Research Article

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Preliminary Wound Healing Activity of Polyherbal Formulation Containing *Cinnamon zeylanicum, Centella asiatica* and *Moringa oleifera*

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ABSTRACT

In the present work, a polyherbal gel formulation for wound healing activity was developed using 70% methanolic extract of *Centella asiatica* leaves, methanolic extract of *Cinnamomum zeylanicum* Nees stem bark as well as oil of *Moringa olifera* seed. The developed formulation was evaluated for organoleptic parameters like colour, odour, texture and physicochemical parameters like a loss on drying (LOD), pH, viscosity, spreadability, skin irritation study, etc. *In-vitro* antibacterial activity of both the extracts and oil of *M. oleifera* was carried out against two pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* by disc diffusion assay using tetracycline as a standard. Apart from that, the wound healing activity of a polyherbal formulation was evaluated by an incision model on wistar rats. The results showed that the polyherbal formulation exhibited dark brown colour with a smooth texture and cinnamon-like odour. The gel showed 9% LOD, pH was found to be 7.5±0.3, viscosity was 4609.5± 13.44 cps at 10 rpm with spindle number 63, and spreadability was found to be 19.44±0.26 (g.cm/s), and no irritation was found on the healthy volunteer's skin. In the wound healing activity, polyherbal did not affect the normal feed and water uptake. The wound did not show any pus formation throughout the treatment period. In the Planimetry assessment, wounds treated with polyherbal formulation showed a good score at the end of the treatment. Results showed that polyherbal formulation containing 70% methanolic extract of *C. asiatica* leaves, methanolic extract of *C. zeylanicum* stem bark as well as oil of *M. olifera* seed have good wound healing activity.

Keywords: *In-vitro* antibacterial activity, Polyherbal gel, wound healing, herbal, medicinal plants

1. Introduction

In the current market, the available formulations for wound healing, especially the gels, contain synthetic Active Pharmaceutical Ingredients that may cause serious side effects related to skin. Also, the wounds which are at their initial stages do not go through a correct cycle of wound healing and may lead to impaired healing. In addition, several bacteria including *Staphylococcus aureus* (SA), *Salmonella Typhimurium* (ST), *Escherichia coli* (EC), *Pseudomonas aeruginosa* (PA) etc. are considered notorious bacteria and require higher antibiotics and a combination of the same to treat the infection, leading to the emergence of Multidrug resistance (MDR) which is a challenging problem in the field of antibacterial chemotherapy for humans [1-3].

For thousands of years ago, medicinal plants are considered to be the safest source of medication to treat certain sorts of diseases. They carry multiple natural bioactive phytochemicals that accelerate the wound healing cycle and regenerate tissue at the wound site which provides certain anti-bacterial activities. As India is rich in biodiversity and is home to many such plant species, for example, It has been demonstrated that an isolated asiaticoside from the *Centella asiatica* promotes epithelization and collagen synthesis in the open wound. The glycosaminoglycan synthesis and collagen remodelling are improved by isolated triterpenes from *Centella asiatica*. *Moringa oleifera* seed oil has been studied for its antioxidant properties as well as anti-fungal properties and activity against human infection-causing pathogenic microorganisms [4]. Ventura *et al*. studied the effect of *M. oleifera* oil on chronic skin wound and concluded that oleic acid is the major constituent of the oil which is mainly responsible for the activity [5]. *Cinnamon Zeylenicum* is hypotensive, spasmolytic and increases peripheral blood flow; and it inhibits cyclooxygenase and lipoxygenase enzymes of arachidonic acid metabolism. Cinnamaldehyde, a bioactive component of cinnamon, has been demonstrated to have strong antibacterial effects on both Gram-positive and Gram-negative bacteria [6]. In the present work, a simple polyherbal gel containing various extracts of all these plants was prepared and evaluated for its antibacterial and wound healing activity. Thus, the present work aimed to prepare polyherbal gel containing extracts of *C. asiatica, C. zeylanicum* and oil of *M. olifera.*

2. Material and Methods

2.1. Collection and authentication of the plant materials

Leaves of *C. asiatica*, bark of *C. zeylanicum* and seed oil of *M. olifera* were purchased from the local market (S. Dipak & Co., Nadiad (Gujarat) India). The plant materials were authenticated by one of the authors (PRB) and voucher specimens of *C. asiatica* (no. FOP/PCOL/01/2021-22) and *C. zeylanicum* (no. FOP/PCOL/02/2021-22) were submitted to Department of Pharmacology, Dharmsinh Desai University, Nadiad (Gujarat) India for future reference. Leaves and bark of *C. asiatica* and *C. zeylanicum*, respectively were shade dried and then subjected to grinding for powdering. All the solvents and chemicals were of analytical grades.

2.2. Preparation of extract

100 grams of powder of *C. asiatica* leaves and powder of *C. zeylanicum* bark were macerated with 70% and 100% methanol, respectively for 7 days. After 7 days, solvents were evaporated under reduced pressure in the rotary evaporator below 40°C. All the extracts were dried and weighed properly [7].

Per cent extractability was calculated by the following formula:

$$\% \text{ Extracted} = \frac{Total\ amount\ of\ extract\ obtained}{the\ total\ amount\ of\ powder\ used\ for\ extraction} \times 100$$

2.3. Preparation and evaluation of polyherbal gel

The polyherbal gel was prepared by using carbopol 934 as a gelling agent which was added to 50 ml of water, with stirring and kept aside to soak for 24 hours. Accurately weighed equal quantities of extracts of *C. asiatica* and *C. zeylanicum* and oil of *M. olifera* were mixed in the beaker with a small quantity of water. Propylene glycol (10%) was placed in a glass mortar pestle and the mixture of extracts and oil were mixed thoroughly in propylene glycol. Triethanolamine was added dropwise till gel consistency was obtained. The colour of the gel was recorded by visual observation. The pH of the gel was measured using a digital pH meter. A 10% dispersion of gel was prepared in distilled water and subjected to me-

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asurement of pH, the gel was placed in a glass container and allowed to settle. Homogeneity was assessed by visual inspection of the gel for its appearance and existence of any clog. A skin irritation test was performed on human volunteers with their informed consent. The gel was applied to the back of the hand of each volunteer and observed for the presence of any irritation on the lesion. The viscosity of gel was measured using a Brookfield viscometer at 10 rpm and spindle no. 63 [8, 9].

2.4. In vitro anti-microbial activity

2.4.1. Bacterial culture

Bacterial cultures of *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 9144) were obtained from J. & J. Science College, Nadiad (Gujarat) India.

2.4.2. Chemicals and reagents

Nutrient broth, nutrient agar, sterile blank disc and antibacterial discs and media were prepared for subculturing and to test the antibacterial activity of plant extracts and standard antibacterial drugs as per the specifications of the manufacturer.

2.4.3. In-vitro antibacterial activity

In-vitro antimicrobial activity of different concentrations like 50, 100, 150 and 200 mg/ml of extracts of *C. asiatica* and *C. zeylanicum* and as well as for *M. olifera* oil, was evaluated against *E. coli* and *S. aureus*, as a test organism by using Disc Diffusion assay. Tetracycline (10 µg/ml) was used as a standard antibacterial drug. Discs were impregnated with 100 µl of sample solutions and placed inverted on the agar plates. The plates with discs were incubated at 37°C for 12 h and zone of inhibition for each samples were measured. Antibacterial activity was compared in terms of measuring the zone of inhibition [1-3].

2.5. Wound healing activity

A preliminary wound healing activity of polyherbal formulation was evaluated using an incision model on *Wistar* rats having weights of 250 to 350 g. The experiment was carried out on 6 animals equally divided into 3 groups comprising 2 animals in each group (Table 1). Two wounds were created on each animal at the dorsal side. So each treatment consists of a total of 4 wounds on two animals. The treatment protocol was approved by the Institutional Animal Ethics Committee of the Faculty of Pharmacy, Dharmsinh Desai University, Nadiad (India) with protocol No.DDU/FOP/03/2021 dated 24 December 2021.

2.5.1. Surgical procedure and treatment

Wound healing activity of polyherbal formulation was evaluated by incision model of the full-thickness wound. The rats were housed in cage with free access to water and feed. The temperature and relative humidity of the animal house was maintained at 25.0 ± 2.0°C and below 55-60%, respectively. Before commencement of wound infliction, rats were anesthetised with Isoflurane (30% in Propylene glycol) in the desiccator. Thereafter, animals were restrained in sternal recumbency and the dorsum was prepared for aseptic surgery. On the shaved skin a cut of 6 mm was created using a Bard-Parker blade (B.P. blade) with minimal depth (approx. 2mm). The skin, including the panniculus carnosus muscle, was cut with a blade. Hemorrhage was controlled by sterile surgical sponge compresses. After completion of the surgery, digital photographs were taken. Approximately 0.5 g of each treatment was applied and wounds were left open till the treatment. The day of surgery was considered as baseline. Post-surgery, the wounds were dressed with freshly prepared normal saline solution and fresh treatments were applied every day till the healing. Day-to-day macroscopic examination (suppuration, scar formation, shape, irregularity and

Table 1. Treatment protocol

Sr. No.	Group Name	Type of Treatment	No. of Rats
1.	No treatment	No treatment was given	2 (each having two wounds)
2.	Control	Blank gel without herbal extracts	2 (each having two wounds)
3.	Treatment	polyherbal gel once a day	2 (each having two wounds)

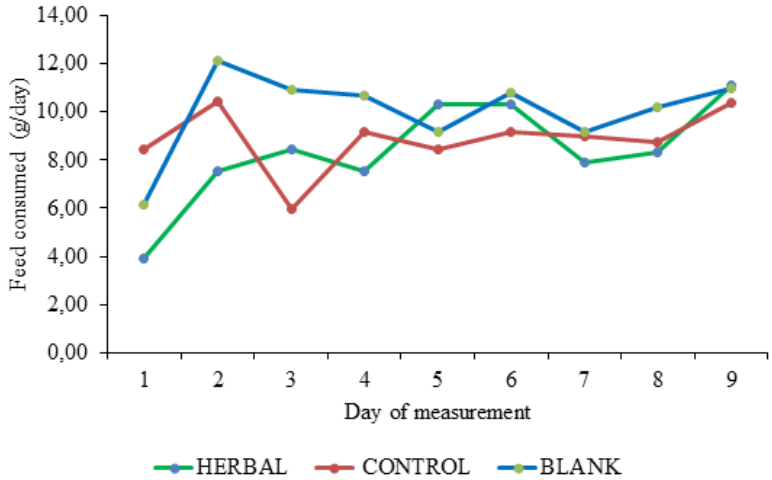

Figure 1. Feed consumption of rats under different treatments

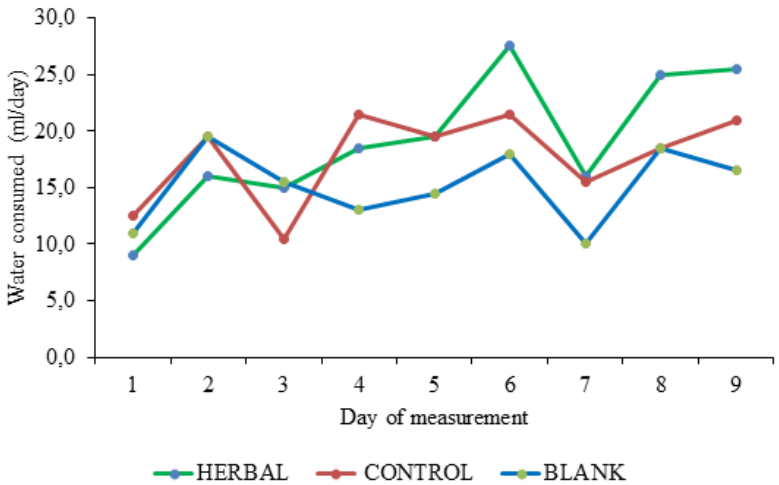

Figure 2. Water consumption of rats under different treatments

colour of wound and epithelization), was done and photographic images were recorded. Data for daily feed and water consumption was also recorded. Each rat was kept in a separate cage throughout the study period [10, 11].

3. Results and Discussion

Per cent yield, appearance and type of extract for both extracts are shown in Table 2. The extract of *C. asiatica* leaves showed a slightly lower yield as compared to *C. zeylanicum* stem bark extract. Extract of *C. zeylanicum* showed dried free-flowing consistency with a characteristic odour while the extract of *C. asiatica* was sticky.

Various organoleptic characters were shown in Table 3. The gel showed a very dark brown colour with the characteristic cinnamon volatile oil-like smell with a very smooth consistency. The spreadability was found to be 19.62 g.cm/s which was also good. The pH of the polyherbal gel showed near 7, so no irritation was produced upon application of gel on the skin. The viscosity of the polyherbal gel was also determined. The polyherbal gel showed loss on drying about 9% w/w which was also good. The gel was easily washed out from the skin. The results of physicochemical parameters were identical to previously reported data [12].

Both the extracts and oil were evaluated for *in-vitro* antibacterial activity against various pathogenic bacteria. *In-vitro* antibacterial activity of all the extracts

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Table 2. Per cent yield and appearance of the extracts

Sr. No	Name of plant	Type of extract	Appearance	Colour	Extractability
1.	***C. zeylanicum***	Methanolic	Dry	Dark brown	13%
2.	***C. asiatica***	Hydroalcoholic	Sticky	Dark green	8%

Table 3. Various physicochemical parameters of wound healing polyherbal gel

Sr. & No.	PARAMETERS	RESULT
1	Organoleptic properties	Colour: Dark brown Odour: Cinnamon like Texture: smooth gel-like
2	Spreadability	19.44±0.26 (g.cm/s)
3	pH	7.53 ± 0.3
4	Viscosity	4609.05±13.44 cps (at 10 rpm and Spindle No. 63)
5	Skin irritation	No irritation as such
6	Loss on Drying	9%
7	Washability	Washable

Table 4. Zone of inhibition (mm) of various extracts and combinations of various formulations against different pathogenic bacteria

plant extract	Concentrations (mg/ml)	Zone of inhibition (in mm)	
		E. Coli	***S. aureus***
C. zeylanicum methanol extract	50	ND	ND
	100	ND	ND
	150	18.3±0.7	14.95±0.07
	200	17.50±2.12	17.40±0.57
C. asiatica hydroalcoholic extract	50	15.95±0.05	ND
	100	16.15±0.15	15.50±0.71
	150	16.95±0.15	15.00±1.41
	200	17.2±0.2	17.50±0.71
M. oleifera seed oil	50	16.85±0.15	ND
	100	15.25±0.25	14.90±0.14
	150	15.85±0.15	16.70±0.42
	200	17.35±0.21	16.20±0.28
Tetracycline	10 µg	20.75±1.06	23.25±0.35

ND denotes not detected; Data are expressed as mean ± SD (n=2).

and oils, at different concentrations, showed good antibacterial activity against various pathogenic bacteria (Table 4). Almost all the bacteria showed sensitivity against all the extracts and oil. Wound healing may be delayed by a bacterial infection in the wound. Suppuration is the most common sign of the wound [13]. Medicinal plants are the richest source of phytochemicals which are structurally diverse molecules and may have strong antimicrobial activity against a wide range of bacteria [14]. *C. asiatica* is reported to have antibacterial action against a wide range of bacteria. It is also used as an antibacterial by tribes in Muruthamallai Hills in Tamil Nadu [15]. *M. oleifera* contains a number of phytochemicals which are responsible for its variety of pharmacological actions including antibacterial action against various bacteria [16].

The wounds of each treatment group were observed daily and photographs were taken for gross examination. At the end of the treatment, the wound treated with polyherbal gel showed complete healing as well as white skin around the wound area. Growth of hair was also observed around the shaved skin. This showed that polyherbal gel increased the healing as well as did not show any allergic reaction to the skin. The wounds of each treatment group were observed for gross observation throughout the experiment. Results showed that, at the end of the treatment, wounds treated with polyherbal gel showed the highest epithelization as well as contraction. Scar formation was also found to be good as compared to wounds of the no-treatment group (Table 5; Figure 3). Wound healing is characterized by granulation, neo-vascularization, wound contraction and epithelization. Epithelization is only possible if the proliferation of epithelial cells is promoted by the treatment. Wound contraction may be a sign of the anti-inflammatory and antioxidant potential of the medicinal plants used in the polyherbal gel. Scar formation and granulation are important processes of wound healing action. They generally happen concurrently but independently. The stronger the scar formation faster the wound healing process will be there [17, 18, 19].

Feed consumption of the rats treated with polyherbal gel did not show any drastic change (Figure 1). Water consumption of the rats treated with polyherbal gel showed the highest water consumption at the end of the treatment (Figure 2). Rats treated with blank gel showed less water consumption as compared to other treatment groups. Normal feed and water intake in the treatment group showed good health condition of the animals [20].

4. Conclusions

Based on preliminary experiments and observations, it was concluded that the formulation with the extracts of *C. asiatica* leaves, *C. zeylanicum* stem bark and oil of *M. oleifera* seed showed good wound healing activity, however, a detailed study with more evaluation parameters is needed.

Table 5. Gross observation of wounds of various treatments at different time intervals

Day	Type of treatments	Suppuration	Scar Formation	Shape, irregularity and colour of the wound	Epithelization
3	Polyherbal Gel	Not observed	++	Irregular, reddish	-
	Blank gel	Not observed	-	Irregular, reddish	-
	No Treatment	Not observed	-	Irregular, reddish	-
6	Polyherbal Gel	Not observed	++	Irregular contracted,	++
	Blank gel	Not observed	+	Irregular, whitish	+
	No Treatment	Not observed	-	Round, brownish	-
9	Polyherbal Gel	Not observed	++	Contracted, White	+++
	Blank gel	Not observed	++	Contracted, white	+
	No Treatment	Not observed	+	Contracted, brown	+

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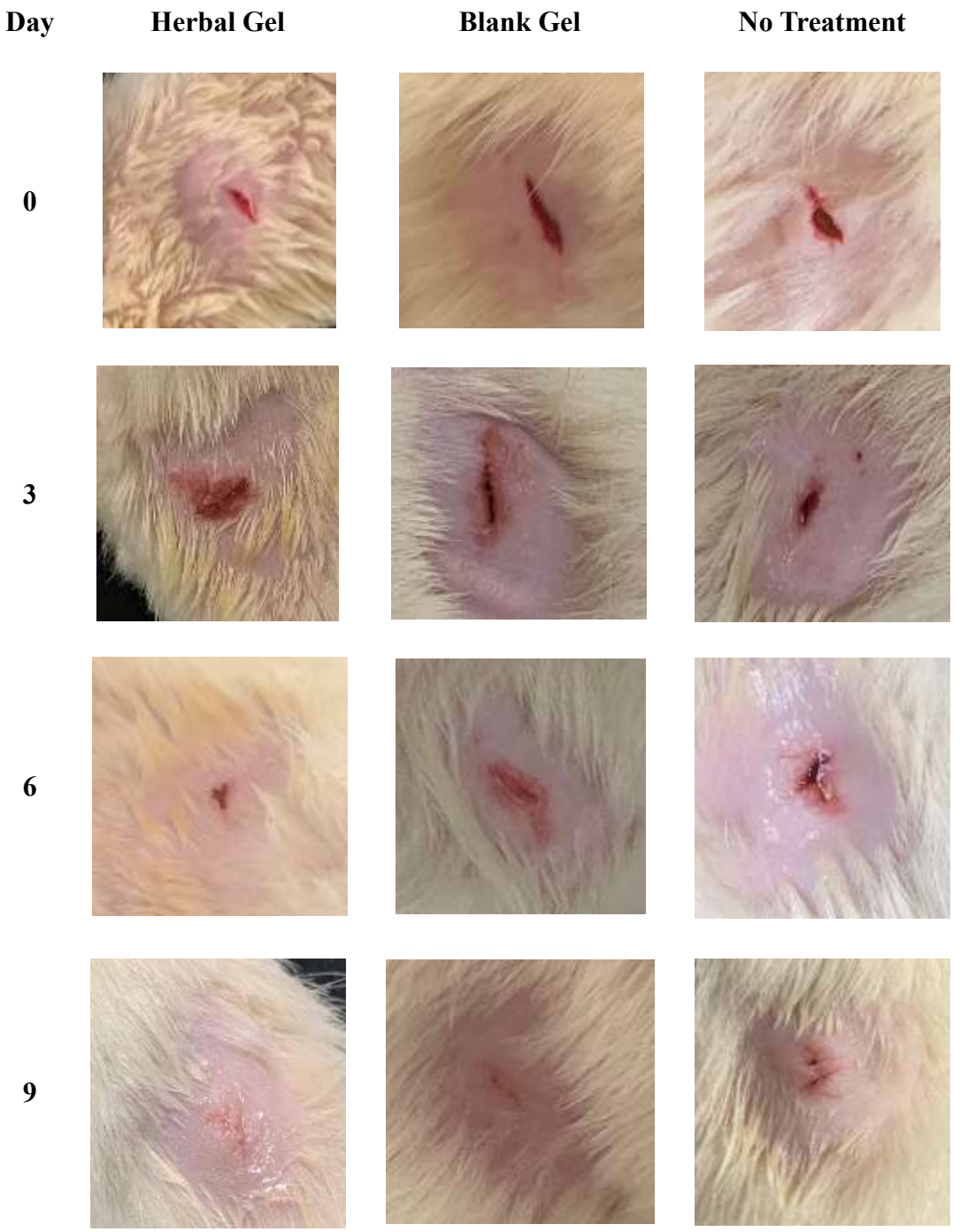

Figure 3. Gross observation of the animals under various treatments at a different time intervals

Statement of Contribution of Researchers

Experimentation - GDU and HJT; Experimentation, manuscript writing, manuscript revision - PRB and KRS; Concept and suggestions – BNS

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Statement of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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