Zones were measured to a nearest millimeter using a ruler, whichwas held on the back of the inverted Petri plate. The diameters of the zone of completeinhibition (as judge by unaided eye) were measured, including the diameter of thewell **(Shah et al., 2021).**

2.9Pharmacological study

- **Animal**

Animals Healthy Wistar albino rats weighing between 180 and 200 g were used for the present study. The study protocol was approved by the Institutional Animal Ethics the whole preclinical experimentation was also done at the authorized animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal. The animals were acclimatized to the standard laboratory conditions at 25 ± 2 °C, relative humidity of 44–56%, and light and dark cycle of 12:12 h and fed with standard diet and water ad libitum during the study.

- **Acute dermal toxicity**

The acute dermal toxicity test of polyherbal extract was determined according to the OECD guidelines no. 402. Adult wistar rats of either sex were used. Animals were divided in five groups, each group comprising of six animals. Approximately 24 h before the test, 10% hairs of the body was depilated from the dorsal area of the test animals by suitable depilatory preparation (Veet). All animals were monitored for 21 days for changes in fur, eyes, behavior and toxic dermal reactions.

- **Grouping of animals and treatment scheduled**

The back of the animals was shaved and sterilized with 70% ethanol before 7 X 7 mm excision wound going to be create by a surgical blade from a predetermined shaved area on the back of each animal. The wound left undressed to the open environment and no local or systemic antimicrobial agents used **(Walker et al., 2015).** This model is used to monitor the rate of wound contraction. A progressive decrease in the wound area was monitored periodically at every 4^{th} day interval. The actual value is converted into percentage value taking the size of the wound at time of wounding as 100 %. The animals were randomly divided into 5 groups and each group containing 6 animals. The treatments of each gel were applied topically once a day**.**

Group-I: Negative control (Gel base applied topically)

Group-II: Rats were treated with Aystasiagangetica extracts gel (1%) Formulation 1

Group-III: Rats were treated with Euphorbia cyathophora extracts gel (1%) Formulation 2

Group-IV: Rats were treated with Polyherbal formulation of extracts gel (1:1)

Group V: Rat were treated with Gentamicin Standard gel (1%)

- **Excision wound model**

Excision wound was created as per the method described with some modifications**.** Animals were shaved on the dorsum portion using depilatory cream (Veet) and were anesthetized using ketamine hydrochloride (100 mg/kg, body weight). An impression was made on shaved dorsal region and area of the wound to be created was marked. A full thickness excision wound with a circular area was created along the marking using toothed forceps, a surgical blade, and pointed scissors. The 500 mg of each simple gel base, formulated extract gel, and standard drug were applied once daily from the same day (Zero days) of the operation until the complete healing. In this model, wound contraction and epithelialization period were evaluated**(Bogalhão, 2017).**

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Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494

III. RESULT AND DISCUSSIONS

3.1 Collection of plant material

Table 2: Percentage Yield of plant material

Plant name	Solvent	Theoretical weight	Yield(gm)	% yield
AsystasiaGangetica	Methanol	400	19.63	4.90
Euphorbia Cyathophora	Methanol	350	17.02	4.25

3.2 Phytochemical Test

Table 3: Phytochemical analysis of Leaves extracts of Asystasiagangetica

The phytochemical screening of methanolic leaf extracts of Asystasiagangetica and Euphorbia cyathophora reveals notable differences and similarities in their secondary metabolite profiles. These findings are essential as phytochemicals are known to contribute to the therapeutic and biological properties of medicinal plants.

Alkaloids: Both Asystasiagangetica and Euphorbia cyathophora tested positive for all four alkaloid tests (Dragendorff's, Mayer's, Wagner's, and Hager's), suggesting that alkaloids are abundant in both species. Alkaloids are well known for their antimicrobial, analgesic, and anticancer activities, indicating that both plants may have potential pharmaceutical applications.

Glycosides: Asystasiagangetica showed positive results for all glycoside tests (Borntrager's, Legal's, and Killer-Killiani), indicating the presence of cardiac or anthraquinone glycosides. In contrast, Euphorbia cyathophora tested negative for all glycoside tests, suggesting a lack or negligible presence of glycosides. This indicates that Asystasiagangetica may have more pronounced cardioprotective or laxative properties, typically associated with glycosides.

Carbohydrates: Both species showed positive results in all carbohydrate tests (Molisch's, Fehling's, Benedict's, and Barfoed's), confirming the presence of monosaccharides, reducing sugars, and complex carbohydrates. This indicates a good nutritional value and potential energy source in both plant extracts.

Proteins and Amino Acids: Asystasiagangetica tested positive for both Biuret and Ninhydrin tests, indicating the presence of proteins and free amino acids. Euphorbia cyathophora was negative for these tests, suggesting an absence or low concentration of proteinaceous compounds.

This suggests that Asystasiagangetica might offer greater nutritional and enzymatic potential compared to Euphorbia cyathophora.

Flavonoids: Both plants were positive in alkaline reagent and Lead acetate tests, confirming the presence of flavonoids, which are known for antioxidant, anti-inflammatory, and antimicrobial properties. This indicates that both species possess significant therapeutic potential in oxidative stress-related conditions.

Tannins and Phenolic Compounds: Both species tested positive for Ferric Chloride test, suggesting the presence of tannins and phenolic compounds, which contribute to astringent, antimicrobial, and antioxidant activities.

Saponins: Asystasiagangetica was negative, indicating an absence of saponins. Euphorbia cyathophora was positive, suggesting the presence of saponins, which are known for hemolytic, expectorant, and immune-boosting properties.

This implies that Euphorbia cyathophora may have additional antimicrobial and surface-active potential compared to Asystasiagangetica.

Triterpenoids and Steroids: Asystasiagangetica showed a negative result for Salkowski's test. Euphorbia cyathophora tested positive, indicating the presence of triterpenoids and/or steroids, which are important for their anti-inflammatory, anticancer, and hormonal activity.

- Asystasiagangetica exhibits a broader range of phytochemicals, particularly glycosides, proteins, and amino acids, which may make it more versatile in medicinal and nutritional applications.
- Euphorbia cyathophora, although lacking some constituents, uniquely contains saponins and triterpenoids/steroids, suggesting a different therapeutic profile, possibly more inclined towards anti-inflammatory and wound healing uses.

Both plants have promising pharmacological potential, but their differing phytochemical profiles suggest that they may serve roles in traditional and modern medicine.

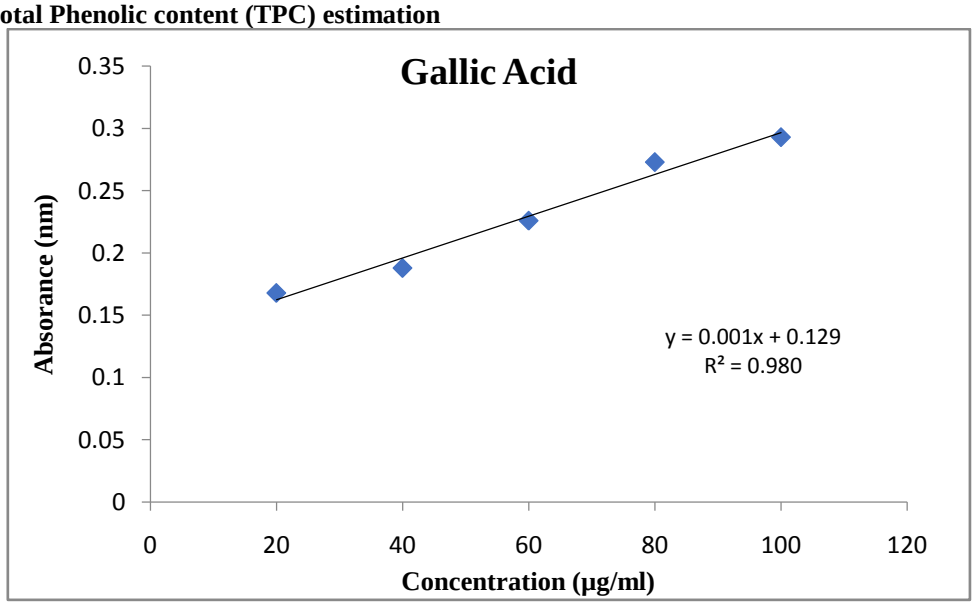
International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494

Figure 2: Phytochemical Testing of AsystasiaGangetica

Figure 3: Phytochemical Testing of Euphorbia Cyathophora

3.3 Quantitative Estimation of Phytoconstituents

- **Total Phenolic content (TPC) estimation**



Graph 1: Represent standard curve of Gallic acid

- **Total Phenolic Content**

Table 5: Total Phenolic Content in AsystasiaGangeticaextract

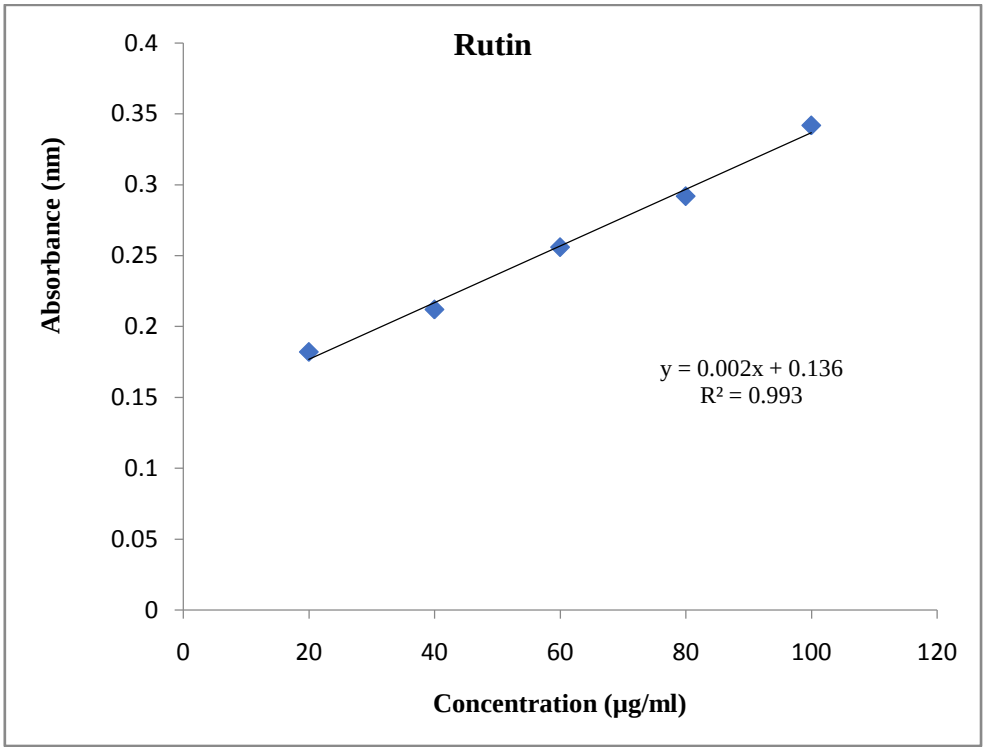
Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.171	92 mg/gm
0.236	
0.265	

Table 6: Total Phenolic Content in Euphorbia Cyathophoraextract

Absorbance	TPC in mg/gm equivalent of Gallic Acid
0.161	60 mg/gm
0.198	
0.209	

- **Total Flavonoids content (TFC) estimation**

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494



Graph 2:Represent standard curve of Rutin

- **Total Flavonoid Content**

Table 7: Total Flavonoid Contentin AsystasiaGangeticaextract

Absorbance	TFC in mg/gm equivalent of Rutin
0.176	29.5 mg/gm
0.198	
0.212	

Table 8: Total Flavonoid Content in Euphorbia Cyathophora extract

Absorbance	TFC in mg/gm equivalent of Rutin
0.151	21 mg/gm
0.179	
0.206	

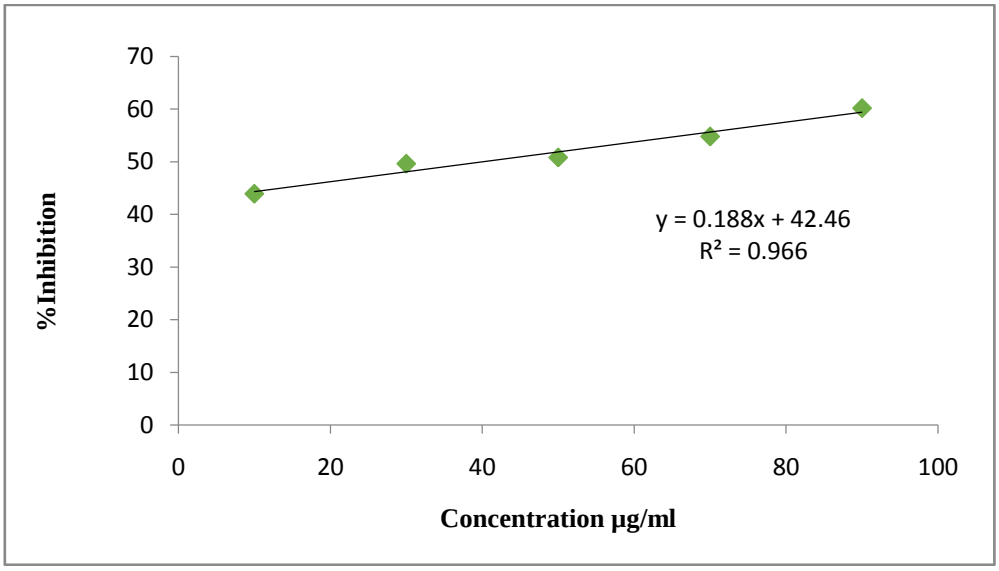
3.4 Anti-Oxidant Activity

- DPPH 2, 2- diphenyl-1-picryl hydrazyl Assay

Table 9: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.486	51.204
40	0.439	55.923
60	0.395	60.431
80	0.343	65.562
100	0.298	70.080
Control	**0.996**	
IC50		21.75

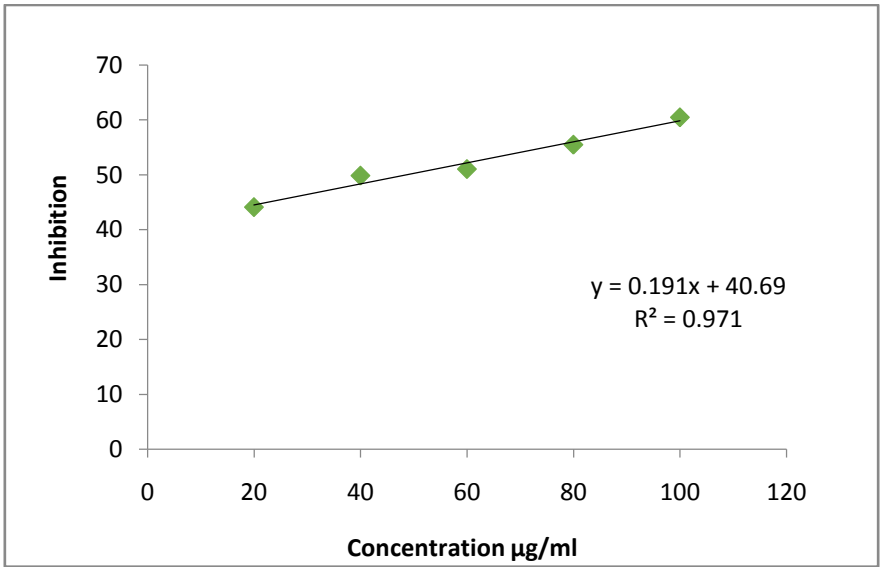
International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494



Graph 3:DPPH radical scavenging activity of Std. Ascorbic acid

Table 10:DPPH radical scavenging activity of methanol extract of Euphorbia cyathophora

Concentration(µg/ml)	Absorbance	%Inhibition
20	0.518	44.108
40	0.464	49.837
60	0.453	51.027
80	0.412	55.459
100	0.366	60.432
Control	**0.925**	
IC5048.74		



Graph 4: Represents the Percentage Inhibition Vs Concentration of extract

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494

3.5 Quality control parameters of formulation

- **Organoleptic properties**

Table 11: Organoleptic properties

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

The gel was evaluated based on color, appearance, and homogeneity. When tested, gel turned out to be a somewhat yellowish color.

- **Measurement of pH, Viscosity and Spreadability test**

Table 12: pH, Viscosity and Spreadability test

Formulation	pH	Viscosity determination (cps)	Spreadability test (gm.cm/sec)	skin irritation study
Formulation1	6.1	5455±0.71	11.99	Not irritation observed
Formulation 2	6.5	5509±0.56	11.19	Not irritation observed
Formulation 3	6.8	5691±0.82	13.20	Not irritation observed

3.6 In-vitro Antimicrobial activity

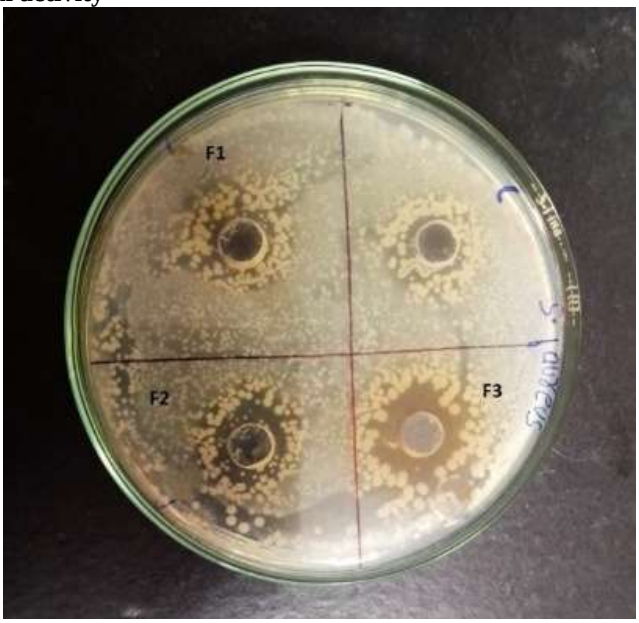
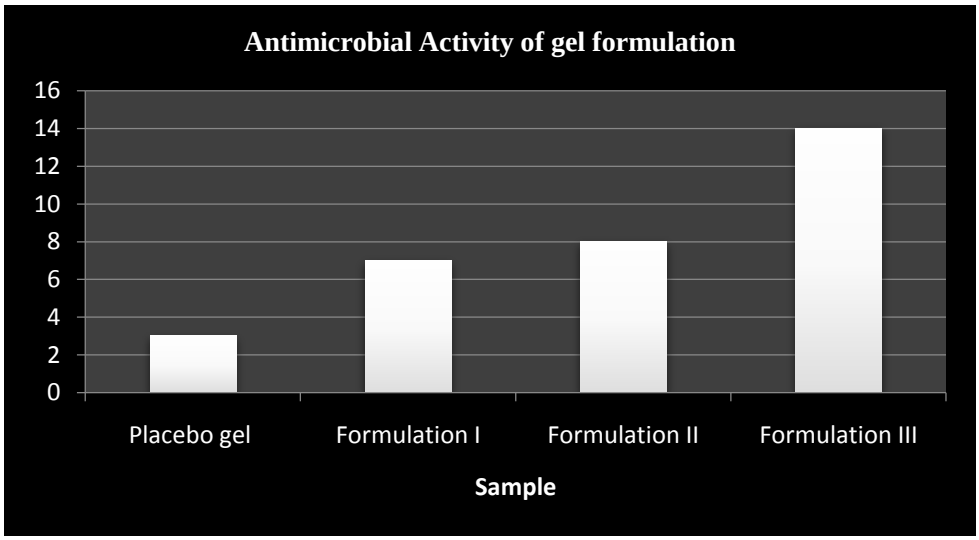


Figure4: Antimicrobial Activity of gel formulation

Table 13: Zone of Inhibition of Antimicrobial Activity

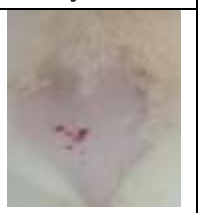
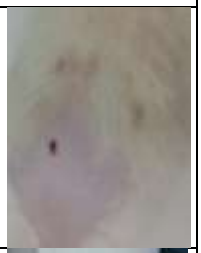
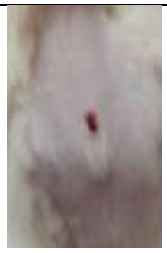
Sample Name	Zone of Inhibition (mm)
Placebo gel	3 mm
Formulation I	7 mm
Formulation II	8 mm
Formulation III	14 mm

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494



Graph 5: representation of anti-microbial activity

3.7 Wound Healing

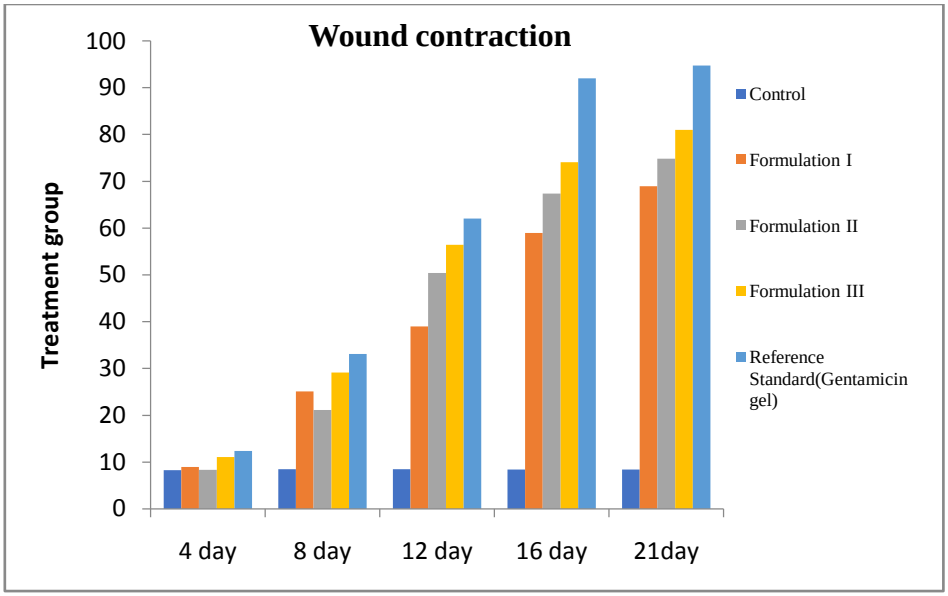
Group	4 Day	8 Day	12 Day	16 Day	21 Day
Control					
Formulation I Aystasia Gangetica gel					
Formulation II Euphorbia Cyathophorgel					
Formulation III Polyherbal gel					

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 4 July-Aug 2025, pp: 94-105 www.ijprajournal.com ISSN: 2456-4494

Reference Standard(Gentamicin gel)					

Table 14: Percentage wound closure in various treatment groups

S. No.	Formulation	Area of wound during different days of observation (%)				
		4 day	8 day	12 day	16 day	21day
1	**Control**	8.31±0.7120	8.45±0.8143	8.49±0.7820	8.39±0.8848	8.42±0.9819
2	**Formulation I**	8.99±0.4048	25.11±0.4355	38.99±0.7070	58.99±0.5421	68.94±0.5532
3	**Formulation II**	8.37±0.7823	21.11±0.5239	50.44±0.5519	67.40±0.5532	74.86±0.5824
4	**Polyherbal formulation**	11.07±0.8599	29.12±0.9523	56.43±0.6679	74.10±0.3359	80.99±0.3826
5	**Reference Standard (Gentamicin gel)**	12.41±0.7437	33.08±0.7279	62.02±0.6429	91.98±0.6431	94.74±0.2523



Graph 6: Evaluation of wound healing activity

Discussion

The pH of all prepared formulation ranged from 6.1- 6.8. The pH of the prepared gel formulation was considered to be acceptable to avoid the risk of irritation upon application to the skin. The measurement of viscosity of the prepared gel was done with Brookfield viscometer with spindle no: 61. The results were found to be Formulation 1- 5455±0.71, Formulation II - 5509±0.56 and Formulation III- 5691±0.82 cps.

The in vitro antibacterial activities of the Formulation I, Formulation II and Formulation III standard gelhave been investigated. Antibacterial activity was performed against Staphylococcus aureus by well diffusion assay. Formulation III showed best zones of inhibition.

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IV. CONCLUSION

The findings of this study suggest that the Polyherbal formulation of Asystasiagangetica and Euphorbia cyathophora extracts possesses significant wound healing properties. The combination of these plants accelerated wound closure, reduced inflammation, and enhanced tissue regeneration. The presence of bioactive phytochemicals contributed to its efficacy, making it a promising natural remedy for wound management.

Considering the effectiveness of this herbal formulation, further research, including clinical trials, is recommended to validate its therapeutic potential. The development of topical applications such as ointments or gels from these extracts could offer a cost-effective and natural alternative for wound care in traditional and modern medicine. Thus, this study underscores the importance of herbal medicine in advancing wound healing therapies and encourages further exploration of plant-based formulations for medical applications.

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