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Formulation And Evaluation of Pergularia Daemia Ointment by Leaf Extract

Syed Gouse Firoz[1*], A. Arun Kumar[2], K. Sudha Rani[3], A.Venkata Padma[4], M. Ramya Brahmini[5], S. Jagan Mohan[6], N. Adilakshmi[7]

[*1]Department of pharmaceutics, AM Reddy Memorial college of pharmacy, Petlurivaripalem, Narasaraopet, Palnadu-522601 Andra Pradesh, India.

[2,3,4,5,6,7] B. Pharm Students AM Reddy memorial college of pharmacy, Petulurivaripalem, Narasaraopet, Palnadu-522601 Andra Pradesh, India.

[*]**Corresponding author:**

Syed Gouse Firoz

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ABSTRACT

The study formulates and evaluates an herbal ointment using Pergularia daemia leaf extract for wound healing and antimicrobial use. The extract contains flavonoids, alkaloids, and tannins, enhancing therapeutic effects. Results show strong antimicrobial action, wound contraction, and epithelization, supporting its potential as a natural alternative. Clinical trials are needed for further validation.

Keywords: *Pergularia daemia, Herbal ointment, Wound healing, Antimicrobial activity, Phytochemicals, Topical formulation, Ethnomedicine, Natural therapeutics, Plant-based medicine.*

1. INTRODUCTION

India stands among the leading global producers of medicinal and aromatic plant materials. With around 20,000 identified medicinal plant species spread across 15 agro-climatic zones, only about 7,000 to 7,500 species are utilized commercially.[1] India's ancient healthcare systems like Ayurveda and Unani have long relied on herbal remedies for treatment.[2] The use of medicinal plants varies across traditional Indian systems as follows: Ayurveda utilizes about 2,000 species, Siddha employs 1,300, Unani uses 1,000, Homeopathy depends on 800, Modern medicine utilizes 200, and Folk medicine includes around 4,500 species. Across the country, nearly 25,000 herbal-based formulations are used, especially in traditional and indigenous medical practices. [3] These herbal preparations are derived from various plant parts such as leaves, roots, stems, bark, flowers, and seeds, as well as exudates like resins, gums, and latex.[3] Medicinal plants are known for producing secondary metabolites biologically active compounds synthesized from primary metabolites. These include alkaloids, flavonoids, glycosides, tannins, terpenoids, phenolic acids, and phytosterols, all of which contribute to various therapeutic effects.[4] However, these bioactive phytocompounds are not universally present in all plants and are often limited in availability. Herbal plants used in traditional medicine systems like Ayurveda and Siddha tend to exhibit low toxicity and contain active chemical structures, which makes them suitable candidates for alternative drug development.[5]

IMAGE:-1 (Pergularia Plant)

Parts of plants:-

- ✓ The flowers of Pergularia daemia are greenish-yellow or dull white and appear in lateral cymes. The corolla forms a tubular shape.
- ✓ The fruit is a follicle that matures after 13 to 14 months, releasing ovate seeds covered with velvety hairs. [5]

IMAGE-2(Fruit And Flower)

2. METHODS AND MATERIALS

1. **Collection and Processing of Pergularia daemia:-**

- Fresh Pergularia daemia leaves and stems were collected from a specified geographical region.
- Plant material was authenticated by a botanist and cleaned to remove impurities.
- Air-dried in a shaded environment for a specific duration to retain phytoconstituents.
- Crushed into a fine powder and extracted using solvent extraction methods (e.g., methanol, ethanol, or aqueous extraction).
- The extract was filtered and concentrated using a rotary evaporator for further formulation.

2. Formulation of the Ointment

- The concentrated plant extract was incorporated into a ointment base (e.g., petroleum jelly, beeswax, or emulsifying agents).
- Active concentrations of the extract were determined based on preliminary studies.
- The mixture was homogenized to ensure uniform distribution.
- Stability tests were conducted to evaluate physical characteristics like texture, spreadability, and pH.

3. Experimental Design and Methodology

- In vitro and in vivo studies were conducted to assess wound healing efficacy.
- Animal models (e.g., rats) were selected based on ethical guidelines for wound healing studies.
- Incision and excision wound models were created, and treatment groups were assigned.
- Healing progression was monitored using histological analysis and wound contraction measurements.
- Control groups included standard wound healing ointments for comparative analysis.

4. Analytical Techniques Used for Evaluation

- Phytochemical Analysis: Identification of bioactive compounds responsible for healing effects.
- Antimicrobial Activity: Evaluating the ability of the ointment to prevent infection in wounds.[6] Skin Irritation Tests: Ensuring safety for human application

Traditional Uses:

- ➢ **Antipyretic**: Used to reduce fever.

- **Anti helminthic**: Effective against parasitic worms.
- **Laxative**: Helps in relieving constipation.
- **Hepatoprotective**: Protects the liver from damage.
- **Anti-inflammatory**: Reduces inflammation.
- **Antioxidant**: Helps in combating oxidative stress.
- **Anticancer**: Shows potential in inhibiting cancer cell growth.
- **Antidiabetic**: Assists in managing blood sugar levels.
- **Antifungal and Antibacterial**: Effective against fungal and bacterial infections.[7]

Objectives:-

1. To identify, extract, and analyze the phytochemical constituents of Pergularia daemia and correlate them with its pharmacological potential.
2. To investigate the plant's pharmacological activities, such as antimicrobial, antiinflammatory, antioxidant, antidiabetic, and wound-healing properties, through invitro and in-vivo studies.
3. To study the cytotoxicity, potential anticancer effects, and safety profile of Pergularia daemia extracts using preclinical models.
4. To evaluate the plant's traditional medicinal uses in ethnobotanical practices and compare its effectiveness with other plants of similar applications.

Anti Microbial Property:

Pergularia daemia ointment has shown promising antimicrobial properties. Research indicates that it is effective against a range of pathogens, including Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli. These are common wound pathogens, and the ointment has demonstrated inhibitory effects through methods like the agar well diffusion technique.

The antimicrobial activity is attributed to the plant's bioactive compounds, such as flavonoids, alkaloids, and tannins, which disrupt microbial growth and survival. Additionally, studies suggest that Pergularia daemia could serve as a natural and cost-effective alternative for treating microbial infections.[8]

Wound Healing Property:

Pergularia daemia has demonstrated significant wound-healing potential in various studies. Its bioactive compounds, such as flavonoids, tannins, and alkaloids, contribute to its ability to promote tissue repair and reduce inflammation. Research has shown that ointments formulated with Pergularia daemia leaf extracts can accelerate wound closure and minimize scarring.

In experimental models, wounds treated with Pergularia daemia ointment exhibited faster healing rates compared to untreated wounds or those treated with standard ointments. This is attributed to its antimicrobial properties, which help prevent infections, and its antioxidant activity, which combats oxidative stress at the wound site .[9]

Formulation procedure:

S.NO	INGRIDENTS	RAASPJ- 1	RAASPJ- 2	RAASPJ- 3	RAASPJ- 4
1	Pergularia daemia extract[Active Ingredient]	5-10g	-	5-10g	5-10g
2	Biomass residue of pergularia daemia	-	5-10g	-	-
3	Bees wax [thickening agent]	12.5g	12.5g	-	-
4	Coconut oil [base,emollient,carrier]	QS	QS	-	-
5	Vitamin-E{Tocopherol} [Antioxidant]	0.25g	0.25g	-	-
6	Methyl paraben [preservative]	0.1g	0.1g	-	-

7	White Petroleum Jelly-Petrolatum [occlusive agent,base &protect skin]	-	-	20g	-
8.	Liquid paraffin [moisturizer,emollient &enhance spreadability]	-	-	4.5g	-
9.	Peppermint oil [cooling effect, soothes irritation]	-	-	0.5g	-
10.	Gelucire® 44/14 {glycerides&PEG Esters of fatty acids} [Lipid-based penetration enhancer,base]	-	-	-	25g
11.	Phytosqualane -from Olive[Natural emollient, skin penetration enhancer]	-	-	-	10g
12.	Isopropyl Myristate [penetration enhancer,emollient]	-	-	-	7.5g
13	Geogard Ultra™ {Gluconolactone and Sodium Benzoate} [Natural preservative, moisturizing properties]	-	-	-	0.5g

3. PREPARATION

Start preparation of the ointment, weigh 12.5 g of beeswax and transfer it into a clean beaker. Heat the beaker using a water bath until the wax is fully melted. Once molten, add 0.1 g of methyl paraben and 0.25 g of vitamin E to the mixture, ensuring thorough stirring for uniform distribution. In a separate mortar, take 5 g of Pergularia daemia extract and slowly incorporate the hot molten mixture while continuously stirring. Gradually add oil to achieve the desired ointment consistency, maintaining continuous stirring to obtain a smooth, homogeneous blend. After mixing, allow the ointment to cool at room temperature before transferring it into a clean, labeled container for storage.[10]

TABLE-1: formulation table

Evalution of pergularia daemia ointment:

4. INVITRO STUDIES

1. Dissolution studies:-

Dissolution studies play a crucial role in pharmaceutical research by analyzing how efficiently a drug dissolves in a specific medium. These evaluations help assess bioavailability, optimize formulation stability, and determine drug release characteristics. Understanding dissolution rates allows researchers to enhance therapeutic effectiveness and ensure consistent drug delivery.[11]

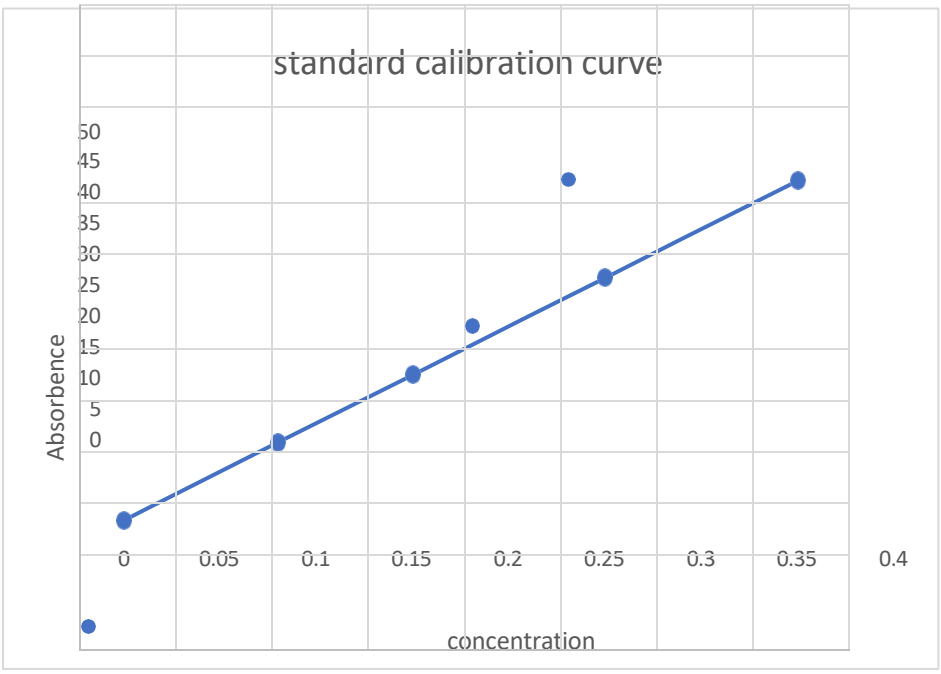
Dissolution Using Franz Diffusion Cell:

The Franz diffusion cell is a widely used **i**n vitro model for studying drug permeation through biological membranes. It consists of a donor compartment (where the drug formulation is placed) and a receptor compartment (containing the dissolution medium**).** A semi-permeable membrane separates the two compartments, allowing controlled diffusion of the drug**.** [11]

Image-4: Franz Diffusion Cell Apparatus

Conc	Abs
0	0
10	0.12
20	0.188
30	0.244
40	0.312
50	0.45

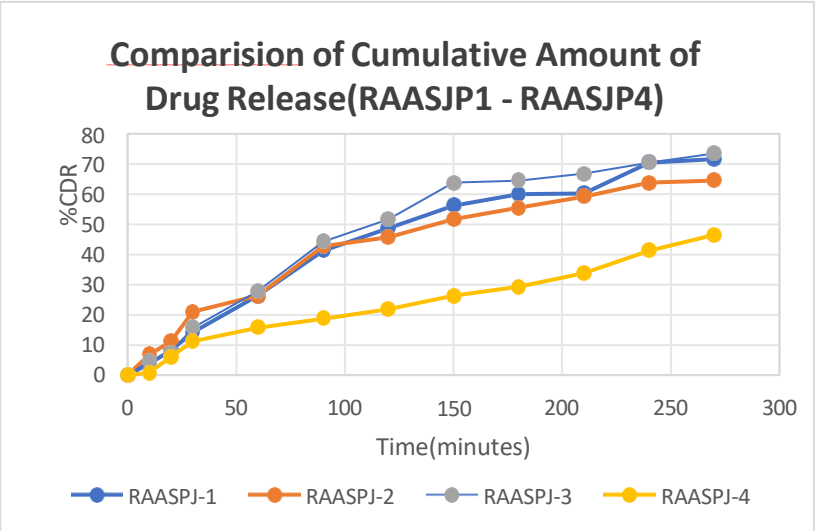
Table-2 Standard Calibrationcurve



Graph:1 standard calibration curve

s.no	time(min)	RAASPJ-1	RAASPJ-2	RAASPJ-3	RAASPJ-4
1	0	0	0	0	0
2	10	3.75	6.75	4.5	0.75
3	20	8.25	11.25	7.5	6
4	30	14.25	21	15.75	11.25
5	60	26.25	26.25	27.75	15.75
6	90	41.25	42.75	44.25	18.75
7	120	48.75	45.75	51.75	21.75
8	150	56.25	51.75	63.75	26.25
9	180	59.925	55.5	64.5	29.25
10	210	60.3	59.25	66.75	33.75
11	240	70.5	63.75	70.5	41.25
12	270	71.625	64.5	73.5	46.5

Table-3 Comparision of %Cumulative amount of Drug Release(RAASPJ-1 RAASPJ-4)



Graph:-2 comparision of cumulative amount of drug release sample-1 to 4

5. ANTI MICROBIAL ACTIVITY

Well Diffusion Method:- To prepare the nutrient agar medium, dissolve 2.8 g of agar in 100 mL of distilled water and heat it to 90°C to completely dissolve the agar. Sterilize the agar by autoclaving at 121°C for 15 minutes. Meanwhile, sterilize the workspace, specifically the laminar airflow chamber, using UV rays for 10 minutes to prevent contamination.[12] Sterilized Petri dishes, which have been treated in a hot air oven at 160°C for 2 hours, are then used to pour the agar, allowing it to solidify aseptically. Once the medium is ready, uniformly spread the Lactobacillus culture over the agar surface. Using a sterile cork borer, create three wells with a diameter of 6 mm in the agar. Prepare an ointment suspension by mixing one spatula of ointment with 20 mL of distilled water, then carefully add the suspension into the wells using a micropipette. [13]The Petri plates are incubated at 37°C for 72 hours, after which the zones of inhibition surrounding the wells are observed and measured. Clear zones of inhibition indicate the antimicrobial activity of the Pergularia daemia ointment against the tested Lactobacillus strain.[14]

Image:-5 Anti Microbial Activity

2. **Wound healing activity:-** Wound healing activity of some selected new compounds was assessed using excision wound model. The albino rats of either sex (150-200 g) were divided in to three groups of five animals each. Test compounds (RAASPJ-2) were formulated as 2.5% w/w ointment for local application, using simple ointment as vehicle. [15]The various groups were treated as follows:

Group-I : Control (0.5 g,2.5 % w/w simple ointment applied locally) Group-II : Standard (5 % w/w povidine iodine ointments applied locally)

Group-III : RAASPJ-2

(0.5 g of 2.5% w/w ointment of test compounds applied) applied locally once a day till complete epithelization. Percentage of wound closure = 1-(AD/AO)*100

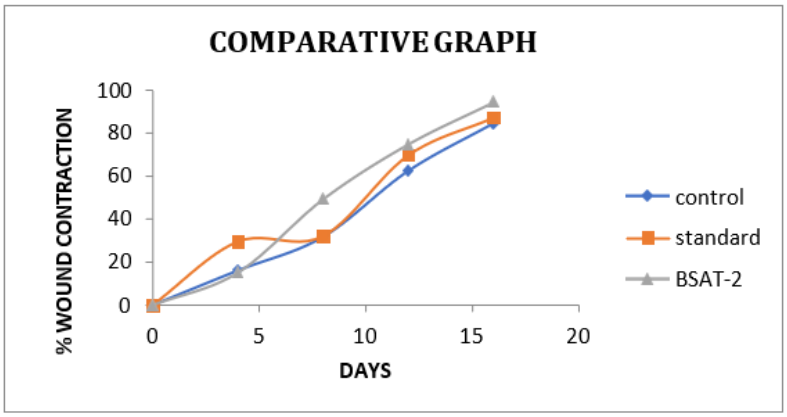
Where,

Ao = Wound area on day zero (500 sq. mm) Ad = Wound area on corresponding days.

The results are tabulated in Table-. The photographs showing percentage wound closure at different days. The results obtained were subjected to statistical analysis using ANOVA followed by Turkey-Krammer Multiple Comparison Test.wound parameter.[16]

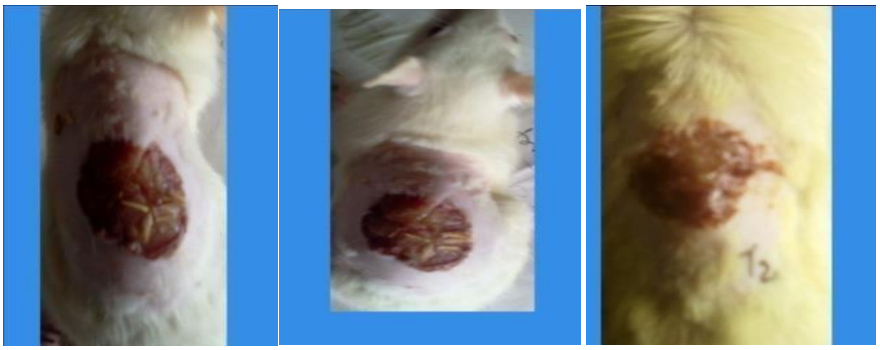
Group	% Contraction of wound on different days (sq.mm)				Epithelization time in days
	4th day	8th day	12th day	16th day	
Control	16±	30.5±14.50	62±2	84.5±	24± 1.42
Povidine iodine	29.5± 0.002***	32± 0.003***	69.5±0.002***	87±0.001***	18.1± 0.69***
RAASPJ-2	15± 0.001***	49.± 0.002***	74.5± 0.002***	94± 0.001***	17.9± 0.87***

Table 4- Comparission Of Samples

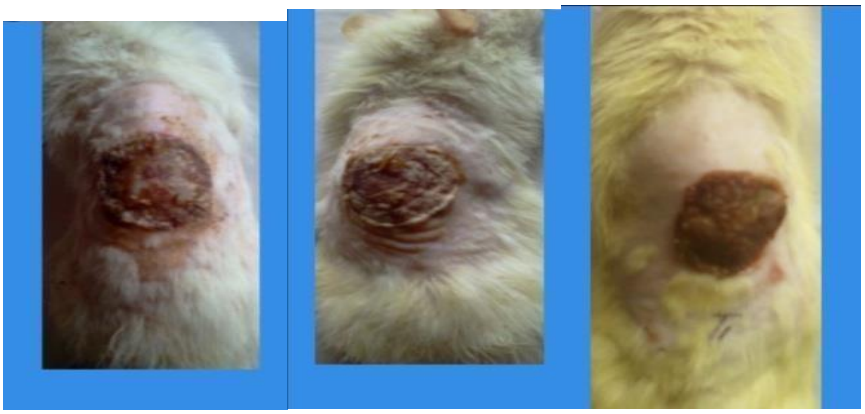


Graph-3: comparison of % wound contraction between RAASPJ-2, standard povidin-iodin and control.

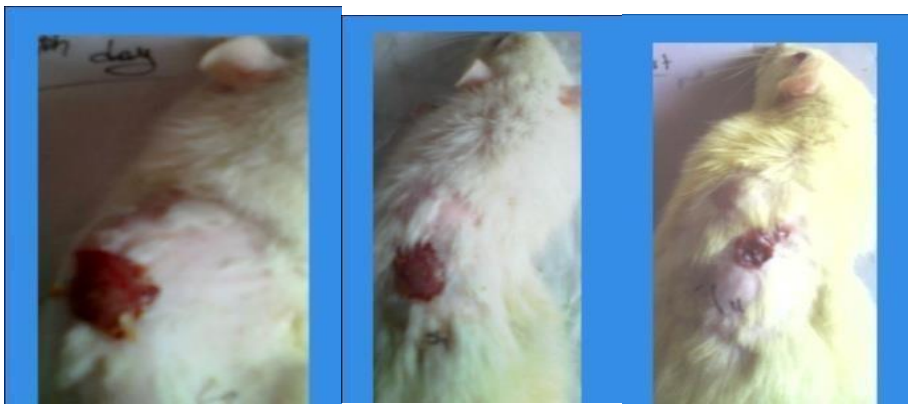
0 Day



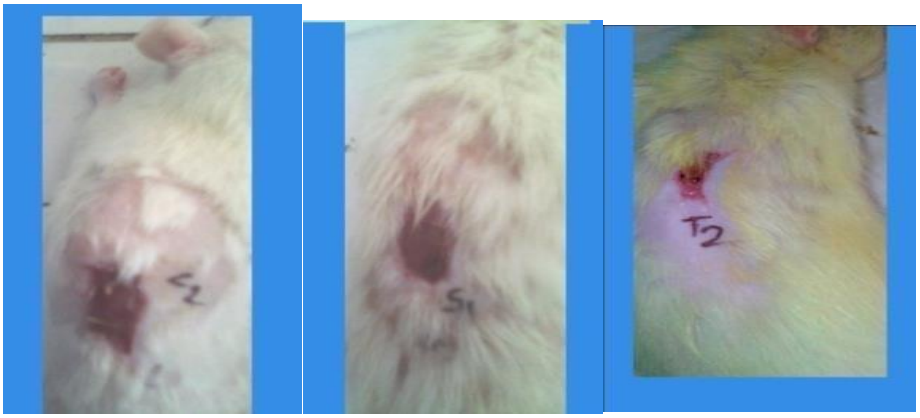
4th day C **4th day S** **4th day T**



8th day C **8th day S** **8th day T**



12th day C **12th day S** **12th day T**



16th day C **16th day S** **16th day T**

Image:-5 Wound Healing Activity By Using Albino Rats

- Wound Contraction: RAASPJ-2 achieved 94% wound contraction by the 16th day, demonstrating significant healing effectiveness. This was superior to the control and closely matched the effectiveness of povidone-iodine, a well- established standard treatment.[17]
- Epithelization: Faster epithelialization was observed, confirming accelerated tissue repair due to the herbal formulation.[18]

6. RESULTS &DISCUSSION

Phytochemical Screening

The preliminary phytochemical screening of Pergularia daemia methanolic extract revealed the presence of biologically active compounds such as flavonoids, tannins, alkaloids, glycosides, steroids, terpenoids, and saponins, all of which contribute to wound healing and antimicrobial activity.

Formulation of Ointment

Four different ointment formulations (F1, F2,F3andF4) were prepared using 5%-10% concentration of Pergularia daemia extract. These formulations exhibited smooth consistency, skin-friendly pH, and stable physical properties, making them suitable for topical application.

Physicochemical Evaluation

All formulations were analyzed for color, consistency, spreadability, pH, washability, and stability, ensuring their effectiveness and ease of application.

Stability Study:

Stability studies confirmed that the ointment remained physically and chemically stable over a three-month testing period, with no significant changes in appearance, odor, or pH.

Invitro studies

1. Dissolution Study

The dissolution profile demonstrated gradual drug release, with the RAASPJ-3 formulation showing the highest bioavailability when compared to other formulations.

Drug Release and Bioavailability:

A key factor in assessing topical formulations is the release profile of the active compound, which determines absorption and therapeutic response. The Franz Diffusion Cell Test provided essential insights into the bioavailability of Pergularia daemia in different ointment formulations.

RAASPJ-3 exhibited a cumulative drug release of 73.5% at 270 minutes, proving efficient bioavailability and rapid therapeutic action.

RAASPJ-1 and RAASPJ-2 showed moderate drug release, maintaining a steady absorption rate beneficial for sustained healing.

RAASPJ-4 demonstrated slower drug release, suggesting potential use in extended therapeutic applications where prolonged effects are needed.

These variations in release profiles indicate that the ointment formulation caters to both acute wounds requiring fast action and chronic conditions benefiting from sustained release.

2. Anti-microbial study

The herbal ointment displayed strong antimicrobial activity, particularly against Staphylococcus aureus and Pseudomonas aeruginosa, common pathogens responsible for skin infections.

RAASPJ-2 formulation exhibited the largest inhibition zone, confirming its high potency against bacterial infections. Mild inhibition against ***Lactobacillus*** **species** suggests selective antimicrobial action, preserving beneficial skin microbiota.

Unlike broad-spectrum synthetic antibiotics that eliminate both harmful and beneficial microbes, the ointment specifically targets pathogenic bacteria while maintaining natural bacterial balance.

7. INVIVO STUDY

Wound Healing Efficacy

The excision wound model in albino rats demonstrated the ointment's ability to accelerate healing and enhance tissue regeneration

- RAASPJ-2 ointment showed significant wound contraction compared to the control and standard groups.
- On the 16th day, wound closure was 94% in the RAASPJ-2 group, compared to 87% in the standard group and 84.5% in the control group.

Epithelization time was 17.9 days for RAASPJ-2, indicating faster healing compared to the standard treatment.

8. CONCLUSION

The present study successfully formulated and evaluated a herbal ointment using Pergularia daemia leaf extract, demonstrating its potential as an effective natural therapeutic agent for wound healing and antimicrobial applications. Phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, alkaloids, tannins, and saponins, which are primarily responsible for the plant's therapeutic properties. Among the four formulations tested, RAASPJ-2 exhibited superior wound healing and antimicrobial efficacy, as evidenced by significant wound contraction, enhanced epithelization, and notable zones of inhibition against pathogenic microbes.The in vitro dissolution studies revealed favorable drug release profiles, particularly in RAASPJ-3, ensuring good bioavailability, while RAASPJ- 2 showed the best performance in both antimicrobial and in vivo wound healing assays. These findings support the traditional use *of* Pergularia daemia in ethnomedicine and validate its potential integration into modern topical therapeutics. However, to fully establish safety and efficacy, further clinical investigations and toxicological evaluations are recommended. Overall, Pergularia daemia ointment presents a promising, eco-friendly, and cost-effective alternative to synthetic wound healing agents.

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