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Development and Evaluation of a Shata-Dhauta-Ghrita Based Formulation Enriched with Curcumin, α -Bisabolol, and Honey for Wound Healing: An In Vitro Scratch Assay Study

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ABSTRACT: Shata-Dhauta-Ghrita (SDG), a traditional Ayurvedic formulation made by washing clarified butter 100 times in a copper vessel is known historically to have skin rejuvenating and wound healing properties. In this study a novel topical formulation containing the active ingredients curcumin, α -bisabolol, honey, and stabilizing excipients were developed and evaluated in terms of physicochemical properties and in vitro wound healing efficacy. The formulation showed favorable organoleptic characteristics, consistency (smooth), homogeneity (uniform), pH compatibility with the skin (6.24-6.35), spreadability and stability in centrifugation, indicating that it could be suitable for dermal administration. Wound healing activity was determined by using in vitro scratch assay for L929 fibroblast cell lines. Results showed that both test formulations significantly improved fibroblast migration and wound closure compared with the untreated control (2.49% closure). The optimized batch resulted in closure of 72.42% which was better than the basic formulation (61.59%) and slightly less than the standard (silver sulfadiazine, 87.53%). Enhanced efficacy of the optimized batch was attributed to the joint action of curcumin, α -bisabolol and honey that exhibit antioxidant, anti-inflammatory and tissue regenerator effects. These results provide evidence for the potential of SDG-based formulations as promising wound healing agents that have less cytotoxicity concerns than conventional antiseptics. Furthermore, in vivo and clinical studies are warranted to determine safety, efficacy, and therapeutic applicability in wound management care.

1. INTRODUCTION

Shata-Dhauta-Ghrita (SDG), a traditional Ayurvedic formulation made by washing clarified butter 100 times in a copper vessel is known historically to have skin rejuvenating and wound healing properties.[1] The repeated washing process is believed to change the physicochemical properties of ghee, making it more bioavailable and easier for topical applications [2].

Curcuma longa is a perennial herbaceous plant distributed throughout India. [3] The *Curcuma longa*'s rhizomes comprises of curcuminoids (2-6%), essential oils, resins, and other constituents.[4] Traditional applications of the rhizome includes culinary use as a spice alongside therapeutic applications for anti-inflammatory, anthelmintic and wound healing purposes.[5] Chemically, curcumin is defined as 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, also known as ferroylmethane. This compound is a major natural polyphenolic constituent found in the rhizomes of *Curcuma longa* (turmeric).

Throughout Asian countries, *Curcuma longa* has been used as a conventional therapeutic plant owing to its beneficial medicinal characteristics, encompassing antioxidant, anti-inflammatory, anti-mutagenic, antibacterial and anticancer properties.[6] Clinical investigations have validated that curcumin presents no toxicity concerns for human use.[7]

The process of wound healing is a meticulous coordination of biological mechanism that reestablishes both structural and functional integrity of damaged tissue. This process undergoes through four phases: haemostasis, inflammation, proliferation, and remodelling [8]. Among these phases, the proliferation phase is very important and difficult as fibroblasts migrate into the wound bed then deposit collagen and promote re-epithelialization. Any disturbance in these phases may delay healing. [8,9]

Conventional wound management therapies include antiseptics and antimicrobial agents such as povidone iodine, which have good effect in preventing infection. However, some researchers suggest that possible cytotoxic effects of prolonged use of povidone-iodine on fibroblast and keratinocytes lead to delayed wound closure [10,11]. This highlights the necessity for alternative or complementary therapeutic agents designed to protect wounds from microbial contamination as well as to improve the naturally occurring wound healing process by promoting healing by fibroblast migration and tissue regeneration.

The study is being done to study the wound repair efficacy of an innovative formulation based on in vitro scratch assay methodology using L929 fibroblast cell lines. Its effect on cell migration and wound closure was compared with the standard drug; the control was untreated cells. The study will provide validation of the wound healing efficacy of this formulation and its ability as a promising alternative to traditional wound care therapeutic approaches.

2. MATERIALS AND METHODS

2.1 Materials

Curcumin is gifted by Bio-gen Extracts Pvt. Ltd., Bangalore, India, and tert-butyl hydroxy quinone (TBHQ) was received from Aarnee International Pvt. Ltd., Ahmedabad, India. Methyl paraben and propyl paraben were purchased from Modern Science, Nashik, India. SDG (Shata Dhauta Ghrita) was lab made. α -Bisabolol was obtained from Sigma Aldrich, Mumbai, India, and honey was obtained from Dabur India Ltd., Baddi, Himachal Pradesh, India. Cow ghee was obtained from Nearby farms, Vadner Bhairav, Chandwad, Nashik. The Copper vessels were purchased from local market, Nashik. All other chemicals and reagents used were of analytical grade.

2.2 Preparation of SDG and Formulation

A traditional Ayurvedic technique is used to prepare Shata Dhauta Ghrita (SDG). a copper vessel is cleaned and rinsed with distilled water to remove impurities. In the copper vessel 50 g of previously standardised cow ghee was measured. 50 mL of distilled water was added to this and manual mixing of the mixture was done using a copper agitator. The mixture was thoroughly mixed and allowed to settle and then the water was carefully decanted to avoid any ghee being lost. After washing of ghee, a fresh amount of 50 mL distilled water was added and trituration procedure was performed again. To reach SDG, the washing, triturating, settling and decanting cycle was repeated 100 times.[12] The formulation was prepared by mixing Shata Dhauta Ghrita (SDG), Curcumin (g), α -Bisabolol (μ L), tert-butyl hydroxyquinone (g), butylated hydroxytoluene (g), disodium EDTA (g), methyl paraben (g), propyl paraben (g), and honey (g). Shat Dhauta Ghrita, obtained traditionally by washing clarified cow's ghee, a hundred times with purified water, was used as the base. Curcumin included for its antioxidant and anti-inflammatory effects, α -Bisabolol for antimicrobial and skin permeation properties, tert-butyl hydroxyquinone and butylated hydroxytoluene as stabilizing antioxidants, disodium ethylenediamine (EDA) as chelating agent, methyl (paraben) and propyl (paraben) as preservatives, and honey for its humectant, antibacterial, wound healing properties and act as a carrier for drug. These ingredients enhanced the traditional base to stable the wound healing formulation.

Table 1: Formulation development

Ingredients	F0	Optimized batch
Curcumin(g)	0.2	0.2
α -Bisabolol(μ L)	00	54
Shata Dhauta Ghrita(SDG)(g)	7.79	7.736

tert- butyl hydroxy quinone(g)	0.002	0.002
Butylated hydroxytoluene	0.002	0.002
Disodium EDTA	0.002	0.002
Methyl paraben(g)	0.001	0.001
Propyl paraben(g)	0.003	0.003
Honey(g)	2	2
Total Weight (g)	10	10

2.3 Evaluation of Formulation

The prepared cream is evaluated for the physicochemical and stability parameters. The color and odor of the formulation is observed visually and organoleptically to check uniformity and patient acceptability[13]. Consistency is examined manually to check smoothness and ease of application. The pH of the formulation was determined by dissolving 1 g of cream in 100 mL of distilled water and recording the value using a digital pH meter at room temperature [14]. Spreadability was assessed by placing 1 g of formulation between two glass slides and applying weight on the upper slide and recording the time required for the slides to separate under a specific load[15]. Homogeneity was checked visually for the presence of aggregates, lumps, and phase separation. Washability was tested by applying the cream to the skin surface and washing with tap water to check ease of removal. The centrifugation test were done by centrifuging the formulation at 5000 rpm for 30 minutes to check phase separation. The viscosity is measured using a Brookfield viscometer at room temperature[16].

2.4 In vitro Wound healing activity

The wound healing activity of the formulation is checked using in vitro cell migration experiments with L929 cells according to a previous established methodology. Cells is seeded at a concentration of 2×10^5 cells/mL in 6-well culture dishes and incubated overnight. a scratch is created using a sterile pipette tip after rinsing with Dulbecco's Phosphate Buffered Saline (DPBS). Detached cells and cellular debris is remove through DPBS rinsing steps. Similarly, cells are treated with 100 μ L of Sample F0 and Optimized batch, alongwith control and standard preparations, following 24-hour incubation. Silversuladiazine used as the reference standard. Non-treated cells considered as the control group. Cellular migration pattern and morphological alteration is documented using an inverted microscope fitted with a digital imaging system. All experiments are conducted three times(n = 3). Scratch width measurements and wound closure evaluation at decided time points (48hrs) are analyzed using SAGLO software. [17].

3. RESULTS AND DISCUSSION

3.1 Evaluation of formulation

The prepared Shata Dhauta Ghrita (SDG) based cream enriched with curcumin, a-bisabolol, honey and stabilizers is evaluated for its physicochemical and stability parameters.

Table 2: Evaluation of Formulation

Sr. No.	Characteristics	F0	Optimized batch
1	Color	Bright yellow	Bright yellow
2	Odor	Characteristic	Characteristic
3	Homogeneity	Good	Good
4	Washability	Good	Good
5	Centrifugation	NO phase separation	NO phase separation
6	pH	6.35 ± 0.05	6.24 ± 0.035
7	Viscosity	2257 ± 0.58 mPa·s	2127 ± 0.6 mPa·s
8	Spreadability	15.78 ± 0.08 g·cm/s	20 ± 0.42 g·cm/s

The color and odor of the cream were found to be uniform, with no phase separation, indicating good physical stability and patient acceptability. The consistency was smooth and semi-solid, implying ease of application on the skin surface.

The pH of the cream is checked and found within the skin compatible range (6.35-6.24), The spreadability study showed that the cream spread easily under a small applied load, confirming good patient compliance, as well as uniform application over the skin.

The formulation showed an excellent homogeneity with no visible aggregates and lumps, which indicate good mixing of ingredients and uniform distribution. Washability was satisfactory as the cream was easily removed with water.

The centrifugation test showed no signs of phase separation and instability at 5000 rpm for 30 minutes, indicating good physical stability under stress conditions. The viscosity measurement showed that the cream had enough viscosity to have uniform application and holding to the skin surface yet be spreadable. A balance between viscosity and spreadability is very important for optimum topical performance.

This evaluation confirmed that the SDG-based formulation satisfied the requirements for application in topical wound healing. The addition of curcumin, α -Bisabolol and honey Increased its therapeutic potential and stabilizers and preservatives contributed to its physical stability and shelf life. These results support its furthermore investigation in biological assays and in vivo wound healing models.

3.2 In Vitro Scratch Assay

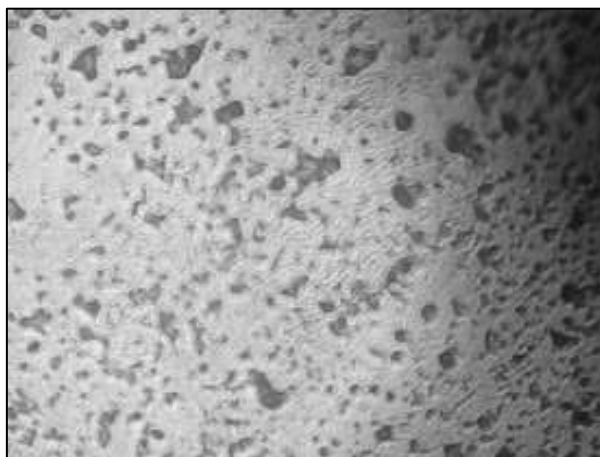


Fig. 1: Normal Morphology of L929



Fig. 2: Standard Wound scratch

Fig. 3: Standard after 48 Hours



Fig. 4: Control Wound Scratch



Fig. 5: Control after 48 Hours



Fig. 6: F0 wound scratch



Fig. 7: F0 after 48 Hours F0



Fig. 8: Optimized batch wound scratch



Fig. 9: Optimized batch after 48 Hours F0

Table 3: Percentage (%) of cells migrated towards the wound and involved in wound closure.

Sample	Reading 0 hours (mm)	Mean	Reading 48 Hours (mm)	Mean	Percentage of cell Reduction
Control	10.00 09.98 10.05	10.01 $\pm$ 0.04	9.79 9.80 9.70	9.76 $\pm$ 0.06	
Standard (5 μ g/mL)	10.00 09.98 10.05	10.01 $\pm$ 0.04	1.22 1.20 1.24	1.22 $\pm$ 0.02	87.53
Sample F0	10.00 09.98 10.05	10.01 $\pm$ 0.04	3.90 3.80 3.60	3.76 $\pm$ 0.15	61.59
Optimized batch	10.00 09.98 10.05	10.01 $\pm$ 0.04	2.60 2.70 2.80	2.70 $\pm$ 0.10	72.42

The wound healing activity of the developed formulations is checked using a scratch test on L929 cells. The initial scratch width is measured at 0 hours. Shows consistency among all groups, which is 10.01 mm. This consistency validates that the experimental parameters are uniform. After 48 hours of incubation a notable decrease in scratch width is observed in both the experimental samples compared to the control group. The control group shows a reduction in wound width from 10.01 mm to 9.76 mm, which corresponds to only 2.49% cell migration. The standard group, which uses 5 μ g/mL shows wound closure. The mean scratch width is reduced to 1.22 mm, which corresponds to 87.53% closure. This result is consistent with its established wound healing efficacy. Among the test formulations the Optimized batch shows activity. It reduces the scratch width to 2.70 mm. Achieves 72.42% closure.

Sample F0 reduces the scratch width to 3.76 mm, which corresponds to 61.59% closure. These results indicate that both formulations promote migration and wound closure over time. The Optimized batch shows efficacy than Sample F0. The enhanced activity of the Optimized batch may be due, to the combined effects of bioactive components. These components include curcumin, α -bisabolol and honey which are known for their -inflammatory, antioxidant and tissue-regenerative properties. Compared to the Standard the formulations have reduced activity. However, they show promising wound healing potential without the cytotoxicity often associated with agents. The scratch assay results provide preliminary data to support the wound healing efficacy of the SDG-based formulations. These formulations should be further studied in in vivo wound models.

4. CONCLUSION

The present study is carried out for the development and evaluation of novel Shata Dhauta Ghrita(SDG) based wound healing formulation enriched with curcumin, alpha bisabolol, honey and stabilizing excipients. The formulation showed desirable physicochemical characteristics, suitable pH, smooth consistency, good spreadability, homogeneity and stability against centrifugation, which is appropriate for topical application. In vitro wound healing evaluation using the scratch assay on L929 fibroblast cells showed that both formulations (F0 and Optimized batch) improved cell migration and wound closure compare to the untreated control. Among these, optimized batch showed superior activity (72.42% closure) over F0 (61.59% closure), highlighting the synergistic role of α -bisabolol, curcumin and honey in improving fibroblast migration and tissue repair. Although the standard drug achieved higher wound closure (87.53%), the SDG-based formulations showed promising wound healing property while minimizing the cytotoxic effects associated with synthetic antiseptics. This study showed that the combination of traditional Ayurvedic base (SDG) with modern bioactive agents can provide a safe and effective alternative for wound care. Furthermore, in vivo studies and clinical validations is required to confirm the therapeutic efficacy, safety and long-term stability of this formulation for potential application in dermatological and wound care therapies.

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AUTHOR CONTRIBUTIONS

Shubham S. Pachorkar: Investigation, Methodology, Laboratory Analysis, Data Curation, Formal Analysis Writing—Review & Editing.

Ritesh R. Karmarkar: Conceptualization, Supervision, Review & Editing.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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