Section A-Research paper
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"BIOACTIVE SYNERGY: IN-VIVO AND IN-VITRO ASSESSMENT OF AN HERBAL FORMULATION DERIVED FROM ALOE VERA ROOTS AND CURCUMA LONGA RHIZOMES EXTRACTS"

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Abstract:

Introduction: Various systems of medicine have been practiced worldwide for centuries, each with its unique principles, philosophy, and treatments.

Objective: This study aims to develop, evaluate, and compare an antimicrobial herbal lotion using curcuma and Aloe vera extracts for dermatological applications.

Materials and Methods: The importance of quality control in herbal medicine and the global preference for traditional medicines and herbal remedies are recognized. The formulation preparation involved the use of ethanolic extracts of Curcuma longa and Aloe vera roots, along with other ingredients. The lotion formulations were evaluated based on organoleptic properties, consistency, pH, spreadability, extrudability, viscosity, diffusion, hardness, loss on drying, solubility, washability, drug content, in vitro diffusion, ex vivo permeation, irritancy, and stability. Additionally, an in vivo experimental study using an excision wound model in Wistar albino rats was conducted to evaluate the prepared formulation. The study included group division, dose schedule, treatment period, and assessment of wound closure time, percentage of wound contraction, time of epithelialization, and histological studies.

Results: The results of this study provide valuable insights into the development of an effective antimicrobial herbal lotion for dermatological applications.

1. INTRODUCTION:

Various systems of medicine have been practiced worldwide for centuries, each with its unique principles, philosophy, and treatments. However, their goal remains the same: "Ensuring Perfect Health for All." It is important not to dismiss these systems as outdated, as they are as scientific as modern medicine when approached with an open mind and without prejudice [1]. The importance of quality control in herbal medicine has gained significant attention, with some herbs included in the British Herbal Pharmacopoeia and many others in the Ayurvedic Pharmacopoeia of India. The Ayurvedic Formulary of India has made commendable efforts to establish quality standards for medicine preparation. Currently, there is a growing global preference, including the World Health Organization (WHO), for the use of traditional medicines and affordable, safe herbal remedies for the general population [4].

In the field of dermatology, it is desirable to develop products that exhibit not only physical and chemical stability but also possess cosmetic appeal. These products should create an optimal environment for the active ingredients to reach their intended target site. They must be non-irritating to the skin, easy to apply and remove, and, when necessary, capable of incorporating buffers, co-solvents, antioxidants, polymeric stabilizers, and preservatives. Consequently, many formulations are complex and consist of multiple components, including interacting surfactants, polymers, and additives. A comprehensive understanding of the microstructure of such systems is crucial for optimizing the formulation and manufacturing processes of existing products, as well as for designing innovative dermatological delivery systems [36].

2. **OBJECTIVE:** Develop, evaluate, and compare an antimicrobial herbal lotion using curcuma and Aloe vera extracts.

3. MATERIALS AND METHODS:

Aloe vera roots were collected from Dr. K. N. Modi University, Newai, and Curcuma longa was obtained from nearby farms. The authentication process was conducted by the Botany Department of Rajasthan University, Jaipur (Authentication no. for Aloe vera: RUBL 21211, Curcuma longa: RUBL 21219).

Preparation of Herbal Formulation

After phytochemical and minimum inhibitory concentration studies, the lotion preparation followed. The extract was dried in a rotary dryer and stored at -8°C for further formulation. Specific formulas were designed for preparing the antibacterial lotion, ensuring a suitable order of ingredients. To prevent water and oil phase separation, the non-polar phase was melted and added to the hot polar phase. The chosen formula considered the compatibility

of P.E. with water and A.S. with the oil phase [4].

Formulation-I: - It involved mixing an undisclosed percentage of Curcuma ethanolic extract and an undisclosed percentage of Aloe vera root ethanolic extract to prepare the lotion. The specific formula used for the preparation is as follows:

Table: Chemical composition of formulation in percentage

Sr. No.	Component	Function	F1 Content % (w/w)	F2 Content % (w/w)
1	Extract of Aloe vera root	Antibacterial	5	4
2	Extract of Curcuma rhizomes	Antibacterial	2.5	2
3	Bentonite	Thickening agent	5	5
4	Methyl Paraben	Preservative	0.1	0.1
5	Glycerine	Humectant	3	3
6	Triethanolamine	Neutralizer	0.9	0.9
7	Water	Diluent	66.8	68.3
8	Cetyl Alcohol	Co-emulsifier	3	3
9	Stearic Acid	Emulsifier	1.2	1.2
10	Coconut oil	Occlusive	5	5

EVALUATION PARAMETERS:

The lotions were evaluated based on the following parameters:

Organoleptic Evaluation: Blank formulations (without active ingredient) and drug-loaded formulation were visually assessed for physical appearance, color, texture, phase separation, and homogeneity. The texture and homogeneity were determined by pressing a small quantity of the lotion between the thumb and index finger to check for consistency and the presence of coarse particles. The immediate skin feels, including stiffness, grittiness, and greasiness, was also evaluated [5, 6].

Consistency: Consistency is a crucial factor to consider in lotions, particularly for their desired application. A more solid consistency enables the lotion to remain on the skin surface for a longer duration, which is beneficial for covering open wounds. The absorption of components into the skin is influenced by the consistency of the semisolid preparation, which is measured using the Penetration Unit (PU). The PU represents the material's consistency and is expressed as a distance in tenths of a millimeter [7].

When testing the consistency of creams using the PNR 12 instrument, there are two potential outcomes for surface detection. The testing process follows the Standard Operating Procedure provided by Anton PAAR Prove Tec GmbH.

pH Analysis: For each formulation, approximately 2.5 g of the lotion was placed in a dry beaker and 50 ml of water was added. The beaker containing the lotions was then heated on a water bath maintained at 60-70°C. The pH of the lotions was measured using a pH meter (pH Tutor, Eutech Instruments). The measurements were performed in triplicate, and the average of the three readings was recorded [7].

Spreadability: The spreadability of the formulation was determined using a modified apparatus suggested by Multimer [8]. A wooden block with a pulley and fixed glass slide was used. An excess of lotion (3 g) was placed on a ground plate and sandwiched between this plate and another glass plate with a hook. A 1 kg weight was applied for 5 minutes to expel air and ensure a uniform film of lotion. Excess lotion was removed, and the top plate was subjected to a 240 g pull. The time taken for the top plate to cover 10 cm was recorded. A shorter time interval indicates better spreadability. The spreadability (S) was calculated using the formula: $S = M \times L / T$, where M is the weight tied to the upper slide, L is the length of the glass slide, and T is the time taken to separate the slides [8].

Extrudability: The extrudability of the formulations was assessed using a simple method preferred by researchers. The formulations were filled into collapsible tube containers. Extrudability was measured as the weight of formulation required to extrude a 0.5 cm ribbon of lotion within 10 seconds [7].

Viscosity: Rheological studies were conducted using a Brookfield Synchro-Lectric Viscometer (Model RVT) with a Helipath Stand. A sample of 50 g was placed in a beaker and allowed to equilibrate for 5 minutes before measuring the dial reading using a T-D spindle at various speeds (10, 20, 30, 50, 60, and 100 rpm). The corresponding dial readings were recorded at each speed. The spindle speed was then gradually decreased, and the corresponding dial readings were noted. The measurements were performed in triplicate at ambient temperature. The viscosity in centipoises (CPS) was calculated by multiplying the dial readings with the factors provided in the Brookfield Viscometer catalog [9].

Diffusion Studies: The diffusion study involved preparing an agar nutrient medium with a central hole. The formulations were placed in the hole, and the time taken for the formulations to diffuse through the medium was recorded (after 60 minutes) [7].

Hardness/Strength: The hardness of the formulations was determined using a Texture Pro CT V1.3 texture analyzer (Brookfield Engineering Labs, Inc.). The probe was displaced into the lotion sample at a speed of 2 mm/s until a 7 g surface trigger was reached. Then, the probe continued to penetrate to a depth of 4 mm at the same speed. The penetration depth of a standard 4 mm needle (P/2N) at a constant 10 kg load force was measured as an

indication of formulation hardness. The peak load, representing the maximum force, was recorded in grams, reflecting the firmness of the product. The tests were conducted twice at room temperature (25±2°C) [9].

Loss On Drying: Loss on Drying (LOD) was determined by drying the formulations at a specified temperature of 105°C. The samples were placed in a glass stopper LOD bottle and dried in a drying oven for 30 minutes. After cooling, the bottles were weighed before and after drying to calculate the loss on drying. If the substance melted at a lower temperature, it was dried at a slightly lower temperature for a specific time.

Solubility: Formulation solubility was determined in various solvents to assess its dissolution characteristics and compatibility [10].

Washability: The ability of the formulation to be easily washed off from the skin with water was evaluated for user convenience [10].

Drug content: UV-visible spectrophotometry was employed to quantify the content of Aloe vera roots and curcuma rhizomes in the formulation [10].

In vitro diffusion study: Franz diffusion cells were utilized to investigate the controlled release and permeation of the drug from the formulation over time [10, 11, 12, 13].

Ex vivo permeation study: Rat skin was employed to evaluate the transdermal absorption and permeation of the drug through the skin using Franz diffusion cells [11, 12, 13, 14].

Irritancy Test: In the skin irritation study, healthy albino rats weighing 150-200 grams were used. They were housed in polypropylene cages under standard conditions with access to food and water. Dorsal hairs were removed prior to the study. The rats were divided into four groups, with formulations applied to their skin. Aqueous formalin solution served as the standard irritant. The animals were observed for 7 days, and signs of edema and erythema were documented. The study was approved by the Institutional Animal Ethical Committee and conducted in accordance with CPCSEA guidelines. Skin irritancy was evaluated based on observations and photographic evidence [14].

Stability Study: The lotion formulations were evaluated for stability following ICH guidelines. Samples were stored at different temperature and humidity conditions for 3 months. Appearance, pH, viscosity, and spreadability were monitored. Storage conditions included 25°C±2°C /60%±5% RH, 30°C±2°C /65%±5% RH, and 40°C±2°C /75%±5% RH [13,14].

IN -VIVO EXPERIMENTAL STUDY:

In medical research, preliminary studies are conducted on lower animals before human trials to confirm the therapeutic effectiveness of herbal/drug treatments. Pilot experiments help

identify potential formulations and save time while minimizing risks to higher animals. Albino Wistar rats, commonly used in wound healing research due to their availability and ease of handling, were chosen for this study involving an excision wound model. To ensure robust statistical evaluation, six rats were assigned to each group. The experiment took place at KNMIPER, Ghaziabad, in accordance with established protocols.

Housing conditions:

Animals were housed under controlled conditions to ensure consistent environmental factors during the study. Temperature was maintained at 25±2°C and relative humidity between 60-70%. A 12-hour light/dark cycle was followed. The animals were housed individually in clean, sterile polyvinyl cages with stainless steel top grills. Sterilized paddy husk was used as bedding material. Animals were provided with sterile laboratory feed and RO water ad libitum. Bedding materials were changed every alternate day, while water bottles were changed daily to maintain cleanliness. A five-day acclimatization period was provided before the start of the experiment [15].

Feed details:

The animals were provided with balanced commercial brown colored pelleted rat feed to ensure adequate nutrition and prevent any nutritional deficiencies that could affect wound healing. The feed was available ad libitum, allowing the animals to consume food as per their requirement. Water was also provided ad libitum to ensure proper hydration throughout the study[15].

Procurement and Maintenance of Animals:

Male Wistar albino rats weighing 200-250g were procured and housed individually in cages at KNMIPER's animal house. A one-week acclimatization period was provided under standard conditions of temperature (25±2°C), relative humidity (60-70%), proper ventilation, and a light-dark cycle. The rats had free access to water and were fed a standard commercial pellet diet. All experimental procedures followed the guidelines of CPCSEA (Committee for the Purpose of Control of Supervision of Experiments on Animals) [16].

EVALUATION OF THE PREPARED FORMULATION BY IN-VIVO STUDY

Skin Irritation Studies

Skin irritation studies were conducted on Wistar rats to evaluate the prepared formulations. The animals were treated topically with each formulation daily for seven days. Visual evaluation of the treated skin was performed to assess the presence of erythema and edema as indicators of irritation [17].

Group division of animals:

A total of 21 animals were selected 3 were used for skin irritation studies and rest divided into three groups for this study. Group I served as the normal control group and received only a simple base treatment. Group II was the experimental group treated with the best formulation based on evaluation. Group III was treated with a standard commercially available povidone iodine 5% lotion for comparison. The formulations were applied topically twice daily for 18 days or until complete wound healing. The antimicrobial effects and healing time of the wounds were recorded and compared between the herbal formulations and the reference formulation. The faster healing observed indicated positive antimicrobial effects of the selected herbal drugs [15-17].

Sr. No.	Group No.	Group Name	Animals in Group	Treated with
1	Group I	Normal Controlled Group	6	Simple Lotion Base
2	Group II	Experimental Group	6	Best Formulation after Evaluations
3	Group III	Reference Treated Group	6	Povidone Iodine lotion 5%

Dose schedule and treatment period:

The lotions were applied topically twice a day for a duration of 18 days or until complete epithelialization occurred from the day of the operation. The animals were anesthetized during wound infliction, and the surgical interventions were performed under light ether anesthesia. Sterilized surgical materials were used, and the skin was prepared by clipping the fur. Animals showing signs of infection were excluded from the study and replaced. No external or internal chemotherapeutic cover was provided to the animals, except for the drug under test. Close observation was maintained for any signs of infection.

In the excision model, the following parameters were assessed to evaluate the antimicrobial activity: wound closure time, percentage of wound contraction, time of epithelialization, and histological studies. The wounds were measured in mm^2 using scale and graph paper at three-day intervals until complete healing. The percentage of wound contraction was calculated based on the reduction in wound size. The period of epithelialization was determined by the number of days it took for the scar to fall off without any remaining raw wound. Histological studies were conducted to examine the tissue healing process [13, 15, 16, 17].

Assessment of Contraction of wound: The contraction of the wounds was assessed by

measuring the changes in wound area over time. Measurements were taken every three days for a period of up to 18 days or until complete healing. The raw excision area was traced onto a transparent polythene paper, and the traced area was measured using a graph paper. The percentage of wound contraction was calculated by comparing the initial wound area with the wound area on specific days. The formula used for calculation was:

% Wound contraction = [(Initial wound area - Specific day wound area) / Initial wound area] * 100. [18]

The period of epithelialization was determined by noting the number of days it took for the scar to fall off without any remaining raw wound. The data obtained from the experimental groups were subjected to statistical analysis. The results are expressed as mean ± standard deviation (SD). The difference between the experimental groups was compared using one-way Analysis of Variance (ANOVA). Statistical significance was considered at a p-value less than 0.05.

RESULTS:

In the following table parts of both plants with their authentication no. along with their collection details are mentioned.

Table: Plants and their Parts with Authentication No. and Collection Details

Sr. No.	Group No.	Group Name	Animals in Group	Treated with
1	Group I	Normal Controlled Group	6	Simple Lotion Base
2	Group II	Experimental Group	6	Best Formulation after Evaluations
3	Group III	Reference Treated Group	6	Povidone Iodine lotion 5%

Organoleptic parameters: Organoleptic evaluation of aloe vera and curcuma rhizomes done by observing roots, and rhizomes with naked eyes and microscope.

Plant/Parts	Aloe vera Roots	Curcuma Rhizomes
Condition	Fresh/Dried roots	Fresh/Dried Rhizomes
Colour	White	Orangish yellow

Odour	Characteristic	Aromatic fragrance
Shape	Straight and branched	Ovate shape
Dimensions	5" (11-13 cm) long	Up to 4 cm long and 3 cm thick
Taste	Bitter, tangy	Bitter and strong, camphoraceous
Surface	Soft, skinny	Dark brown, bluish black, or buff in colour

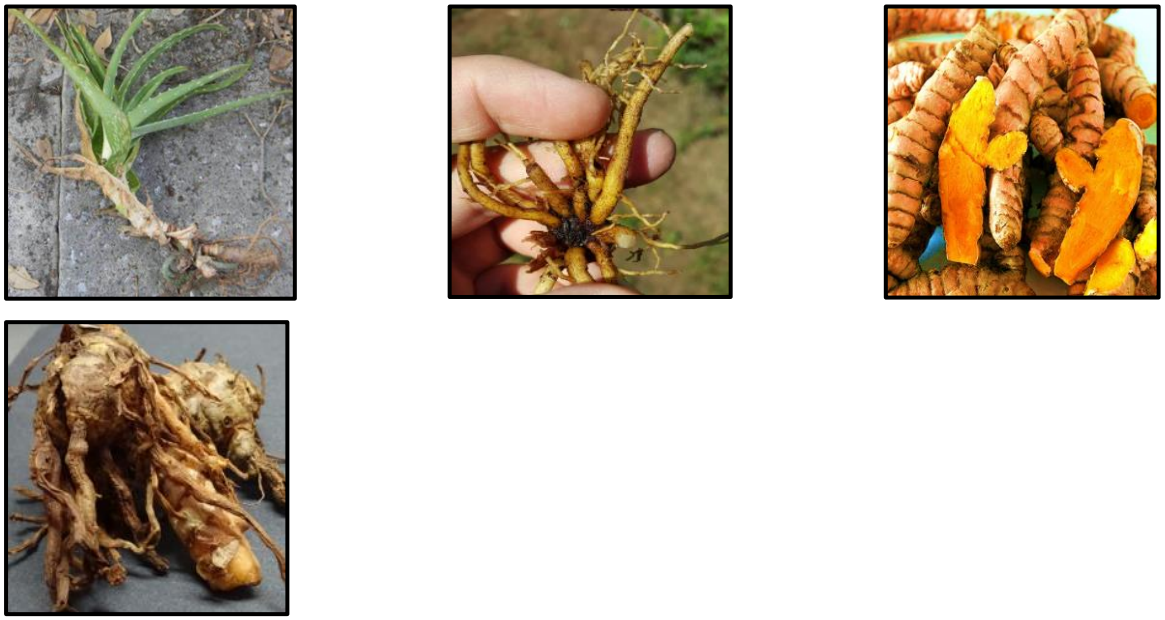


Figure 1: Overview of Aloe vera Roots and Curcuma Rhizomes

Organoleptic Evaluation: The physical appearance, color, consistency, phase separation, homogeneity, and immediate skin feel of the ointment formulations were assessed. The results are presented in the table below. All formulations exhibited an appealing opaque appearance with a smooth consistency. There was no phase separation observed, indicating homogeneity. The color of all formulations was light yellow, and they had a characteristic odor. Furthermore, the ointments had a pleasant immediate skin feel, with no grittiness or greasiness.

Sr. No.	Parameters	Formulation I	Formulation II	Formulation III
1	Physical appearance	Opaque	Opaque	Opaque
2	Colour	Light yellow	Light yellow	Light yellow
3	Consistency	Smooth	Smooth	Smooth
4	Phase separation	NO	NO	NO

5	Homogeneity	Homogeneous	Homogeneous	Homogeneous
6	Immediate skin feel	No grittiness or greasiness	No grittiness or greasiness	No grittiness or greasiness

pH: The pH values of the formulations were measured and found to be 5.20 and 6.11, respectively, which fall within the desired range of 4-7. These pH values are presented in the following table. The formulations exhibit a pH range that is suitable for optimal results and compatible with the normal pH of the skin.

Viscosity: The viscosity of the formulations was determined and recorded in the range of 2314±6.13-2851±9.93 CPS at 10 rpm, as shown in the table above. All formulations exhibited pseudoplastic flow behavior. The average of three readings was calculated, and the standard deviation was determined (n=3).

Spreadability: The spreadability of the ointments was evaluated and categorized into three groups: low, moderate, and high. After screening, it was observed that the spreadability was inversely proportional to the concentration of hard paraffin. As the amount of paraffin increased, the ointment became thicker, resulting in decreased spreadability. Among the formulations, formulation F2 showed greater spreadability compared to the others.

Loss on Drying: The loss on drying, which indicates the amount of moisture content, was found to be very low due to the greasy nature of the formulation. It was less than 1% of the total weight of the formulation.

Solubility: The formulations were not completely soluble in normal water but could be easily removed from the skin by using lukewarm water for cleaning.

Washability: The formulated preparations exhibited good washability and were easily washed off with lukewarm water, indicating convenience in application and removal.

S. No.	Characteristics	Formulation 1	Formulation 2
1	pH	6.11	5.2
2	Viscosity	7635	6743
3	Spreadability	8.4	8.5
4	Loss on Drying	0.20%	0.30%
5	Solubility	Soluble in boiling water, miscible with alcohol, ether, chloroform	Soluble in boiling water, miscible with alcohol, ether, chloroform
6	Washability	Easily washable with normal water	Easily washable with normal water

Irritancy Test:

The optimized lotion formulation, F1, did not exhibit any signs of erythema or edema when applied topically to the skin of animals throughout the study period. It received a score of 0, indicating no redness or swelling. Based on the positive results obtained in all the tests mentioned above, formulation F1 demonstrated good efficacy and results. It was found to be safe and well-tolerated, making it a promising candidate for further evaluation and potential use.

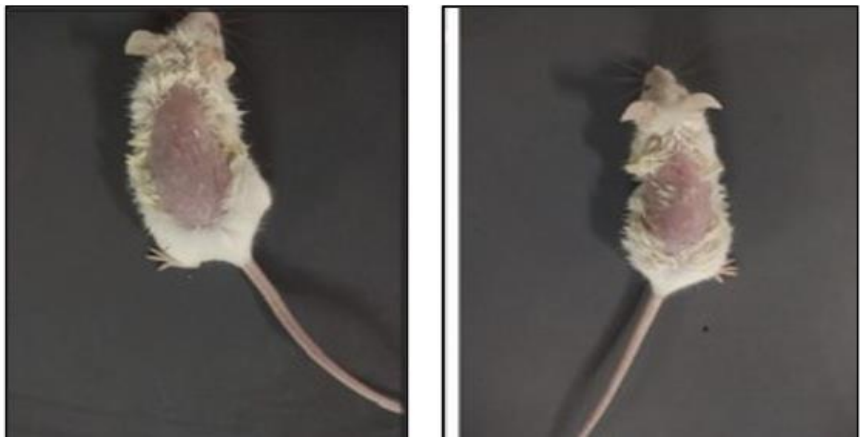


Figure : Irritancy test performed for formulations

Stability study: The table summarizes the stability testing results for Formulation I over a period of 90 days. The mean values with their corresponding standard deviations (SD) are provided before stability testing and after stability testing at 1 month, 2 months, and 3 months. The observations include pH, viscosity, and spreadability of the formulation. No significant variation was observed in these parameters during the stability study.

Sr. No.	Observation	Before Stability Testing (mean±SD)	After Stability Testing (mean±SD)
		1 month	2 months
1	pH	6.11±0.185	6.05±0.91
2	Viscosity	2612±8.13	2606±7.45
3	Spreadability	8.4	8.5

Evaluation of wound healing activity by excision wound model: - The evaluation of wound healing activity was conducted using the excision wound model, and the results are presented in the following table. A boarer was used to create wounds of equal size for the study.

Figure: Borer for wound creation

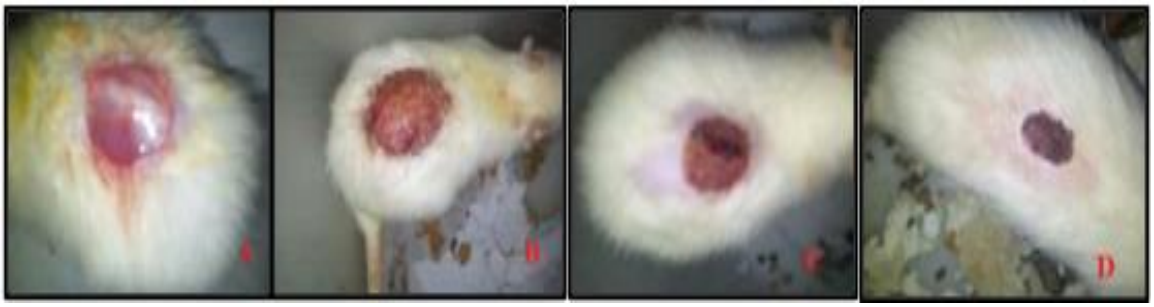
Excision antimicrobial wound model: -

In this study, two parameters were evaluated: microbial inhibition and wound contraction. Wound contraction can be used as an indicator of microbial inhibition. To measure wound contraction, the raw wound area was traced on a transparent polythene sheet at specific time intervals (0, 4, 8, 12, 16, and 18 days). The results of these measurements are presented in the table below.

The aim of the experiment was to assess the progress of wound healing using different formulations. The formulations included a reference drug marketed lotion at 5% concentration, an herbal test formulation (Formulation I) consisting of 5% Aloe vera root extract and 2.5% curcuma extract, and a normal control group treated with a lotion base. The excision wound model was utilized for this investigation.

Sr. No.	Application	Group No.	Group Name	Days/ Wound area in mm^2					
				0	4	8	12	16	18
1	Simple Lotion Base	Group I	Normal Controlled Group	10.1	9.18	8.35	6.6	5.3	2.67
2	Formulation I (10%)	Group II	Experimental Group -I	10.14	8.42	5.4	2.8	0.6	0
3	Povidone Iodine Lotion 5%	Group III	Reference Treated Group	10.11	8.5	5.3	2.7	0.5	0

Group I (Normal Control Animal Group)



Antimicrobial effects were assessed as the wound healed from day 0 to day 18 in the normal control animal group treated with a simple lotion base.

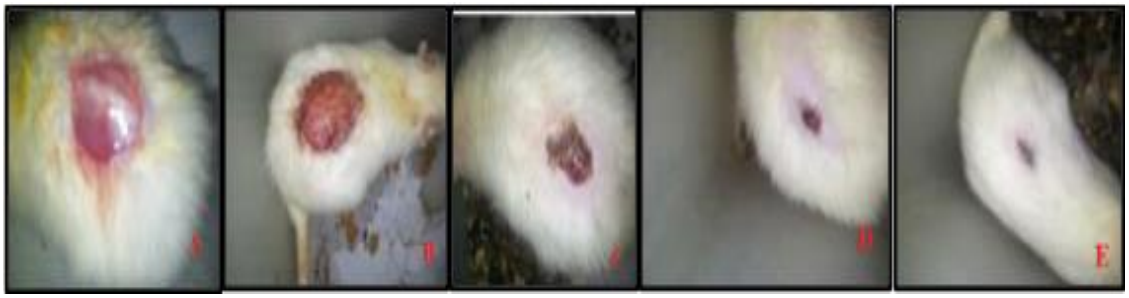
Here; A: Normal control group at Day 0, B: Normal control group at Day 4, C: Normal control group at Day 12, D: Normal control group at Day 18

Group II (Experimental Rats Group treated by formulation I)

The wound healing process from day 0 to day 18 in the experimental animal group I, treated with Formulation I, demonstrated antimicrobial effects.

Here; A: Group I (Day 0), B: Group I (Day 4), C: Group I (Day 8), D: Group I (Day 14), E: Group I (Day 18)

Group III (Experimental Rats Group treated by Marketed 5%)



The antimicrobial effects of the marketed preparation were observed as the wound healed from day 0 to day 18 in the experimental animal group II.

Here; A: Group II (Day 0), B: Group II (Day 4), C: Group II (Day 8), D: Group II (Day 14), E: Group II (Day 18)

Average mean wound area closure: - In this study, Wistar Albino Rats were utilized to assess wound healing. The wound area was measured periodically, and the healed area was

calculated by comparing it to the original wound area. The closure of the wound is indicative of microbial inhibition. Based on the results, it can be inferred that the effectiveness of Formulation I (consisting of 5% Aloe vera root extract and 2.5% curcuma extract) is comparable to the marketed formulation.

DISCUSSION: This research study shows the development, evaluation, and comparison of an antimicrobial herbal lotion using curcuma and Aloe vera extracts. The introduction highlights the importance of traditional medicines and herbal remedies in achieving perfect health. The field of dermatology requires products that are stable, cosmetically appealing, and effective in delivering active ingredients. The materials and methods section describes the collection and authentication of Aloe vera and Curcuma longa extracts, as well as the formulation process. Various evaluation parameters were used to assess the lotion, including organoleptic evaluation, consistency, pH analysis, spreadability, viscosity, diffusion studies, hardness, solubility, washability, drug content, in vitro and ex vivo permeation studies, irritancy test, and stability study. The in vivo experimental study involved using albino Wistar rats to evaluate the lotion's effects on excision wounds. The animals were divided into different groups, and the healing time and antimicrobial effects were compared with a reference formulation. Various parameters such as wound closure time, wound contraction, and epithelialization time were assessed.

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