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DEVELOPMENT AND EVALUATION OF POLY-HERBAL FORMULATION FOR ESCHAR REMOVAL AND WOUND HEALING

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ABSTRACT: The objective of this work was to create a polyherbal formulation and evaluate its antibacterial and wound-healing properties in an *in-vitro* setting. Physicochemical properties of a topical polyherbal formulation were assessed with the chitosan, gelatin, neem, pectin, honey, and aloe vera were combined in different proportions. Polyherbal formulations were assessed for their tensile strength, swelling behavior, moisture retention, microbial penetration, honey release, pH, folding endurance, thickness, water vapor transmission in the prepared formulation. *In-vitro* activity revealed that F5 formulation had the better wound healing potential as compared to F4 and F3. Thus, we can conclude that enhanced dressings with good wound healing capabilities can lead to new possibilities in the treatment of wound healing related to burns. The outcome of this investigation indicates that the film made with various herbs and 5.4 ml of honey meets all the specifications for wound dressings, including thickness, weight, and folding endurance. The polyherbal formulation has tensile strength of 26N/mm², moisture uptake of 17.14 ± 1.12 to 26.7 ± 2.17%, and swelling behavior of 85.38. Its antibacterial properties against *S. aureus* and *E. coli*, which also prevent microbes from migrating to the wound bed from the surrounding environment. The results of the microbiological penetration test were positive. The mechanisms behind wound healing will be more clearly defined by additional research on polyherbal formulation.

INTRODUCTION: The skin is a largest body organ that helps in maintenance of the physical and immunological protective barrier against the physical harm, bacterial invasion and UV radiation. Skin consists of the various nerve cells and receptor cell that detect receptor related to temperature, touch, pressure and feel of sensation, exogenous aggressors such as pain and infection may lead to the damaged some of all these function.

Skin is very sensitive to various types of lesions, burns, ulcers, or other physical abrasion. As a natural healing process skin initiates a complex cascade for regeneration of the damaged or lost tissue. However, self-regeneration ability of skin may be declined under some conditions such as extensive skin loss, non-healing ulcers, diabetic state, or deep burns. Such un-managed wounds may increase the risk of infection and mortality rate⁴².

In addition, these wounds represent one of the most serious and life threatening in various stages, painful, and this skin conditions, become critical to both medical and as well as to the society for both patients and the countries^{1, 7}. The issue for burn specialists has always been managing burn injuries.

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The primary goals of burn specialists are patient isolation, infection control, and a hopeful functional recovery. Burn injuries are complicated and exhibit serious issues that call for lifelong rehabilitation or late prevention, thus difficulties are common. Many inflammatory mediators and releasing agents, including histamine, oxygen free radicals, nitric oxide, TNF- α , and interleukins, are produced at the wound site. Furthermore, the majority of burn injuries damage almost every organ system. Although a lot of work has been done to address all of these issues, the mortality rate has decreased to a certain level, but the morbidity rate from burn infections is rising daily.

Traditional dressings like cotton, wool, gauze, hydrogel, ointment, cream, and natural and synthetic bandages are used to protect the wound site from contamination, but they are also used to deliver the bioactive molecule to the wound site. Topical therapeutic agents available in different formulations like solution, cream, and ointment for drug penetration to the wound site are insufficient because of their poor rheological characteristics. The proposed medication can play a beneficial role in the wound healing process by either directly acting as debrided agents to clear the necrotic tissue or indirectly acting as an antimicrobial drug that helps prevent the growth of microbes⁴³. Thus, advanced dressings should be designed to have therapeutic properties either by themselves or by releasing active constituents present in the dressing⁷.

Gold standards for an ideal dressing include clear transparent sheet where without removal of sheet wound surface can be easily visualized, non-toxic, non-irritant to the biological tissue, should also provide soothing, cooling effect, and may be easily removed from wound surface⁴⁴. Due to their high porosity power and superior oxygen penetration power, polyherbal sheets are becoming more and more popular in the current clinical setting. Sheets based on herbal ingredients in particular are thought to be safer, more effective, and more affordable. In order to create a cutting-edge dressing for wound healing that can enhance patient compliance and therapeutic outcomes⁴¹. The skin is the largest organ in the human body and performs a wide range of intricate tasks that are vital to our existence.

In addition to providing a barrier of defense against physical abrasion, it also acts as a barrier against pathology. Furthermore, skin plays a vital role in preserving vitamin D and regulating body temperature¹⁶.

Burn injury to the skin provides a challenge, as wound healing was a complicated process during the burn injury. Burn injuries are of two types local and systemic response. Jackson describes the three zones of burn injury:

Zone of Coagulation: Maximum area covered at the center of the wound and irreversible tissue loss. Coagulation and degradation of protein and loss of plasma hemostasis was visualized and necrotic tissue observed in the center of the wound.

Zone of Stasis: In this zone tissue perfusion decreases. This zone transformed to necrosis due to accumulation of fluid at the wound site and bacterial infection.

Zone of Hyperemia: In the outer zone tissue perfusion of the blood was increased, the tissue will invariably have recovered if there was more sepsis or prolonged hypo-perfusion^{3, 40}.

In the zone of status and hyperemia contribute to local pathology of burn wound. An activation of inflammatory media such as oxidants and proteases further damage skin. Immediate after burn micro vessel in these zones of hyperemia loss their capacity to keep fluid apart from the interstitial area loss of protein and fluid result in ischemia due to which the aggressive of tissue loss. The burn shock which increases the entire body inflammatory response and results in multi-organ failure. Thermal injury may increase the activation of kinin system may result in the increases in bradykinin leads to local edema formation, increases capillary permeability. On the other hand, biosynthesis of metabolites from arachidonic acid cascade this increases the prostaglandin (which leads to burn edema formation) thromboxane A₂ increase leads to vasoconstriction and local ischemia in burned tissue¹⁴. When leucocytes reach to the site of wound, they release mediator which control the accumulation and activation of another cell. Cytokines are involved in inflammation and immunity.

No. of cytokines released by activated platelets, IGF-1 was to be reduced impaired wound healing. Superficial burn damaged the epidermis which

damage the keratinocytes activate was shown in Fig. 1.

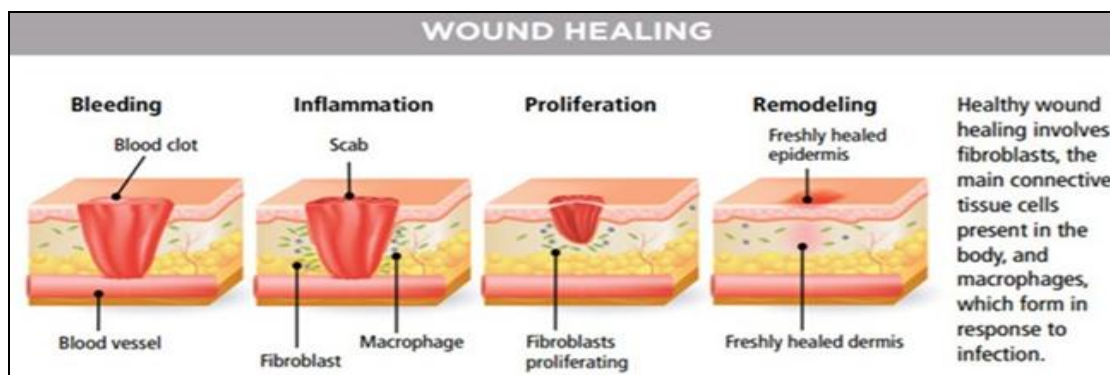


FIG. 1: STAGES OF THE WOUND HEALING

Since ancient times, numerous efforts have been made and various herbs have used to treat wounds infection, to prevent bleeding, absorb burn fluid, and significantly help in healing. Some of materials used in healing consist of animal fats, honey, mud or animal oil. Some of these readily available as a natural substance that provide less beneficial effect, but are having some limitations.

MATERIAL AND METHODS:

Preparation of Polyherbal Formulations:

Procedure: Chitosan 2 gm was added to 20 milliliters of distilled water, and then 0.2 ml acetic

acid was dropwise into the chitosan solution until it dissolved completely, homogenously stirred at 60°C, at 250rpm for 1 week. Weighed the 2gm gelatin and dissolved in 3.6ml minimum volume of *Aloe vera* extract and warm at 40°C for 20 min. Then 5.4ml honey and 1 gm neem and pectin powder were added dropwise by continuous stirring for 30 min. At last, the 1 gm chitosan was added into it and stirred for 30 min, then mixed fluid was spread over Petri dish and allow it to air dry for two hours and then kept in fridge for 72 hr^{7, 39, 20, 45, 52}. Prepared formulation was shown in Fig. 2, 3 and 4.



FIG. 2: IMAGE SHOWING PREPARED FILM F1 AND F2



FIG. 3: IMAGE SHOWING PREPARED FILM F3



FIG. 4: IMAGE SHOWING PREPARED FILM F4 AND F5

Preparation and Coding: A total five film were made using different composition and were given

codes ^{17, 20, 48, 54}. The composition and codes allotted to formulation are as in **Table 1**.

TABLE 1: COMPOSITION AND CODES ALLOTTED TO FORMULATION

S. no.	Sheet code	Ingredients used
1.	F-1	2gGE(B) + 3.0MLAV + 0.5gCH + 4.40gH + 1gm Neem
2.	F-2	2gGE(B) + 3.5MLAV + 1gCH + 4.5gH + 1gmNeem
3.	F-3	2gGE(B) + 3.9MLAV + 1.5gCH + 4.53gH + 1gmNeem
4.	F-4	2gGE(B)+ 4MLAV + 2gCH + 4.60gH + 1gm Neem
5.	F-5	2gGE(B) + 5 MLAV + 2.5CH + 5.4gOIH + 1gmNeem

Parameter Evaluated for Polyherbal Formulation:

Thickness and Weight Variation: A digital vernier caliper that has been used to measure the thickness of the prepared Polyherbal formulation. The samples were first weighed separately for the weigh measurement. Every experiment was conducted three times, and the standard deviations were calculated based on observation ^{7, 20}.

Folding Endurance: Folding endurance provides a measure the durability of formulation when repeatedly folded under constant load. It determines that how many times a film may be folded in a single plane before cracking or breaking. Three duplicates of experiment were carried out ⁴⁷.

Swelling Ratio: A Completely dry polyherbal formulation was pre- weighed and the immersed in a distilled water. At various time intervals of 30 minutes the formulation was removed and weighed after blotted the excessive water from the surface of the polyherbal formulation. Each experiment was done in triplicate ^{1, 20, 47}. Result was calculated by the help of equation:

$$Q = (Ma-Mb)/Mb$$

Q = Swelling Ratio

Ma = Mass after the Swelling State

Mb = Mass before the swelling

Tensile Strength (Mechanical Properties): Polyherbal formulation of required dimension were cut 1cm x 1cm and sheet were fixed with two clamps in a vertical position and preloaded at 150gm. During the measurement, the top clamp was pulled at a rate of 100mm/min and force and elongation were measured. Data were collected till the sample rupture. The experiment was conducted

in triplicate stress and strain were recorded by young's modulus ⁵.

Tensile Strength = Force at break / Initial cross-sectional area of the sample

Elongation% = Increase in length at breaking point / Initial length (mm)

Water Vapors Transmission: Polyherbal formulation were cut and placed on top of the vial containing 1gm of silica determined by weighing the vial at every 1 hour respectively. The vial was kept at 30°C in a desiccator with CaCO₃ (at room temperature ^{13, 14}. The equilibrium vapors penetration ^{2, 7, 30}.

$$WVTR = W2-W1/AX100$$

Here, W1- Initial weight of sheet

W2-Final weight of sheet

A - Area of the film sample covered

Microbial Penetration Test: A study on microbial growth employed nutrient broth media. The prepared polyherbal formulation was sterilized by gamma radiation in laminar air flow for 45 minutes. Then 5ml broth was poured in pre-autoclaved test tube. The sterilized sheet was tied over the mouth of test tube and the edges were sealed with parafilm. The microbial growth was observed daily ^{6, 19, 46, 49}.

Tube Conditions:

1. Standard without any cover
2. Controlled with cotton plug
3. Covered with F3 formulation
4. Covered with F4 formulation
5. Covered with F5 formulation

Moisture Retention (Content): Pre-weighed 0.2 g dry sheet was kept in the incubator at 37°C and then after each interval of 1 hr. the sheet was weighed respectively ¹⁸. The moisture content was determined as per the following equation:

Moisture Content (%) = (Wi - Wd) / Wd x 100.

The moisture content determination experiment was performed in triplicate ⁷.

Moisture Uptake: The films were first weighed and then left in a desiccator with activated silica gel for twenty-four hours. After being gathered, the films were moved to a different desiccator with saturated sodium chloride solution for a further 72 hours while the relative humidity was kept at 75%. After the final weight of the films was noted, the following formula was used to calculate their moisture-uptake capacity.

Moisture uptake (%) = (Wm - Wi) / Wi x 100.

The experiment on moisture uptake was carried out in triplicate ².

Scanning Electron Microscope (SEM) Study: The morphology and surface topography of the film was examined by Scanning Electron Microscope. Spherical samples (5 mm to 2 mm) were mounted on the SEM sample stab using a double-sided sticking tape and then coated. The coated samples were examined using a scanning electron microscope and photomicrographs of suitable magnifications were obtained ^{7, 32}.

RESULTS:
Swelling Behavior: The swelling ratio measured to examined the capacity of the film to absorb the wound exudates at wound site. The Result shows in **Fig. 5** the highest absorbance value was 85.38% seen in the F-5 and the lower absorbance value was 41.06% seen in the F-1. It was shown in **Table 2** and **Table 3** that swelling ratio decrease as the concentration of honey and chitosan decrease and vice versa ^{20, 49}.

TABLE 2: DATA OF SWELLING BEHAVIOR OF DIFFERENT SAMPLE PROPORTION

Sheet code	Initial weight of sheet (gm)	Final weight of sheet (gm)	Weight change (gm)
F-1	0.2253	0.3537	0.1284
F-2	1.0791	1.2958	0.2167
F-3	0.3282	0.7994	0.4712
F-4	0.7083	1.3726	0.5643
F-5	0.9022	1.8291	0.9269

TABLE 3: SWELLED FORMULATION OF DIFFERENT SAMPLE PROPORTION

Sheet Code	Percentage (Sheet Swelled)
F-1	41.06%
F-2	60.08%
F-3	63%
F-4	71.69%
F-5	85.38%

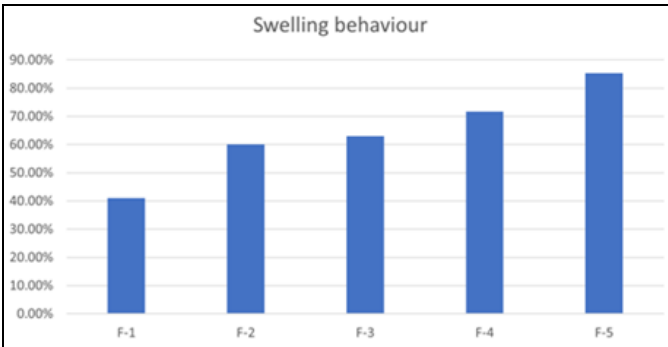


FIG. 5: SWELLING RATIO OF POLYHERBAL FORMULATION

Moisture Retention of Prepared Sheets: The wound dressing retains the moisture and help in

providing the moist environment to the wound site due to which faster healing occur and help to reduce scar formation as compare to the dry environment ⁷. The **Fig. 6** shows that highest percentage of moisture retention was seen in the F-5 and lower retention was seen in the F1. It was shown in **Table 4** that film can retain the moisture for a long time so frequent dressing change is not required.

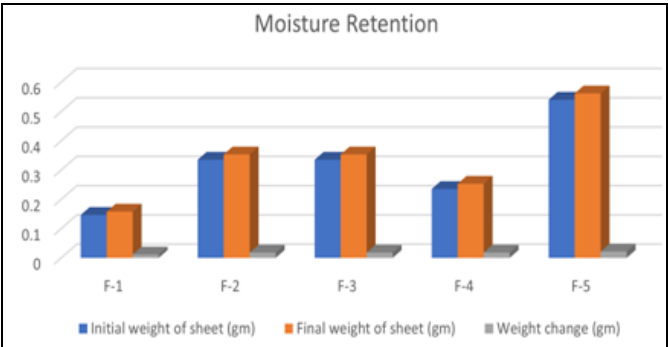


FIG. 6: MOISTURE RETENTION OF POLYHERBAL FORMULATION

TABLE 4: DATA OF MOISTURE RETENTION OF DIFFERENT SAMPLE PROPORTION

Sheet	Initial weight of sheet (gm)	Final weight of sheet (gm)	Weight change (gm)
F-1	0.1465	0.158	0.0115
F-2	0.3352	0.3532	0.018
F-3	0.3352	0.3532	0.018
F-4	0.2352	0.2531	0.0179
F-5	0.5400	0.5616	0.026

Tensile Strength of Prepared Formulation:**Young's Modulus of the Prepared Formulation:**

The **Table 5** shows that young's modulus of different honey concentration sheet prepared. The

young's modulus was found to be the highest in the F5 and lowest in the F1 and F4. Honey concentration decrease the young's modulus of the film²⁰.

TABLE 5: DATA OF STRESS VERSUS STRAIN OF THE DIFFERENT PROPORTION OF SAMPLE USED IN THE HERBAL FORMULATION

Sheet	Initial length (cm)	Final length (cm)	Change in length (cm)	Strain (m/m)	Force (N)	Area (m ²)	Stress (m/m)
F-1	1	2	1	1	5	1	5
F-2	1	2	1	1	7.641	1	7.641
F-3	1	1.5	0.5	0.5	7.641	1	7.641
F-4	1	2	1	1	5	1	5
F-5	1	2	1.1	1.1	26.36	1	26.36

pH of Different Honey used: The pH of honey was studied to ensure the potential of honey treatment for lowering wound pH that leads to reduction in protease activity, increase oxygen release help in wound healing. The **Table 6** displays the pH values of the F3, F4, and F5 base sheets, which were determined to be 4.6, 4.5, and 4 respectively. This indicates that the F5 honey film has a higher acidic pH, making it superior to the F3 and F4 in terms of wound healing properties¹¹.

TABLE 6: THIS SHOWS THE DATA OF PH OF THE 3 SELECTED HONEY

Formulation	pH
F3	4.6
F4	4.5
F5	4

Moisture Content and Moisture Uptake: It was observed that the films moisture content percentage increased from 17.14±1.12 to 26.7±2.17% between batches F1 and F5. Films with a higher moisture content provide better water vapor permeability and UV barrier performance. The water absorption ability of films is caused by the abundance of hydrophilic amino and hydroxyl groups found in chitosan and honey. Consequently, the moisture content of the generated films rise in proportion to the increase in chitosan concentration. Furthermore, the moisture content of the films rise in a comparable manner such as 10.28±0.04 to 15.47±0.08%. Moisture content in itself is an

important parameter for films, as it is related to the exudate soaking capacity of hydrogels.

Water Vapor Transmission: To keep the wound moist and promote quicker healing, moisture must permeate the film. It was observed that the rate of water vapor permeation increased more quickly between one and three days and followed by decrease. This result shows in **Table 7**. Weight gain during the period of 8 days by the selected film F5 found to be more permeable to the moisture up to 3 days, so it represents that moisture permeability was seen better in F5 for faster wound healing³⁰.

TABLE 7: THIS SHOWS THE DATA OF WATER VAPOR PERMEATION

Days	Weight of the sheet (gm)
1	0.006
2	0.0185
3	0.0243
4	0.021
8	0.0285

Thickness, Weight Variation and Folding

Endurance: The developed polyherbal compound was assessed using a number of parametric physiochemical tests. The physical analysis shows how much cross-linking there is between the various polymers employed in the formulation. The thickness was found to vary 0.032 ± 0.004 to 0.054 ± 0.005mm while the weight of dried films varied from 0.417 ± 0.03 to 0.470 ± 0.02.

It indicates that the concentration of honey and chitosan increase from batch F1 to batch F5²⁰. The folding endurance was observed to rise from 343±11 to 470±14. The results are tabulated in **Table 8**.

TABLE 8: THIS DISPLAYS THE DATA OF THICKNESS, WEIGHT VARIATION AND FOLDING ENDURANCE

Formulation code	Thickness	Weight variation	Folding endurance
F1	0.032 ± 0.004	0.417 ± 0.03	343 ± 11
F2	0.041 ± 0.005	0.420 ± 0.05	410 ± 14
F3	0.048 ± 0.007	0.453 ± 0.04	431 ± 10
F4	0.056 ± 0.006	0.470 ± 0.03	433 ± 12
F5	0.054 ± 0.005	0.490 ± 0.02	470 ± 14

Microbial Penetration Test: The microbial penetration test was performed to ensure the honey included in the polyherbal formulation has a positively charged amino group, in the chitosan bind with the negatively charged bacteria and protect the wound from infection the microbiological penetration test was conducted to make sure it has a good chance of being used as wound dressings. The result shows in the **Fig. 7** that the control tube so the bacterial growth after 3 days but in the negative control tube is free from bacterial contamination and the honey film covered on the test tube do not show the bacterial contamination^{19, 47, 53}.



FIG. 7: MICROBIAL PENETRATION TEST

Antimicrobial Test:

Agar Diffusion Test: As shown in **Table 9**, the agar disc diffusion techniques zone of inhibition for *S. aureus* and *E. coli* utilizing various films varied. Compared to polyherbal films, the bacterial inhibition of chitosan films natural antibacterial activity is lower. The mean diameter of the zone of inhibition for polyherbal films rise in tandem with the content of honey. *S. aureus* exhibits greater bacterial inhibition than *E. coli*^{12, 20, 56}. Therefore, it is evident that polyherbal enhances the antibacterial properties of produced films and that adding honey expands the zone of inhibition^{50, 51, 55}.

TABLE 9: ZONE OF INHIBITION OF THE SAMPLE AGAINST S. AURUES AND E. COLI

Sample	<i>S. aureus</i>	<i>E. coli</i>
F1	20	19
F2	22	-
F3	24	22
F4	29	24
F5	30	25

SEM (Scanning Electron Microscopy): SEM images showing the surface methodology are presented in **Fig. 8**.

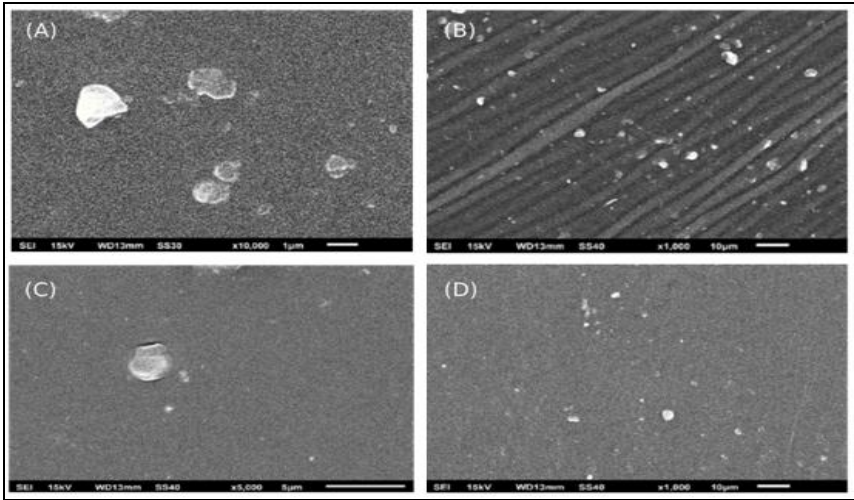


FIG. 8: SEM IMAGES OF POLYHERBAL FILM. (A) F1 UNDER 50X, (B) F2 UNDER 50X, (C) F4 UNDER 50X, (D) F5 UNDER 50X

The F5 batch formulated film presents a smooth surface with low protrusions. The chitosan molecules are spread on film, resulting in a mixture with good adhesive properties, while formulations containing lesser amounts of Chitosan have more abrasive surfaces. Progressing from for formulations F1 to F5 the surface becomes much smoother, as the neem particles are trapped inside the void spaces of the polymeric network³².

CONCLUSIONS: The results of this investigation indicate that the film made with various herbs and 5.4 ml of honey meets all the specifications for wound dressings, including thickness, weight, and folding endurance. The polyherbal formulation has tensile strength of 26N/mm², moisture uptake of 17.14 ± 1.12 to 26.7 ± 2.17%, and swelling behavior of 85.38%. Its antibacterial properties against *S. aureus* and *E. coli* prevent pathogens from penetrating the wound from the surrounding environment to the wound bed. The results of the microbiological penetration test were positive. The mechanisms behind wound healing will be more clearly defined by additional research on polyherbal formulation.

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CONFLICT OF INTEREST: Nil

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