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Development and evaluation of herbomineral ointment from *Bauhinia variegata* L. for wound healing effects

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The present study has been undertaken to formulate and evaluate the herbomineral ointment containing *Bauhinia variegata* L. extract. The ointment formulation was designed by using aqueous extract of stem barks of *Bauhinia variegata* (Linn.), mineral and other excipients needed for formulation. Wound healing activity of herbomineral ointment was evaluated in excision and incision wound models in rats. The ointment was prepared by using *B. variegata* extract, prawal bhasma, propylene glycol, stearyl alcohol, white petrolatum and required amount of distilled water. The prepared herbomineral ointment was evaluated for physical appearance, pH, spreadability, skin irritation to observe side effects and also for wound healing activity. The herbomineral formulation was found to be good in appearance, homogeneity and it possessed significant (P <0.001) wound healing effect compared to the positive control group and nearly comparable with a standard group. In the incision wound model of rats, the formulation showed a significant increase in the skin breaking strength compared to control and reference standard. The results suggested that herbomineral formulation possessed significant wound healing potential.

Keywords: *Bauhinia variegata* L., Herbomineral, Ointment, Wound healing.
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Introduction

Medicinal plants have been used as alternative sources of medicine in virtually all cultures. According to WHO, herbal medicines play a vital role to serve the healthcare needs of about 80% of the world's population, especially for millions of people in the rural areas of developing countries[1]. India has great environmental and biological diversity[2] and its one of the largest producers of herbs and herbal products. Herbal medicines are prepared from various parts of a plant like leaves, stems, roots, barks and seeds which usually contain various bioactive compounds and are used primarily for treating mild and chronic ailments of well being[3]. Ayurveda herbs have a very imported role in the wound healing process. Plants are more potent healers because they promote the repair mechanisms in natural ways. The healing process can be physically monitored by assessing the rate of contraction of the wound[4]. In the natural product form herbomineral formulation of Ayurveda consist of the substance of herbal, mineral/ metal and animal origin which is processed to have therapeutic effects[5].

Bauhinia variegata L. belonging to family *Caesalpiniaceae* is an important medicinal plant distributed and cultivated throughout India, in areas up to 1800 m elevation. A modern sized deciduous tree with vertically cracked grey bark, wood moderately hard, greyish brown with irregular darker patches; leaves of 2 leaflets, connate about two-thirds up, leaflets ovate, rounded at apex, 10-15 cm long, pubescent beneath when young, coriaceous; flowers white or pink, the uppermost petal darker and variegated, usually appearing before the leaves in short axillary or terminal racemes, stamens 5, fruits flat dehiscent pods, seeds 10-15. The barks are astringent, acrid, cooling and useful in leprosy, tumours, wound, ulcer and diabetes[6]. The pharmacological activities reported so far from this plant are anthelmintic and antimicrobial[7,8], anti-inflammatory[9], antidiabetic[10], hepatoprotective properties[11], wound healing activity[12], anticancer[13]. The phytochemical screening revealed that *B. variegata* contained terpenoids, flavonoids, tannins, saponins, reducing sugars, steroids and cardiac glycosides[14]. The stem bark is reported to contain 5, 7 dihydroxy and 5, 7 dimethoxyflavanone -4-O-L

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rhamnopyrosyl-β-D-glycopyranosides, Kaempferol -3-glucoside, lupeol, and beta-sitosterol[15,16].

The present investigation involves the development of herbomineral formulation as a hydrophilic ointment of *B. variegata* stem bark (aqueous extract) and prawal bhasma followed by evaluation for physical appearance, pH, spreadability and wound healing activity.

Materials and Methods

Plant collection and authentication

The stem barks of *B. variegata* was procured from a local market and same was authenticated by Dr Seema Bhadauria, Head of Department of Botany, R.B.S. College, Agra and herbarium sheet has been preserved in the Department of Pharmacognosy, Anand College of Pharmacy, Agra, for future reference (No 91 dated 27.04.2017). The prawal bhasma (Baidyanath, Jhansi) was procured from the local market of Agra, India.

Drugs and Chemicals

Betadine ointment (Huns Digital Home Pvt. Ltd., Maharashtra), Simple petroleum jelly (Hindustan Unilever Ltd., Mumbai), Propylene glycol (Merck Ltd., Mumbai), Stearyl alcohol (Qualikems Fine Chem. Pvt. Ltd., Vadodara), White petrolatum (Loba chem., India) and other chemicals were procured from Thermo Fisher's Scientific India Pvt. Ltd., Mumbai.

Preparation of plant extracts

The collected stem barks of *B. variegata* were washed and dried in a hot air oven at 35 °C to avoid degradation of phytoconstituents. After drying plant material was coarsely powdered with a grinder. About 300 g of powder was subjected to extraction by maceration with water for seven days at 37 °C[17,18]. After that, the extract was filtered and concentrated at room temperature.

Phytochemical investigation

The prepared extract of *B. variegata* stem bark was qualitatively tested for the presence of chemical constituents or phytoconstituents like flavonoids, phenolic derivatives, saponins, tannins, triterpenoids, carbohydrates and phytosterols by adapting standard methods[19]. These were identified by characteristic colour changes using standard procedures[20].

Experimental animals

Albino Wistar rats of either sex weighing between 200-250 g were used in the experiment. The protocol for the animal experiment was approved (CPCSEA/ IAEC/ACP/2017/17) from the Institutional Animal Ethical Committee of Anand College of Pharmacy. Animals were housed under constant conditions (temperature 25±2 °C, humidity 40-60% with 12:12 h (light:dark cycle). During maintenance, the animals received a diet of standard food pellets and water *ad libitum*. The rats were anaesthetized prior to and during inflection of the experimental wounds. The surgical interventions were carried out under sterile conditions by the administration of pentobarbitone sodium 25 mg/kg of body weight (i.p.) and were secured in normal position[14]. After inflection of wound, animals were divided into three groups of six animals each. The group I was considered as the positive control and treated with simple petroleum jelly, the group II served as reference standard group and treated with betadine ointment, group III as formulation group treated with developed herbomineral formulation as a hydrophilic ointment (O/W emulsion base containing drug extract and prawal bhasma) twice in a day for seven days.

Preparation of herbomineral formulation

All ingredients were weighed and in a beaker, melted the stearyl alcohol, white petrolatum on a hot plate and the mixture was heated up to 70 °C in a beaker. In the second beaker, remaining ingredients were dissolved in water and was heated up to 70 °C. The oleaginous phase was added slowly to the aqueous phase with constant stirring until it congeals and prawal bhasma was added to the ointment[21]. The formula for herbomineral formulation (Hydrophilic Ointment) is mentioned in Table 1.

Evaluation of herbomineral formulation[22,23]

Evaluation parameters of herbomineral formulation are given in Table 2.

pH

The pH value of herbomineral formulation was determined by Digital pH meter, Systronic, Ahmedabad. The measurement was performed at 1, 15, 30, 45 days after preparation to detect any pH changes with time.

Table 1 — Formula for herbomineral formulation (Hydrophilic ointment)

Ingredients	Quantity in g/mL
Aqueous extract (powder)	1.5 g
Prawal bhasma	0.5 g
Propylene glycol	3.0 mL
Stearyl alcohol	6.25 g
White petrolatum	6.25 g
Purified water	7.75 mL

Table 2 — Evaluation parameters of herbomineral formulation

Evaluation parameters	Observations
Colour	Light skin
Odour	Sweet characteristic
pH	6.8
Spreadability	21 s
Diffusion study	0.78 cm
Skin and Eye irritation tests	No skin irritation was observed

Appearance and homogeneity

The developed formulation was tested for physical appearance and homogeneity by visual observation.

Diffusion study[22]

The diffusion study was carried out by preparing agar nutrient medium of known concentration. It was poured into a petri-dish and allowed to set. A hole was bored at the centre of the petri-dish and the prepared formulation was placed in it. The time taken for the ointment to get diffused was noted.

Spreadability study[23]

The spreadability was expressed in terms of times in seconds taken by two slides to slip off from ointment placed in between the slides under the direction of a certain load. Spreadability was calculated by using the formula:

$$S = M \times L \div T$$

Where, S= spreadability, M=weight tied to upper slide, L=length of slides, and T= time taken to separate the slide.

Skin irritation test[24]

Healthy albino rabbits were selected and were shaved on the dorsal side area of about 500 cm^2 without making any cut to the dorsal skin. The control group (Group I), group II and group III animals were applied simple petroleum jelly, betadine ointment and herbomineral formulation respectively. About 1 g of ointment was applied daily for 30 days, the skin was observed daily and compared with control and standard groups.

Eye Irritation test[24]

The albino rabbit was selected and herbomineral formulation was applied in the conjunctiva sac of the rabbit's right eye. The left eye was kept as control. The animals were observed for lacrimation, redness, oedema, swelling and pupil size. The test substances were applied daily for 7 days and the animals were observed for 15 days.

Stability studies[22,24]

Stability studies were performed for the wound healing ointment for 45 days by keeping the product at 12 ^oC, room temperature (25 ^oC) and 45 ^oC . On storage, the product was examined physically[21] at an interval of 15 days to assess colour, odour, pH, spreadability and diffusion study.

Assessment of pharmacological activity

Acute dermal toxicity

The acute dermal toxicity was performed according to the OECD guidelines (OECD 410), a limit test of 2000 mg/kg did not show any sign of lethality and the gross behaviour of animal was normal and no signs of dermal toxicity were observed[25].

Creation of excision wound and wound closure area measurement

In the excision wound model, an impression was made on the dorsal thoracic region 1 cm away from the vertebral column and 5 cm away from the ear of the anaesthetized rat. The skin was excised to the full thickness to obtain a wound area of about 500 mm^2. The wound was left undressed to open environment[26,27]. The parameters studied were percentage closure of excision wound and epithelialization time and recorded[28].

Creation of incision wound and tensile strength measurement

In incision wound model[29], two Para vertebral straight incision of 6 cm was made through the entire thickness of the skin on either side of the vertebral column with the help of surgical blade on anaesthetized animals. After complete homeostasis, the wounds were closed using interrupted sutures at equidistance points about 1 cm apart, using four zero silk thread and surgical curved needle. Removal of sutures was done on 8^{th} post wounding day and tensile strength of the healed wound was measured on 10^{th} post wounding day by continuous water flow techniques of Lee[30].

Statistical analysis

The experimental results were expressed as mean±SEM of six animals. Analysis of variance was performed by one way ANOVA followed by Dunnet's test[31]. A probability value less than (P <0.001) was considered statistically significant.

Results and Discussion

The dried powdered of stem bark of *B. variegata* extracted with water. The yield of the concentrated crude extracts was 9.89%. Herbomineral formulation

Table 3 — Effects of topical application of herbomineral formulation on wound healing in rats

Name of groups	Tissue breaking strength (g)	50% Wound contraction in days	Periods of epithelialization in days
Control group	398±0.1	4.0±0.90	8.0±0.36
Standard group	468±0.94*	2.8±0.98*	4.0±0.92*
Formulation	508±0.68*	3.5±0.17*	6.0±0.36*

All values are presented as mean±SEM (n=6), *P <0.001 vs positive control by Dunnet's test.

was prepared in ointment form using herbs and mineral. Composition of the formulation is shown in Table 1. The physicochemical properties of ointment were shown in Table 2. The formulated ointment did not show any skin and eye irritation confirming that they are reasonably safe. The stability study showed no change in colour, odour, spreadability, pH and diffusion study of the formulation after keeping it at various temperatures for 45 days at an interval of 15 days. The present study confirmed the potential value of this developed herbomineral formulation as a wound healing product.

Wound healing comprises of different phases such as contraction, granulation, epithelialization and collagenation[32]. In excision wound model, the data reveals that at the 4th days of treated with formulation 50% wound contraction was observed and complete epithelialization was observed at 6th days which was significant (P <0.001) and nearly comparable with a standard group.

In the incision wound model, developed herbomineral formulation showed a significant increase in the skin breaking strength compared to control and reference standard groups. The wound contraction and tensile strength of experimental ointment under test were nearly comparable with the standard group. The results of herbomineral formulation on the incision and excision wound model are mentioned in Table 3.

A wound may be defined as the loss or rupture of the cellular, anatomical or functional continuity of living tissue[27]. Wound healing is the process of repair that follows injury to the skin and other soft tissues. After an injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production and letter epithelial tissue is regenerated. Wound contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage[25]. Healing of skin wounds is a complex process which ultimately leads to the restoration of the injured skin. The process of wound healing is promoted by various natural products which have been reported and used in Ayurveda, Siddha and Unani systems of medicines. These either promote direct wound repair or exhibit related properties like anti-microbial, analgesic and anti-inflammatory properties which are beneficial in overall wound care[27].

The extract was subjected to the qualitative method of the preliminary phytochemical analysis showed the presence of flavonoids, phenolic derivatives, saponins, tannins, triterpenoids, and carbohydrates. Flavonoids and tannins which are known to promote wound healing process mainly by their astringent, antimicrobial properties[8] and antioxidant potential[33,34]. The presence of flavonoids, tannins and triterpenoids may be responsible for its wound healing activity.

Besides, triterpenoids can increase collagen content, which is one of the factors promoting wound healing. Moreover, the free radicle scavenging activity of flavonoids and triterpenoids can be attributed to wound healing effects. Both these class of phytoconstituents are known to reduce lipid peroxidation, not only by preventing or slowing the onset of cell necrosis, but also improving vascularity. Lipid peroxide is a significant process in several types of injuries like burns, infected wound, skin ulcer etc. Therefore any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibres, by increase the circulation, preventing the cell damage and promoting the DNA synthesis[29]. It is reported that antioxidants, such as vitamin C and vitamin E have been shown to promote wound contraction and epithelization[12]. It is found that there was an increase in the wound breaking and granuloma breaking strength after the applied of the developed formulation. The developed herbomineral formulation showed significant wound healing activity and even comparable with the standard drug.

Conclusion

This investigation was an attempt to study the wound healing effects of herbomineral formulation prepared from bark of *B. variegata* extract and another excipient has wound-healing effects. The preliminary phytochemical investigation of the stem barks showed the presence of flavonoids, phenolic derivatives,

saponins, tannins, triterpenoids and carbohydrates which may be responsible for wound healing activity which was successfully confirmed after the study. Further studies are needed to understand the molecular mechanism at a deeper level for wound healing effects of herbomineral formulation of *B. variegata*.

Conflict of interest

The authors declare there is no conflict of interest.

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