Formulation and Characterization of Poly Herbal Cream with Wound Healing Activity

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Summary

The herbal cream formulations were designed by using ethenolic extracts of *Argemone mexicana, Cassia tora, Evolvulus alsinoides, Ocimum centum, Curcumis sativus*. The ethanolic extracts of herbs were incorporated in a cream base i.e. prepared by a phase inversion emulsification technique. Therefore, an attempt has been made in this study to combine these herbs in an herbal cream to determine the synergistic effects of *Argemone mexicana* L. for wound healing activity on excision wound model.

Physicochemical assessments, microbiological testing, skin sensitivity test using open patch were performed for all formulations according to the Indian Standard Bureau. Excision wound measuring about 177 mm2 was created on the albino rats placed in groups (n=5) and the ointment applied topically on the wounded area which was measured at intervals of 3 days until epithelialization and complete wound closure.

The formulations show all the physicochemical parameters in the range of 5.84-6.23 pH, 6.0-6.29±1.0 acid values, 15.1-18.9±1.3 Saponification value, 95-97±0.1 % spreadability, 95.9-98.1±1.3% thermal stability and 23-36±4 CFU g^{-1} microbial count.

Application of the herbal cream formulation containing the *Argemone mexicana* L. extract (1.0 g/100g ointment) produced the highest rate of wound healing, reducing the epithelialization period to 14.9 days compared to the control formulation treatment with epithelialization period of 18.3 days.

Therefore, it can be concluded that the formulating *A. maxicana* L. extracts (0.1g/100g of cream base) into an herbal cream is most effective and safer usage in wound repairs process

Keywords- Emulsification technique, Indian Standard Bureau, Excision wound, *Argemone mexicana*

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Introduction

Healing of wounds is an important biological process involving tissue repairs and regeneration. It involves the activity of an intricate network of blood cells, cytokines, and growth factors which ultimately leads to the restoration to normal condition of the injured skin or tissue[1].
The aim of wound care is to promote wound healing in the shortest time possible, with minimal pain, discomfort, and scarring to the patient and must occur in a physiologic environment conducive to tissue repair and regeneration[2]. Wound healing processes are known to be influenced by among other factors by infections, nutritional status, drugs and hormones, type and sites of wound, and wasting diseases like diabetes[3].
In fokelore medicine, medicinal plants have been used widely in facilitating wound healing with high degree of successes. This has inspired many researches which are aimed at validating the claims and discovering mechanisms which possibly explains the potentials of these herbs on wound repair processes. The selected herbs described in the present investigation have been utilized medicinally in traditional Indian and Chinese medicine systems to treat various skin ailments like wounds, psoriasis and inflamed joints. These herbs have been selected on the basis of a traditional system, ethanobotanical survey and scientific justification with modern uses of *Argemone mexicana* L. *Cassia tora* L., *Evolvulus alsinoides* L., *Ocimum sanctum* L, *Curcumis sativus* L. Some of these plants either possess pro-wounding healing activities or exhibit antimicrobial and other related properties which are beneficial in overall wound care. Therefore, an attempt has been made in this study to combine these herbs in an herbal cream to determine the synergistic effects for wound healing activity on excision wound model.
The application of juice of seeds of Pili Kateri or *Argemone mexicana* L. (papaveraceae), in case of itching in rural and tribal area of Patalkot valley in Chindwara and Rewa district of Madhya Pradesh. It cures leprosy, skin diseases, inflammations and cutaneous & subcutaneous parasitic infection[4]. *Cassia tora* L. (Leguminosae) seeds and leaves are used in traditional systems for curing skin diseases like psoriasis, leprosy and other cutaneous disorders. Aqueous extracts of plants are used in tribal states of Madhya Pradesh and Chattisgarh in India. They also possess anti-fungal, anti-microbial, anti-oxidant and anti-noiceptive activity[5]. Shankhpushpi, *Evolvulus alsinoides* L (convolvulaceae) the juice of plant is used in treatment of scabies. Different parts of plant stem, roots, leaves, flower and seeds of *Ocimum sanctum* L (Labiatae), holy basil or Tulsi are known to possess therapeutic potential and have been used by traditional medicinal practitioners, as expectorants, anti-inflammatory, anti-bacterial and chemo protective activity. Juice of cucumber, *Cucumis sativus* L (cucurbitaceae) has countless health benefits as well as cosmetics properties. It is an excellent source of vitamin C, folic acids and potassium. It has proved effective in skin eruption and improves skin complexions and skin conditioning[6].
Herbal Cosmetics and creams have been reintroduced into the market in recent years, due to consumer's demand for natural herbal product with more emolliency, less side effects and better safety properties. In this study, different herbal extracts were formulated into herbal creams to measure changes in the physicochemical and wound healing properties of the formulation.

Materials and Method

The plant material leaves of *E. alsinoides* L and *Ocimum sanctum* L., seeds of *C. tora* L. *and Argemone maxicana* L, fruits of *Curcumis sativus* L, oil of *Olea europia, Sesamum indicum,* and honey were produced from a local authentic herbal distributor of Bhopal, Madhya Pradesh. All

plant materials were identified from the herbarium, Department of Pharmacognosy, VNS Institute of Pharmacy, Bhopal, MP, India and tested for percent purity (99.7%) by microscopic methods evaluating characteristics of leaf and seeds. Other quality ingredients were of analytical grade and purchased from Loba Chem, Mumbai, India.

Instruments

In this study, a pH meter (Equip-tronics, India), Brookfield viscometer (RVDVE-330, Brookfield Engineering Laboroatories Inc., Middle-boro, MA, USA), colony counter (M-37, Rolex, India), overhead stirrer (Remi, India) were used.

Preparation of Herbal extracts

The hydro alcoholic extracts of herbs were used in this study because of their acceptability and compatibility with the skin's nature and economy[7]. Plant materials were cleaned to remove the dirt and extra genus material and were ground to a coarse powder separately (particle size~ 0.3 mm) using a laboratory mill and the coarse powders were passed through a sieve number 20. Accurately weighed 250 gm. coarse seed powders of each *C. tora* L. & *A. mexicana* L. were extracted with a hydro alcoholic mixture (500 mL, 60:40 v/v ethanol: water) at 45-55°C for 24 h by a continuous hot extraction, exhaustion of drug using a soxhlet apparatus. Dried and minced leaves powder of *E. alsinoides* L and *Ocimum sanctum* L and fruits of *Cucumis sativus* L (250 gm of each) were extracted with a hydro alcoholic mixture (500 mL, 60:40 v/v ethanol: water) using a cold maceration process according to Indian Pharmacopoeia process for 8 h to make concentrated extract. The obtained extracts were evaporated under reduced pressure (AU 5 psi) at 50 ± 5°C for 5-15 min and concentrated extracts were dried to obtain actual yield.

Preparation of base cream (F 1)

Cream base F1 was prepared by using a phase inversion technique[8]. The internal phase was prepared in the vessels in which the emulsification was carried out (according to the composition depicted in Table 1). Initially, natural oil and other ingredients (olive oil, sesame oil, cetyl alcohol, stearic acid, sorbitanstearate, sorbitan monooleate, propylene glycol, glycerin and honey) were mixed using an overhead stirrer at 200 ± 25 r.p.m. at 60-70°C on a hot plate. After a complete melting and homogenous mixing, a 50 mL portion of distilled water (70± 2°C) was added at a rate of 30 mL min^{-1} at increased speed (250 ± 25 r.p.m.). When the temperature of the internal phase was reduced at 50°C, phase inversion took place and the solution became viscous; the remaining aqueous phase containing propylene glycol was then added. When the temperature was reduced 40°C honey (2% w/w) was added to this mixture.

Preparation of Herbal cream

Different concentrations, i.e. 0.5-1.5% w/w (extracts in propylene glycol), were prepared and incorporated into the base cream formula summarized in Table 1. F1, which contains no extracts, was used as the control base cream.

Evaluation studies

Several physiochemical parameters were measured for each prepared cream formulation (F1-F4) according to the Indian Standard Bureau methods which provided information regarding formula stability and skin compatibility. The pH and thermal stability of the prepared formulation were determined according to Indian Standard guidelines (IS: 6608-1978B-1 IS: 6608-1978B-2 IS:

6608-1978B-3)[9]. Acid values and saponification values were determined according to methods discussed by Lachman[10]. The viscosity was using a Brookfield viscometer (at 3 r.p.m.) and the spreadability and layer thickness was evaluated according to Multimer[11]. Spreadability refers to the % area covered by a fixed amount of cream sample after the uniform spread of sample and layer thickness refers to the thickness of the layer (in microns). All evaluations were carried out in triplicate presented as ± SD. Phytochemical studies were carried out for all the herbal formulations.

Microbial examination

Microbial contamination of prepared cream formulations was tested according to Indian Standards methods for skin creams and cosmetics IS 11648; 1999[12]. The total viable counts, i.e. total bacterial yeast and mould counts were recorded by using a colony counter. A skin sensitivity study using an open patch test design was conducted to make sure that the prepared formulations did not cause any adverse affects[13].

Wound healing studies

Animals

White albino rats (200-300 g) were obtained from the animal house of the Department of pharmacy, VNS Institute of Pharmacy, Bhopal, MP and placed in five groups (n = 5) for the studies. All animals had free access to pellet food and water *ad libitum*. Temperature was maintained at 23± 1^{o}C. The experimental protocol was approved by the Institutional Animal Ethical Committee and the animal regulatory body of the Indian Government (Registration no: CPCSEA/778/03).

Study Protocol

A round seal of 15 mm diameter was impressed on the sides of the central trunk depilated and sterilized with ethanol. Excision wound was inflicted on the rats according to methods described by Morton and Malone under light ether anaesthesia[14]. Full skin thickness was excised from the marked area to get a wound measuring about 177 mm2. After achieving complete homeostasis by blotting the wound with cotton swab soaked in warm saline, the animals were placed singly in individual cages. The wounds of the animals were treated topically depending on the group. Group 1 served as the control and was treated with the control herbal cream formulation (F1) while group 2, 3 and 4 were treated with the herbal formulation F2, F3 and F4 containing plant extract per 100 g of the cream base. Group 5 was treated with the standard gentamycin ointment. The wound area was measured with a translucent paper and thereafter estimated on a 1 mm2 graph sheet every 3 days until epithelialization and complete wound closure was recorded. Wound contraction was calculated as a percentage of the original wound size[15].

Statistical analysis

The significance of differences between the means was analyzed by one way analysis of variance (ANOVA) followed by student's t- test. The *P*-value<0.05 was considered as significant.

Results and discussion

Base cream (F1) and herbal extract containing cream (F2-F4) were prepared with natural ingredients and optimized for their stability at room temperature and physical characteristics, i.e. erythema score, skin pH range and microbial count (Table I and II).

Higher acid and saponification values, less thermal stability, more microbial count and less spreadibility resulted in cracking and phase separation of formulation[16]. Based on physicochemical parameters shown in Table II, the pH, erythema score, thermal stability, viscosity and spreadibility of base cream F1 was found to be 5.84, 0 erythema score, 98.1±1.3, 6105±79, 97±0.1, respectively (Table II). This formulation showed uniform mixing, desired consistency and no sign of bleeding at room temperature. The extract concentration in formulation F2-F4 was selected after optimization of individual extracts based on literature and marketed formulation. The pH must be controlled between 5.8 and 6.2 for all formulations. It was observed that formulation F2 and F4 had higher free acid values which may have caused more irritation to the skin following application. The saponification value of the formulations may influence the formulation stability, pH and cleansing properties. Formulation F4 had low thermal stability (95.9%) which may be due to higher saponification value 18.9±1.3 and pH 6.23. The viscosity of all formulations was between 6105 and 6298 cps (Table II). Spreadbility and layer thickness were found to be in the range between 95-97% and 28.73 -32.43 μm for formulations F2-F4.

Thermal stability, viscosity and spreadibility are the prime parameters which affect the formulation acceptability during storage and handling[17]. Based on the results of physicochemical and stability studies shown in table II, formulation F2 and F3 were the most stable formulation than F4 probably due to the highest concentration of extract.

Microbial evaluation was performed for all formulations including control base cream formulation. The average number of colonies per gram of sample in nutrient agar medium was calculated. It was observed that the formulation F4 had the more microbial count than in the control base cream formulation F1. This Formulation F4 showed the highest susceptibility to microbial growth might be because of the incompatibility of the higher extract content with the base cream.

When performed skin sensitivity test using an open patch test evaluation, an erythema score of 0 indicates the no irritation (no redness) and score of 1 indicates slight redness by visual observation. The formulation F1-F4 showed the erythema score 0 and were found to be non-irritating to the skin.

Medicinal plants have been reported to be very beneficial in wound care, promoting the rate of wound healing with minimal pain, discomfort, and scarring to the patient[18].
In this study, topical application of the base cream F1 and herbal formulation F2-F4 incorporated on the excision wound in rats caused a significantly ($P<0.05$) higher rate of wound healing and reduced the epithelialization period. Application of the herbal cream formulation containing the *Argemone mexicana* extract (1.0 g/100g ointment) produced the highest rate of wound healing, reducing the epithelialization period to 14.9 days compared to the control formulation treatment with epithelialization period of 18.3 days (Table III). Wound healing is a natural process of regenerating dermal and epidermal tissues[19]. These processes have been categorized into phases which include the inflammatory, proliferative, and the remodeling phases[20].

In the inflammatory phase, bacteria and debris are phagocytosed, removed and cytokines & mediators are released that cause the migration and division of cells involved in the proliferative phase. Angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction occur in the proliferative phase. The wound is eventually closed by a combination of all these and by the process of wound contracture. During wound contraction, the wound is made smaller by the action of myofibroblasts, which establish a grip on the wound edges and contract themselves using a mechanism similar to that in smooth muscle cells. In the maturation and remodeling phase, collagen is remodeled and realigned along tension lines and cells that are no longer needed are removed by apoptosis[21]. Although, we have not determined the stage of wound repair process that is affected, it is possible that the *A. mexicana* L. based herbal ointment has significant influence on one or some of the stages resulting in faster rate of wound closure when compared to the untreated group.

Phytochemical analysis of the ethanolic extract of selected herbs used in formulating the herbal cream showed the presence of glycosides, alkaloids, saponins, tannins, flavonoids, resins, sterols, terpenoids, and carbohydrates.

Conclusion

These beneficial effects might be due to synergistic anti-inflammatory and protective properties of herbs with *A. mexicana* L. Therefore, it can be concluded that the formulating *A. mexicana* L. extract (0.1g/100g of cream base) into an herbal cream is most effective and safer usage in wound repairs process

Table-I Herbal Cream composition of formulation

Ingredients	**Incorporated in %w/w**			
	F1 (Base Cream)	**F2**	**F3**	**F4**
Olea europia	7.5	7.5	7.5	7.5
Sesamum indicum	1.5	1.5	1.5	1.5
Cetyl alcohol	3.5	3.5	3.5	3.5
Stearic acid	4.75	4.75	4.75	4.75
Polysorbate monooleate	1.75	1.75	1.75	1.75
polysorbitone monostearate	0.75	0.75	0.75	0.75
Propylene glycol (4000)	4	4	4	4
Glycerin	3.5	3.5	3.5	3.5
Honey	2	2	2	2
Argemone mexicana	-	0.5	1.0	1.5
Cassia tora	-	0.5	0.5	0.5
Evolvulus alsinoides	-	0.5	0.5	0.5
Ocimum centum	-	0.25	0.25	0.25
Curcumis sativus	-	2.5	2.5	2.5
Distilled water q.s. (100 gm)	q.s.	q.s.	q.s.	q.s.

Table-II Physicochemical Parameter of Herbal cream formulation

Formulation Code	pH	Acid value	Saponification value	Spredability (%)	Thermal Stability (%)	Layer thickness (μm)	Viscosity (Cps.)	Microbial count (CFU g^{-1})	Erythemal score
F1	5.84	6.0 ± 0.6	15.1 ± 1.6	97 ± 0.1	98.1±1.3	30.12 ± 1.6	6105 ± 79	23±4	0
F2	6.12	6.2 ± 0.4	17.3 ± 0.8	96 ± 0.4	96.3±1.8	32.43 ± 2.3	6123 ± 73	31±3	0
F3	6.06	6.1 ± 0.8	17.2 ± 1.2	96 ± 0.9	96.8±1.5	28.73 ± 1.3	6153 ± 94	29±6	0
F4	6.23	6.9 ± 1.0	18.9 ± 1.3	95 ± 0.3	95.9±1.2	29.43 ± 2.4	6298 ± 86	36±4	0

All the values are represented as mean ± SD (n=3), shows significant differences between the herbal formulation and base cream (P>0.05), CFU, Colony forming units

Table III: The effect of herbal cream on excision wound healing in rats.

Treatment Group	Wound area in mm^2 (percentage wound contraction in parenthesis)							Epitheliazation Period (Days)
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	
F1 (Base Cream)	147.43±0.4 (16.71)	95.05±0.32 (46.30)	41.86±0.38 (76.35)	28.28±0.16 (84.02)	14.52±0.78 (91.79)	5.31±0.06 (97.00)	3.90±0.28 (97.89)	18.3±0.84
F2	149.63±0.64 (15.46)	78.57±0.35 (55.61)	41.85±0.06 (76.34)	23.77±1.41 (86.57)	11.35±0.84 (93.57)	4.17±0.32 (97.65)	1.32±0.69 (99.15)	17.8±0.98
F3	153.96±0.29 (13.02)	67.94±0.96 (61.62)	14.52±0.5 (91.79)	6.16±0.08 (96.54)	2.55±0.02 (98.56)	0.20±0.19 (99.89)	0.00±0.00 (100)	14.9±0.71*
F4	143.16±0.7 (19.12)	95.06±1.3 (46.30)	41.87±1.5 (76.43)	22.07±0.9 (87.53)	6.16±1.2 (96.52)	1.77±0.89 (99.00)	0.1±0.04 (99.99)	15.1±0.68*
Gentamycin ointment (1%)	147.44±0.5 (16.71)	78.55±0.56 (55.62)	28.28±1.32 (84.02)	17.35±1.16 (90.20)	1.33±0.64 (99.25)	0.39±0.16 (99.87)	0.00±0.00 (100)	14.1±0.95*

*Significant difference between treatment groups and the base cream ($P \leq 0.05$), n=5

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