



Development of Wound Healing Ointment Formulation containing Active Extracts of *Tridax Procumbens*, *Calendula Officinalis*, *Murraya Koenigii*, and *Aloe Barbadensis*

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ABSTRACT

The present research reflected towards exploring the plausible role(s) of the active extracts of *Tridax procumbens* (whole plant), *Murraya koenigii* (leaves), *Calendula officinalis* (flowers), and *Aloe barbadensis* (leaves) formulated as ointment products. The formulations were characterized by determining the pharmaceutical characteristics like skin irritancy test, pH, appearance, viscosity, spreadability, extrudability, swelling index, and washability. Further, wound healing activity was studied on Swiss albino rats. The phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins, carbohydrates, sterols, saponins, proteins, and other miscellaneous phenolic components are believed to play a pivotal role in the healing of the wound in rats by significantly increasing the rate of wound closure and rate of epithelization. In comparison to the standard drug (betadine), both the formulations proved to be quite equi-efficacious. This finding provided an insight into the applications of the polyherbal formulations in the traditional treatment of serious wound conditions and also rejuvenating the ethnopharmacological principles in context to modern medicine.

Key Words: Wound Healing, *Tridax procumbens*, *Murraya koenigii*, *Calendula officinalis*, *Aloe barbadensis*, Honey.

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INTRODUCTION

The process of wound healing is a very complex phenomenon where the skin or the affected organ after injury repairs itself [1]. Under normal conditions, the outermost layer of the skin (epidermis) and the inner or deeper layer (dermis) exist in steady-state symmetry and form a defensive barrier against the exterior environment [2]. If this defensive barrier is broken as a result of any trauma or injury, the normal physiologic function of wound

healing is instantly instigated [3]. The wound healing is initiated by growth factors act by autocrine-, paracrine-, and endocrine-signaling systems [4]. Apart from them, numerous growth factors are present, which aid in wound healing through varied mechanisms. Platelet-derived growth factor (PDGF) is accountable for the stimulation of connective tissue proliferation, epidermal growth factor (EGF) is responsible for stimulating the cutaneous tissue proliferation, and fibroblast growth factor (FGF) stimulates the fibroblast cells proliferation [5]. Several commercial

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products are available with claims that they accelerate the wound healing process but in a long duration, they came up with a number of issues (hypo-pigmentation, scar marks appearance, etc.) [6].

In the developing and developed countries, herbal therapy prevails in traditional medicine as well as in alternative medicine [7]. World Health Organization (WHO), as well as India, has been promoting the use of traditional medicine because they are less expensive, easily available, strong belief among the community in developing countries, etc [8]. Literature reveals that simple traditional plants (*Adhatoda vasica*, *Boerhaavia diffusa*, *Caesalpinia sappan*, *Curcuma longa*, *Cyperus rotundus*, *Eclipta alba*, *Glycyrrhiza glabra*, *Gymnema sylvestre*, *Oryza sativa*, *Piper longum*, *Plumbago zeylanica*, *Santalum album*, *Saussurea lappa*, *Terminalia chebula*, *Tribulus terrestris*, *Vitex negundo*, *Woodfordia fruticosa*, *Zingiber officinale*, etc.) are beneficial in the treatment of several skin-related problems and also for wound healing [9]. The widespread interest in the applications of herbal-based active extracts or phytoconstituents lies due to the belief that plant-based materials are safe and dependable, along with fewer side-effects [10]. In recent practice, several herbal-based wound healing formulations such as ointments, creams, gels, emulsions, suspensions, liquids, sprays, jelly, carbogel, wet dressing, foams, lotions, lipogels, etc. have been investigated [11, 12]. Although diverse forms of creams are considered for wound healing, but these still appear to be very limited in the rate of tissue regeneration [13].

The present research reflected towards exploring the plausible role(s) of active extracts of *Tridax procumbens* (whole plant), *Murraya koenigii* (leaves), *Calendula officinalis* (flowers), and *Aloe barbadensis* (leaves) formulated as ointment product. The formulations were characterized by determining the pharmaceutical characteristics like skin irritancy test, pH, appearance, viscosity, spreadability, extrudability, swelling index, and washability. Further, wound healing activity was studied on Swiss albino rats.

MATERIALS AND METHODS

Chemicals

All the analytical-grade chemicals such as White wax and White petroleum were obtained from SD Fine Chem Ltd., India. PEG 300, Cetostearyl alcohol, and Chloroform were procured from HiMedia Chemicals Ltd., India. Propylparaben and Methylparaben were purchased from Sigma Aldrich Ltd., Germany. Honey (99.96% pure) was purchased from Pachmadi Gold Forest Honey Ltd., India. Double distilled water was obtained from Borosil® distilled water apparatus.

Instrumentation

All weighing was done using Shimadzu® electronic balance (Model AUW220D, Kyoto, Japan). The pH was measured using VSI® digital pH meter of model VSI-1B. The viscosity was estimated by Brookfield Digital DV-II+ Viscometer (using spindle 6). The accelerated stability studies were carried out in a stability chamber (Bio-Technics, India).

Animals

After getting approval from Department Ethical Committee and CPCSEA, the wound healing activity was performed on Swiss albino rats (5 to 6 weeks of age and 150-200 g body weight). The rodents were kept in the animal house under the temperature of 25–26°C, humidity 50–65%, 12 hr light-dark cycle. The rats were kept in polypropylene cage, fed with standard pellets, and given free access to food and water.

Collection of plant materials

Fresh leaves of *Murraya koenigii*, fresh whole plant of *Tridax procumbens*, fresh leaves of *Aloe barbadensis*, and fresh flowers of *Calendula officinalis* were collected from the medicinal plant garden of Kamla Nehru College of Pharmacy, Nagpur, Maharashtra, India. The plants were authenticated by Dr. Dongarwar, Department of Botany, Nagpur University, Nagpur, Maharashtra.

Extraction process

Extraction of *Murraya koenigii*

200 g of the collected fresh leaves of *Murraya koenigii* were washed to remove dirt from the leaves' surface. The leaves were cut in small pieces and placed in a 1000-mL conical flask. The content was macerated with ethanol and water in a 1:1 ratio for 7 days. After 7 days, the solution was filtered, evaporated, and the dried extract was collected.

Extraction of *Tridax procumbens*

500 g of fresh whole plant of *Tridax procumbens* was collected and washed to remove the entrapped dirt. The plant material was immersed directly in the conical flask filled with ethanol: water in a 1:1 ratio for 5 days. The extract was collected and concentrated by using the rotary vacuum evaporator. The crude semi-solid extract was collected and kept in a small vial.

Extraction of *Aloe barbadensis*

200 g of freshly collected *Aloe barbadensis* leaves were taken and washed thoroughly with water to remove the adhered dirt. Yellow juice was extracted by crushing the leaves and extracted with two parts of ethyl acetate at 60°C for 30 min with continuous stirring. The volume of the extract was concentrated to 1/5th of the original volume by

using the vacuum rotary evaporator. The content was distilled at 50°C to obtain a solid brown residue. This residue was dissolved in 50 mL of isobutanol at 70°C and allowed to crystallize to obtain a yellow powder of aloein.

Extraction of *Calendula officinalis*

100 g of freshly collected flowers of *Calendula officinalis* were washed to remove dirt from the surface. The flowers were cut in small pieces and placed in a 1000-mL conical flask. The content was macerated with ethanol and water in a 1:1 ratio for 7 days. After that, the solution was filtered, evaporated, and the dried extract was collected.

Formulation development

Preparation of ointment base

For preparing the ointment base, white wax was melted on a hot plate at 70-75°C. When the wax was completely melted, white petroleum was added and the whole mixture remained on a hot plate to become liquid. Following the liquefaction process, the content was removed from heat and allowed the mixture to congeal. The mixture was stirred until it began to congeal (**Table 1**) [14].

Preparation of polyherbal formulation

The semi-dried extracts were used to prepare ointment. The polyherbal formulations (F1 and F2) were made by using the ointment base. The standard trituration method was used where solid fats were melted and mixed. The required amount of the ointment base was added respectively to the melted base at 40°C and thoroughly mixed. The preparation was stirred gently and continuously until a homogeneous dispersion was obtained (**Table 2**) [15].

Evaluation of polyherbal formulations

Physical Evaluation

The color, general appearance, and the feel on the application of the prepared formulations were noted and the results are described.

pH

The pH of the ointment formulations (F1 and F2) were determined by applying the digital type calibrated pH meter, which was further calibrated before each usage with the aid of buffered solutions at both pH-4 and pH-7. The pH of the formulations was measured by completely dipping the reference electrode and the glass electrode into the ointment [16].

Spreadability

The spreadability of the formulations was determined by a special apparatus comprising of a flat wooden block supported by a pulley at its end. Based on the principle of drag and slip characteristics, the formulations were screened by placing 2 g of the polyherbal product on the

ground slide. Between the two slides of the same dimensions, the formulation was sandwiched and the system was supported by a hook. To expel the entrapped air from the formulations and also to form a uniform film between the two slides, a unit kilogram weight was applied over the slide. Excess formulation extruding outside was removed from the edges. With the help of the hook, 50 g of weight was tied to induce a pulling force and the time required to cover 7.5 cm distance by the top slide was determined [17]. The spreadability of the formulation was determined from the formula:

$$\text{Spreadability} = \frac{M \times L}{T}$$

where, M = weight tied to the upper slide (50 g); L = length of glass slide (6 cm); T = time taken (sec) to separate the glide slides from each other.

Washability

The washability of the polyherbal formulations was determined by applying the ointments over the skin and the extent of easy washing with distilled water was manually observed [18].

Skin irritancy test

Over an area of 6 cm² on the skin, 0.5 g of the formulation was applied and afterward covered with a gauze piece, which was loosely kept in contact by a dressing (semi-occlusive) for 1 hr. The gauze was removed and after 1 hr, the residual content was removed without altering other conditions. A thorough examination was performed regarding the sensitivity characteristics and other signs of rash or reaction. The protocol was performed for 7 consecutive days and grading was done [19].

Viscosity

The apparent viscosity values of the ointment formulations were measured using a Brookfield viscometer with spindle no. 6 at room temperature and 50 rpm using the standard operating procedure as directed in the manufacturer manual (**Table 3**) [20].

Extrudability

The polyherbal ointment formulations were filled in a standard plastic capped collapsible aluminum tube that was sealed by crimping with ointment sealing apparatus. The tubes were placed in between the two slides and clamped further. A weight of 500 g was placed over the slides and the cap was immediately released. The formulation extruded in a ribbon manner for 10 seconds. The length of the extruded ribbon was recorded [21].

Swelling index

Since the ointment comprised of hydrophilic ingredients, the swelling index of the dermal ointment was estimated by pouring 2 g of the content in a beaker containing distilled water (10 mL). After 1 hr, the formulation was removed from the beaker and placed on a Petri dish [22]. The content was weighed and the degree of swelling was established from the formula:

$$\text{Swelling index} = \frac{W_t - W_o}{W_o} \times 100$$

where, W_t = weight of swollen after 1 hr; W_o = original weight of ointment at 0 hr.

Accelerated Stability Studies

Both F1 and F2 were studied for their stability under accelerated temperature and moisture ($40^\circ\text{C} \pm 2^\circ\text{C} / 75\% \pm 5\% \text{ RH}$) conditions for 90 days. The formulations were placed in a PVC container, wrapped with an aluminum foil. After 90 days, the formulations were taken out of the stability chamber and re-tested for the pharmaceutical characteristics such as pH, washability, extrudability, spreadability, appearance, and viscosity [23].

Wound healing activity

The hairs of the rats were removed from the posterior sides using the hair removal cream. The wound (10 mm diameter) on the experimental rat was created using the excision method. The wounded area was measured with a sterile scale and this area was marked with the black marker pen. The rats were anesthetized with chloroform in a closed chamber. After 15 min of anesthesia administration, the marked area of skin was excised using surgical blade number 18 and forceps. After creating the wound, the skin was removed. The polyherbal wound healing ointment and the marketed formulation was applied, starting from the day of the operation. The wound was measured using the transparency paper, scaled, and the area was calculated at days 0 to 15 [24]. The degree of wound contraction was determined by:

$$\text{Percentage of wound contraction} = \frac{\text{Initial wound size} - \text{Specific day wound size}}{\text{Initial wound size}} \times 100$$

RESULTS AND DISCUSSION

Evaluation of formulations

Both the ointment formulations look very elegant, colored, very soft to touch, free from grittiness, non-irritant, and no such defects detected. Formulation-1 has a greenish-brownish colored appearance with characteristic herb-type odor while Formulation-2 has a brownish color with

characteristic herbal odor. Formulation-2 looked more elegant than Formulation-1 in terms of appearance.

From the skin irritation test study, no particular edema or erythema symptoms were seen after application for consecutive 7 days. From simple observation, it was noticed that the Formulation-2 was much lesser non-irritant as compared to the Formulation-1. While looking at the compatibility of usage, numerous synthetic cosmetics in the market contain new synthetic excipients, which lead to skin irritation in sensitive populations, in contrast to it, polyherbal formulations demonstrated better compatibility for human use with no local irritation.

While looking at the rheological aspects of formulations, the viscosity was found to be 3800 cps (Formulation-1) and 4580 cps (Formulation-2). It has been perceived that as the torque augments, the shear stress enhances, and subsequently, the viscosity reduces. The high viscosity of Formulation-2 can be explained by the addition of honey, which imparted hindrance to the Brookfield viscometer spindle. The other reason may be the absence of high emulsifier concentration because it is well known that when emulsifier concentration gets raised, the viscosity reduces. The high viscosity also focused on the retention of the product for a longer duration over the wounded surface and will continue to exert the action.

The pH of the formulations was found to be 5.2 (Formulation-1) and 5.7 (Formulation-2), which is indicated compatibility for dermal application as the formulation pH closely lies with the pH of the skin (5.4-6.0).

The swelling indexes of both the formulations were very low. Formulation-1 showed 1.04% swelling whereas Formulation-2 demonstrated 1.09% swelling. Although hydrophilic excipients are present in the ointment, the degree of swelling was found to be extremely low due to the presence of a high concentration of extract components that prevented the swelling of the formulation. Although the swelling has both positive as well as negative effects, a certain degree of occlusive swelling is required when it comes to dermal applications.

The spreadability of ointment formulations was found to be 6.6 g.cm/sec (Formulation-1) and 4.8 g.cm/sec (Formulation-2). As the viscosity of the developed formulation decreases, the spreadability increases concurrently. Formulation-2 has low spreadability due to high viscosity. The higher activity of Formulation-2 may be due to less spreadability and more retention to the wounded area, which causes a concentrated amount of the product in the focused area.

The extrudability of the formulations from the collapsible tubes were found to be +++ for Formulation-1 and ++ for Formulation-2. The reason for the lesser extrusion of the Formulation-2 as compared to the Formulation-1 may be

due to the higher viscosity, which hindered free extrusion from the collapsible tube.

The washability of the formulations was found to be ++ for Formulation-1 and + for Formulation-2. The higher viscosity, better retention ability, and high stickiness of the Formulation-2 resulted in a lesser washability.

The accelerated stability conditions (40°C±2°C and 75%±5% RH for 90 days), the formulated polyherbal wound healing formulations (F1 and F2) revealed no extensive variations in terms of physical appearance, washability, viscosity, spreadability, pH, and extrudability. The fall in the pH of Formulation-1 may be due to the production of some small fragmented acidic components under the accelerated state. In contrast to it, Formulation-2 was found to be more robust in terms of pH and viscosity (Table 4). The plausible reason may be the presence of honey in the product that prevented the degradation of the chemicals under accelerated stress. Therefore, the prepared ointment formulations were observed to be highly stable.

Wound healing activity

The wound healing activity of the formulations was studied on Swiss albino rats. On day zero, a prominent scarring was observed on the posterior side of the experimental animals. After the application of both formulations over the wounded animals, a reduction in scar was observed in both the cases, which further became less prominent visually on prolonged application from the 1st day (F1 = 0%, F2 = 0%), 3rd day (F1 = 13.93%, F2 = 11.53%), 6th day (F1 = 36.81%, F2 = 46.37%), 9th day (F1 = 63.53%, F2 = 65.38%), 12th day (F1 = 90.67%, F2 = 88.46%), and 15th day (F1 = 95.76%, F2 = 96.15%) (Table 5). The therapeutically active phytochemicals present in the extracts of *Tridax procumbens*, *Murraya koenigii*, *Calendula officinalis*, *Aloe barbadensis*, and also in honey such as alkaloids, flavonoids, glycosides, tannins, carbohydrates, sterols, saponins, proteins, and other miscellaneous phenolic components are believed to play a pivotal role in the healing of wound in Swiss albino rats by significantly increasing rate of wound closure, migration, and epithelization.

Formulation-2 expressed higher activity than the Formulation-1 because of several factors that can be taken into account. Firstly, the pharmaceutical properties such as viscosity and spreadability lead to better concentration of the product over the wounded area, which results in better interaction with the scared components. Secondly, honey has a potential pharmacokinetic modifying activity, which leads to a better epithelization process. Thirdly, honey has several essential known and unknown chemical components that play major pharmacological, pharmacodynamics, and pharmacotherapeutic roles in wound healing activity. In comparison to the standard drug (betadine), both formulations proved to be quite equi-efficacious (Figure 1). Figure 2 describes the visual

progression of the wound healing and reduction in wound contraction (1st day to 15th day) by both the formulations as well as standard drug betadine.

CONCLUSION

This study has positively opened up new avenues for treating wounds of diverse origins. The developed polyherbal wound healing formulations containing active extracts of *Tridax procumbens* (whole plant), *Murraya koenigii* (leaves), *Calendula officinalis* (flowers), and *Aloe barbadensis* (leaves) will have future perspectives of application as wound healing medicament, which will play a role in accelerating the natural healing process. The phytochemical constituents such as alkaloids, flavonoids, glycosides, tannins, carbohydrates, sterols, saponins, proteins, and other miscellaneous phenolic components are believed to play a pivotal role in the healing of the wound in rats by significantly increasing the rate of wound closure and epithelization. Formulation-2 containing honey showed higher closure of wound as compared to Formulation-1, which contains Aloe, because honey has potential pharmacokinetic modifying activity. In comparison to the standard drug (betadine), both formulations proved to be quite equi-efficacious. This finding provided an insight into the applications of the polyherbal formulations in the traditional treatment of serious wound conditions and also rejuvenating the ethnopharmacological principles in context to modern medicine.

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Conflict of Interest

The authors state that there is no conflict of interest regarding the publication of this manuscript.

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