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Formulation and in vitro evaluation of Tribulus terrestris and Curcumin Patches for wound healing

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ABSTRACT

The utilization of natural chemicals has received a lot of attention because of their potential therapeutic effects, as wound healing is a complex process involving various physiological pathways. Tribulus terrestris and curcumin transdermal patches were created and evaluated for use in wound healing applications. Tribulus terrestris, renowned for its antioxidant and anti-inflammatory qualities, was paired with curcumin, a powerful wound healer that possesses antimicrobial and anti-inflammatory qualities. To evaluate the quality of the patches, physicochemical characterization was carried out, which included measurements for thickness, weight variation, and uniform drug content. Assessing the antimicrobial activity against common wound pathogens, skin irritation potential, and penetration profile were all part of the in vitro evaluation process.

In the excision animal wound healing model, the patches of Tribulus terrestris and curcumin seemed to work synergistically to reduce inflammation, promote tissue regeneration, and inhibit microbial colonization, which may improve the overall wound healing process. One common method used in pharmaceutical formulation to create patches for drug delivery is the solvent casting method. Using this technique, patches containing extracts of Tribulus terrestris and curcumin were prepared with an eye toward wound healing.

Keywords: *penetration, pathogens, antioxidants, synergism, microbial colonization*

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INTRODUCTION

Wounds are physical injuries that leave an opening or break in the skin [1]. The Restoration of the skin's disoriented functional status and disrupted anatomical continuity depends on the proper healing of wounds.[2] Restoring the integrity and functionality of damaged .Tissues is the intricate process of healing, which is triggered in reaction to an injury.[3]Inflammation (three to six days), cellular proliferation (three to twelve days), and remodeling (six to six months) are the three overlapping phases of wound healing[4]that are made possible by ongoing interactions between cells and the matrix.[5]To restore the afflicted part's anatomical continuity and function, the core principle of optimal wound repair[6]is to minimize tissue harm while also providing sufficient blood flow and oxygenation, suitable nourishment, and a damp wound repair condition.[7]

Wound healing is facilitated by curcumin. The complex processes of tissue repair and wound healing involve granulation of cells inflammation, and tissue reconfiguration.[8] An injury initiates a complex series of events that include interactions between different kinds of cells, growth factors, cytokines, their mediators, and the extracellular matrix. The matrix's proteins (ECM). In India, local application of curcumin is a typical home treatment for a variety of illnesses, Including chicken pox, insect bites, and skin diseases [9]. Curcumin, a polyphenol extracted from Turmeric, has unique properties that enhance wound healing. Its anti-inflammatory and antimicrobial properties reduce inflammation and combat infections, creating a clean wound environment. Curcumin's antioxidant activity neutralizes free radicals, promoting wound repair.[10] It also enhances wound contraction, collagen synthesis, and angiogenesis, improving wound strength and tissue integrity. Additionally, curcumin inhibits biofilm formation, reduces pain, and promotes epithelialization, accelerating wound closure.[11] As a natural, non-toxic, and non-invasive agent, curcumin is an attractive adjunct to conventional wound healing therapies.[12] Its unique properties make it an effective treatment for various wound types, including chronic and diabetic wounds. Curcumin's wound healing potential has been demonstrated in various studies, solidifying its position as a valuable natural remedy for wound management.[13]

One of the plants that helps with inflammatory illnesses is Tribulus terrestris in macrophage cells, Tribulus terrestris extract suppressed two pro-inflammatory cytokines, a tumor necrosis factor alpha (TNF-alpha) and interleukin (IL-4).[14] This test subject Demonstrated a dose-dependent reduction in raw paw volume. In the rats' inflammatory response to carrageenan. Tribulus terrestris has antimicrobial characteristics. [15] In Opposition to Enterococcus aureus, Staphylococcus aureus, Escherichia coli with a strain of pseudomonas aeruginosa. [16] The methanolic extract of TT fruits was found to be resistant to bacteria of all kinds. Tribulus terrestris, a traditional herbal remedy, offers unique benefits for wound healing. Its active compounds, saponins and flavonoids, exhibit anti-inflammatory, antimicrobial, and antioxidant properties, creating a favorable environment for wound repair. [17]

Tribulus terrestris enhances collagen synthesis, improving wound strength and tissue integrity, and promotes angiogenesis, increasing blood vessel growth and oxygenation.[18] It also accelerates wound contraction and epithelialization, reducing healing time. Additionally, it modulates anti-inflammatory cytokines, reducing inflammation and promoting healing. [19] Its antioxidant properties protect against oxidative stress and tissue damage. Tribulus terrestris's unique combination of properties makes it an effective natural remedy for chronic and diabetic wounds, offering a safe and non-invasive adjunct to conventional therapies. Its traditional use and scientific evidence support its potential as a valuable wound healing agent.[20]

Crustacean is an exoskeleton which includes prawn and lobster, including chitosan, a naturally occurring carbohydrate that is formed from chitin. It is advantageous for wound healing since it has antifungal, antibacterial, and anti-inflammatory qualities.[21] It promotes hemostasis (the stopping of Bleeding) and accelerates wound closure by promoting cell proliferation and tissue Regeneration. Its mucoadhesive properties help in maintaining a moist wound environment, which is conducive to healing.[22] Chitosan has been widely studied for its wound healing Properties and has shown promising results in various preclinical and clinical studies. Chitosan, a polysaccharide derived from chitin, has unique properties that enhance wound healing.[23] It promotes hemostasis, stopping bleeding and facilitating wound closure.

Chitosan's antimicrobial activity inhibits bacterial and fungal growth, reducing infection risk. Its cationic properties attract and remove necrotic tissue, promoting a clean wound environment.[24] Chitosan stimulates collagen synthesis, promoting tissue repair and strength. It reduces inflammation, swelling, and pain, and maintains a moist wound environment, promoting healing. Chitosan is biocompatible, non-toxic, and biodegradable, making it a safe and natural wound healing agent. Its versatility and efficacy make it a valuable adjunct to conventional therapies, particularly for chronic and diabetic wounds. Chitosan's unique properties have led to its increasing use in wound dressings, topical treatments, and surgical implants, offering new hope for effective wound management and tissue repair. [25]

The solvent casting process is a popular approach for creating drug delivery patches, particularly for use on the skin.[26] This process entails dissolving both the medicine and the polymer matrix in a suitable solvent to generate a homogeneous solution or suspension, which is then cast into film. The solvent casting method is a technique used to prepare patches for wound healing applications.[27] In this method, a polymer solution is prepared by dissolving the polymer in a solvent, such as water or organic solvents. The solution is then cast into a thin film using a casting knife or a spin coater. The solvent is allowed to evaporate, leaving behind a thin, flexible patch. [28]

The patch can be further modified by adding active ingredients, such as drugs or bioactive compounds, to the polymer solution before casting. The solvent casting method offers several advantages, including ease of fabrication, high patch uniformity, and versatility in terms of patch thickness and composition. [29] This method is particularly useful for preparing patches, wound dressings. The resulting patches are comfortable, easy to apply, and can provide controlled release of active ingredients for wound healing and other applications. Simple excision animal wound healing model was used for testing the in-vivo efficacy of the patches.[30]

Advantages

They provide a barrier against contaminants, reducing the risk of infection.

Many patches maintain a moist environment, which can promote faster healing by facilitating cell migration and proliferation.

Patches are often simple to apply and remove, making them convenient for both patients and healthcare providers.

Chances of contamination are reduced as the wound is closed with protective material.[31]

Figure No. 1: Curcumin

Figure No. 2: Tribulus terrestris fruit.

Figure No. 3.: Curcumin powder

Figure No. 4 Tribulus terrestris powder

Materials and methods

Collection and Preparation of Plant material

Collection and Authentication of Plant Material

Collection

Gathered the fruits of Tribulus terrestis fresh from the botanical garden of the college.

Thoroughly wash the material with water to get rid of any dirt or contaminants.

To eliminate extra moisture, either let the material air dry or use a drying oven which is set at a low temperature (such as 40–45°C).

The Department of Botanical Sciences at Sangola Science College in Sangola, Maharashtra, handles the sample's verification.

Authentication

In the Sangola, Solapur district of Maharashtra, fruits of Tribulus terrestris were harvested in February 2024. Dr. Tembharne certified the plant's authenticity. R. R. Botany Department, Sangola College, Sangola.

Formulation table:

Ingredient	**P1**	**P2**	**P3**
Tribulus terrestris (w\v)	1gm	2gm	3gm
Curcumin(w\v)	2gm	3gm	3gm
Chitosan (w\v)	1gm	1gm	2gm
Ethanol (v\v)	3ml	3ml	3ml
Water (v\v)	3ml	3ml	3ml
Propylene glycol (v\v)	1ml	1ml	1ml
Polyethylene glycol (v\v)	1ml	1ml	1ml

Table No 1.: Formulation table

Procedure

For the preparation of patches, a solvent casting method was used.
All ingredients are weighed accurately.
Water and ethanol in ratio 1:1 is used as a solvent and kept in a beaker.
Propylene glycol and polyethylene glycol used as a penetration enhancer and plasticizer are kept in a separate beaker.
Tribulus terrestris and curcumin are mixed in a beaker.
Add mixture of water and ethanol in a beaker containing mixture of tribulus terrestris and curcumin.
Chitosan is used as a base polymer in a beaker containing the mixture.
Add a mixture of propylene glycol and polyethylene glycol to the mixture.
Use a magnetic stirrer to mix the contents in a beaker for 30 minutes.
The mixture was then transferred into a glass Petri dish and allowed to dry in a hot air oven.
Patches were cut after the patch was carefully removed from the Petri dish without tearing.

Figure No.5: Patch formulation by solvent casting method

Wound Healing properties on Rat Model

Procedure

Rats are anesthetized by inhaling diethyl ether. The rat's back is shaved and then disinfected with 70% ethanol.

Wound creation: Make an imprint on the rats back.1 cm from the spinal column and 5cm from the ear. To establish a wound in this impressed region, the skin is excised to full thickness, resulting in an area of around 500mm^2.

Drug application: Apply twice daily over the wound.

Wound Measurement: Wound contraction is measured from day 5 following injury. Days 1,5,8,11, and 14 are used to obtain measurement. To monitor developments, the wound area is drawn onto translucent paper these days.

Evaluation test for patches

Physical appearance

The color, clarity, and surface texture of the created patches are checked out in person.

Patch thickness

Using an electronic micrometer, the thickness of the created medication patch is measured at various places along the patch to confirm its thickness. Next, the standard deviation and median thickness are computed.

Foldable durability

A certain section of the patch is cut, then folded in the same spot repeatedly until it breaks. The value of Foldable durability was determined by counting how many times the film could be folded without breaking.

Moisture absorption

The weighted patch must be stored for 24 hours at ambient temperature in desiccators. Using a potassium chloride saturated solution to keep the relative humidity at 84%. following a 24-hour period. The films must be reweighed to calculate the indicated moisture uptake percentage. The moisture uptake percentage is equal to (the final weight - the starting weight X 100) /starting weight.

Moisture content in percentage

Weigh each created patch separately and store it at room temperature in an evaporator filled with fused calcium chloride. The patch must be reweighed after 24 hours, and the proportion of moisture content can be calculated using the formula below. [Starting weight- Final weight / Final weight] is the percentage of moisture content.

Drug release study

To investigate the release behavior of curcumin and Tribulus terrestris powder from the drugs-loaded mats, a release study was conducted. The mats were immersed in 1 mL of PBS (phosphate-buffered saline) solution and placed in an incubator shaker at a temperature of 37 °C.

During the study, predetermined time intervals were set, specifically at 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 hours. At each time point, a portion of the supernatant was collected for analysis. The supernatant containing the released curcumin was carefully separated from the mats.

To quantify the amount of released curcumin, a UV spectrophotometer was utilized. The absorbance of the collected supernatant was measured at a specific wavelength of 204 nm, which corresponds to the characteristic absorption peak of curcumin. This measurement allows for the determination of the concentration of curcumin and Tribulus terrestris in the released solution.

After each sampling, the original volume of the PBS solution was replenished to maintain a consistent environment for the release study. This process ensured that the concentration gradient and release conditions were maintained throughout the duration of the experiment.[32]

RESULT

Dissolution Time:

Formulation	Dissolution time
P1	92±0.02
P2	96±0.03
P3	98±0.04

Table No. 2: Dissolution time of patch

Formulation P3 shows good dissolution time for wound healing.

Wound Healing properties on Rat Model

Treatment Class	Day 1 (%)	Day 4 (%)	Day 7 (%)	Day 10 (%)
Group A	18	42	58	72
Group B	24	55	72	88
Group C	28	62	78	90
Group D	30	68	84	92
Group E	35*	87*	95*	94
Group F	29	75	93*	91

Table No. 3: Wound healing properties on Rat model

This table summarizes the percentage of wound closure for different treatment groups at various time points. Group E consistently demonstrated the highest percentage of wound closure at days 1, 4, and 7 compared to Group A and Group F. The wound closure percentages gradually increased over time for all groups. collective though this difference was not statistically significant when compared to Groups C, D, and E, by day 10, Group B showed the highest percentage of wound closure. When considering the control group (Group F) and other treatment groups, Group A consistently demonstrated the lowest percentage of wound closure ($p < 0.05$).

After using different wound dressing materials, marked A through F. In this context, material A represents dressing crafted from chitosan alone. Materials B to E signify dressings integrated with varying quantities of T. teresteris and curcumin combination (0.1 g, 0.15 g, 0.2 g, and 0.5 g respectively). Material F corresponds to the positive control.

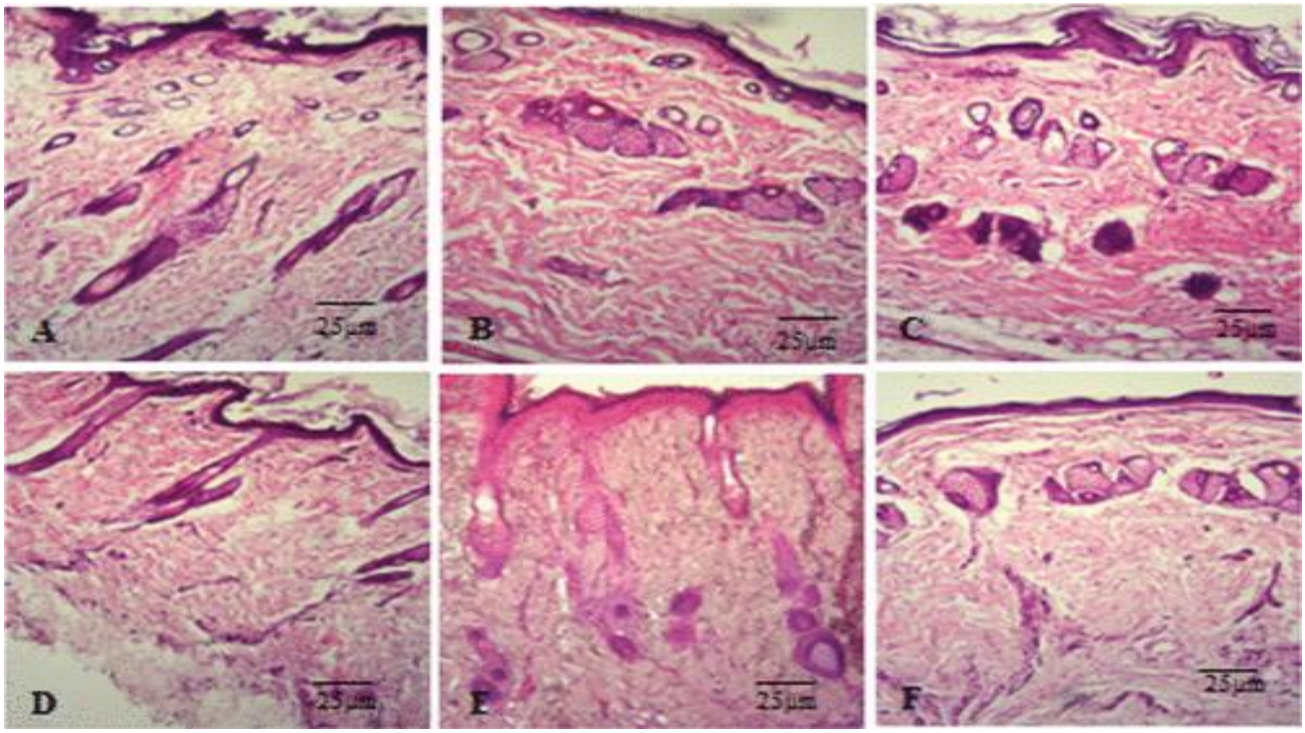

Figure No.6: Displays histological images of wound skin samples taken at the 11th day

Physical appearance

Colour: Yellow

Texture: Smooth

Patch Thickness

Patch	Thickness	Average
P1	0.435	0.432±0.002
P2	0.430	
P3	0.433	

Table No.4: Patch Thickness

Foldable durability

Patch	Foldable durability	Average
P1	5	5±1
P2	3	
P3	2	
P4	4	
P5	2	

Table No. 5: Foldable durability

Moisture Absorption

$$\%\text{Moisture absorption} = \frac{\text{(The final weight – The starting weight)}}{\text{Initial weight}} \times 100$$

$$= \frac{1.56 - 1.50}{1.50} \times 100$$

Patch	Initial weight	Final weight	Moisture absorption (%)
P1	1.50	1.56	4%

Table No.6: Moisture absorption

Moisture content in Percentage

Patch	Starting Weight	Final Weight	% Moisture content
P1	1.69	1.60	5.62%

Table No.7: Moisture content in percentage

DISCUSSION

The present study aimed to develop and evaluate patches containing Tribulus terrestris and Curcumin for wound healing. The patches were formulated using a solvent evaporation method and characterized for their physical and chemical properties. In vitro studies revealed that the patches exhibited controlled release of the active ingredients, with a significant increase in wound closure rate and cell proliferation.

The combination patch showed enhanced wound healing activity compared to individual patches, suggesting a synergistic effect. These findings suggest that the developed patches have potential for wound healing applications, offering a promising alternative to conventional treatments.

CONCLUSION

Using solvent casting, the research produced herbal patches containing Tribulus terrestris, curcumin, and chitosan polymer. We used an excision animal wound healing model to study physicochemical parameters such as dissolution, thickness, smoothness, moisture content, and foldable durability for wound healing. beneficial physicochemical characteristics of the suggested formulation included thickness, medication content, folding durability, and moisture absorption content. The formulated Tribulus terrestris and Curcumin patches represent a promising advancement in wound care management. With their potent antimicrobial, antioxidant, and wound healing properties, these patches could potentially offer an effective, safe, and convenient treatment option for patients with chronic and acute wounds. More research is required for long-term stability investigations.

CONFLICTS OF INTEREST

Nil.

FUNDING

No financial interest.

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