Original article

Formulation and physiochemical evaluation of honey-containing gel mask, PVA, and total hydro-alcoholic extract of *Scrophularia striata Boiss* for the healing of minor wound

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Abstract

Objective: One of the aims of medicine is to heal wounds in a shorter time and with fewer complications. In the studies on the effect of *Scrophularia striata Boiss* on the probability of mitigation of infection, reduction of the wound healing period, inflammation and scar remaining from the wound have been shown. Gel mask formulation, enjoying PVA polymer, creates a natural scaffold on wounds, causing the cells to migrate in an organized fashion towards the wound's bed and proliferate. By introducing the plant's extract in this base, its effect on reduction of the healing time and mitigation of the scar remaining from the wound becomes predictable.

Methods: The total plant was extracted through percolation using ethanol 70%. The plant's standardization was done through determination of the total polyphenols' percentage using folin-ciocalteu reagent. The extract was then concentrated and the amount of the dry extract per each 100 g of the plant's powder was calculated. Next, different formulations with different percentages of PVA, ethanol, isopropyl alcohol, propylene glycol, and glycerin and other substances available in the base were prepared and then evaluated in terms of formation of medicinal film, drying time, pH, and other physiochemical parameters. Thereafter, drug diffusion was investigated using Franz cell and drug diffusion kinetics from the base.

Results: The amount of the plant's polyphenols was 25.1% and the amount of the plant's dry extract was 12.5%. The optimal formulation is a clear gel that forms a medicinal film after application of wound and following 10 minutes of use. It contains 7.5% pva, 15% alcohol, 10% isopropyl alcohol, 0.5% xantam, and 3% glycerin. The pH of the formulation was 5.65 and had the stability required in physiochemical evaluations and stability experiments. Drug release was done within 35 min as much as 90%, with the drug's release model following Higuichi model.

Conclusion;The optimal gel mask formulation containing 10% the extract of *Scrophularia striata Boiss* plant indicated a desirable stability in physiochemical evaluation. Considering the synergistic effect of the base and the extract in shortening the wound healing period and mitigation of remaining scar, clinical studies should be conducted.

Keywords: *Scrophularia striata Boiss, gel mask, wound, film-forming gel*

Introduction

A wound can be described as a defect or a break in the skin, resulting from physical or thermal damage or as a result of the presence of an underlying medical or physiological condition (1). One of the objectives of medical science is to treat wounds in a shorter time and with fewer complications (2). There have been numerous attempts to use novel therapeutic methods through which one is able to achieve aims such as acceleration of wound healing, prevention of wound infection, elevation

of the wound bed elasticity, mitigation of remaining scar and prevention of disability of patient (3). Various Herbs (quince seeds, licorice, and chamomile) and chemical drugs (zinc oxide, dexpantol, and phenytoin) are employed in the treatment of wounds (2). Today, having known the undesirable, untoward, and unwanted effects of chemical medications, consumption of herbs has again increased and numerous factories and centers begun their activity in this field (3). With the advancement of medicine, the adverse effects of chemical drugs become well-known more than ever, whereas in contrast application of herbal medicines and compounds grows rapidly. This goes back to the greater adaptability of the human body with the components of nature, which is itself a part of it (4). In the Western regions of the country especially Ilam Province, Scrophularia striata Boiss plant with the local name of Tashneh Dari is used for healing skin wounds, also it uses in traditional medicine, especially in the Ilam province, such as increase ear and eye pains and inflammations, gastrointestinal disorders treatment, Cold treatment, healing cutaneous wounds , healing burns and Hemorrhoid treatment (5).

Various compounds have been separated, purified, and identified from different strains of Scrophularia striata. These compounds include Iridoid, phenyl propanoid, phenolic acid, flavonoid, and saponin (6).

In the studies conducted on the plant it has been shown that effective components of this plant can cause stimulation of collagen development, faster constriction of wounds, angiogenesis, vasodilation, decreased inflammation, bleeding, and wound edema. Therefore, the wound heals within a shorter time and more effectively (2). In various studies it has been proven that the extract of s.striata plant is effective against gram-positive and gram-negative bacteria together with viruses (7) and the plant has anti-inflammatory and antinociceptive effects are due to flavonoids especially quercetin (8).

Honey is one of the compounds used in this formulation. This substance has long been used as wound dressing. Honey develops a humid environment for wound healing with no risk of infection. It also develops the best conditions for the activity of fibroblasts through creating an acidic environment in the wound's bed (9).

Wound dressing is one of the most important medical and pharmaceutical aspects of wound care around the world, which has been witnessing great advances throughout different periods. These dressings have changed from simple compounds such as plants, fat of animals and honey to engineering scaffolds (1).

The formulation proposed in this study is gel mask formulation. This formulation is composed of a group of dressings of film-forming gels. This formulation is applied to the skin in the form of hydrogel and then through evaporation of the solvent, it develops a film on the skin (10). Containing PVA, this formulation develops a dressing in the form of a mask on the wound which plays an important role in the process of wound healing. In this way, the wound is dressed effectively through formation of a protective natural mask and development of a network between the wound edges, and thus it is kept away from access of infective agents (11).

The aim of this study was to formulate a gel mask containing the extract of the Scrophularia striata boiss plant, enjoying antibacterial, anti-inflammatory, and wound healing promoter properties, for healing of small wounds in a shorter time and with the lower degree of remaning scar.

Methods

1. Collection and identification of plant materials:

whole plant with the scientific name of Scrophularia striata Boiss was collected from surroundings of Ilam, iran in spring of 2014 and authenticated by Pharmacognosy Department of Pharmacy School, Isfahan University of Medical Sciences.

2. Preparation of extract:

The plant's were dried under shadow at room temperature Then dried material was powdered and extracted at room temperature according to percolation method using 70% ethanol (12). The collected extracts are then concentrated by a rotary device at 40°C, followed by Bon Mari at the same 40°C for 4-8 h (13).

3. Determination of the amount of dry extract:

In this method, 2 ml of the extract was removed and following the evaporation of its solvent in steam bath, it was placed in an oven at 100-105°C for 3 h to reach a constant weight. Next, it was put in a desiccator for 1 h. Finally, the remaining weight was obtained. This determination was replicated three times (14).

4. Standardization of the obtained extract based on total poly phenols:

In order to determine the total amount of polyphenols, Folin-ciocalteu colorimetry method is used. The basis of this method is recovery of a reagent of tungsten and molybdenum oxides. 20 µl extract 5gr/L was added to 1.58 ml deionized water into test tubes and 100 µl Folin-Ciocalteu reagents were mixed. Then 300 µl of 20% sodium carbonate solution was added to the mixtures. The tubes were mixed, and then were allowed to incubate for 2 h at 20 °C. The absorbance of the resulting blue color was measured by colorimetric at 765 nm. A

calibration curve of Gallic acid (ranging from 50 mg/L to 500mg/L) was prepared (15).

5. The formulation of gel mask:

To develop a suitable gelmask , the formulations were developed with different compounds, within the determined concentration range with different concentration-percentages. according to the formula given in the Table-1 10 different formulations were fabricated and then evaluated physiochemically in order to select the best formulation.

For preparation of gel formulations, the concentrated extracts of Scrophularia striata Boiss were used. Initially Stoke 15% PVA was prepared. A certain amount of PVA powder was weighed and added to the water on the magnet stirrer at 80 ° C for two hours to reach to a gel-like state then placed in the freezer for 24 hours. After this time PVA was prepared for construction formulation.

Propylene glycol and glycerin was mixed well together in a beaker and xantam powder was then dispersed in it. Ethanol and Isopropyl alcohol were added In a separate beaker and then added Cremophor and tapper plant extracts to the contents of beaker, and then was stirred. The gel PVA, honey and contents of the first beaker was added to it. After stirring the solution was brought to volume with deionized water. The formulation pH was adjusted by Triethanolamine. . Evaluation of gel formulations

6.1. Physical appearance of gelmask formulations in macroscopic investigation:

the products were macroscopically examined following 48 h off the fabrication time and establishment of equilibrium (presence of tangible and follicle particles, color, and transparency (16).

6.2. Homogeneity of the formulation in microscopic investigation:

In order to study the originating of the formulations, after 48 hours following the formulation preparation, a thin layer of the synthesized product was dragged on a slide, and the formulation's homogeneity was examined under a microscope. This experiment was replicated 48 hours, one week, one month, and three months after the preparation time (17).

6.3. Measurement of pH:

To perform this stage, first pH was calibrated using standard buffers. Next, 1 g of the formulation was dispersed in 10 ml of pure water. Before measurement of pH, the electrode was placed inside the sample for 10 min, followed by measurement of the sample's pH for 3 times. The pH of formulations was measured within a time period from 48 h off their preparation time until three months after that (13).

6.4. Centrifuge test :

The prepared samples were transferred after 48 hours to test tubes (length: 10 cm, diameter: 1 cm). They were then centrifuged for 30 min at 1250 and 2500 rpm. The centrifugal force exerted to the products is calculated considering the distance between the centrifuge's center and the middle of the test tube (12.5 cm) and the selected rates by the following equation. The distance and rate calculated by the equation were 218.11 and 872.44, suggesting that the exerted force has been 218.11 and 872.44 times as large as gravity force in normal state. Following 5, 15, 30, and 60 min off the centrifuge time, stability of the prepared formulations was investigated (16).

Table 1: Composition of gel formulations ingerdients formulations

ingerdients	formulations									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
pva	2.5	2.5	2.5	4.5	6	7.5	7.5	7.5	7.5	7.5
xantam	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
cremophor	1	1	2	2	3	3	3	3	3	3
glycerin	5	5	4	4	3	3	3	3	3	3
ethanol	20	20	10	10	10	15	15	15	15	15
Propylene glycol	5	2	5	2	2	3	3	3	3	3
isopropylalcohol	-	-	-	-	-	-	5	5	15	10
honey	5	5	5	5	5	5	5	5	5	5
Extract *Scrophularia*	10	10	10	10	10	10	10	10	10	10
Triethanolamine	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs
water	100	100	100	100	100	100	100	100	100	100

6.5. Thermal variation test :
In this experiment, once the formulations reached equilibrium, three 15-g samples was chosen from each formulation. One of the samples was exposed to 4-6°C. The second one was subject to 25°C, and finally the third one was exposed to 45-50°C with a relative humidity of 65%. Examination of the formulations in terms of apparent properties was carried out after 24 h, one week, one month, and three months, respectively (14).
6.6. Freezing and thaw test :
To do this experiment, 15 g of each formulation was evaluated after reaching equilibrium in six consecutive periods, each of which included 48 hours at -8°C and 48 h at 25°C. By the end of the sixth period, the apparent quality of the formulations was studied (14).
6.7. Cooling and heating test :
To carry out this experiment, 15 g of each formulation was introduced into a suitable container after 48 h off the preparation of

formulations. They were then kept at 45-50°C for 48 h. thereafter, the formulations were kept at 4°C for 48 h. this was replicated six times for each formulation and by the end of the six periods, the apparent quality of formulations was investigated (14).
6.8. Determination of viscosity:
In order to determine the viscosity, a viscometer (Brookfield DV-III) is used. First, the viscometer was calibrated by Brookfield viscal kit. Next, the gel samples that were kept at room temperature for 30 min were poured into a container. The

connected spindle and the relevant viscosity at 25°C were determined to rotate at 100-250 rpm. This was replicated three times for each formulation (13).
6.9.Determination of Spreadability :
One gram of the selected products that have reached equilibrium is placed between two glass planes (with dimensions of 20 * 20 cm and weight of 125 g) in a circular shape with a diameter of 5 mm. Following three replications, the standard deviation and mean were calculated for each formulation (16).
6.10. Determination of the drying time:
A certain amount of the sample was dragged onto a slide and placed inside an oven at 25°C. Next, the drying time of the product and the time of film formation were measured.
7. In-vitro Drug Release Study (Diffusion study):
In order to measure the degree of drug diffusion, synthetic membrane made of acetate cellulose with pore diameters of 0.1 micron and Franz diffusion cell were used. The weight of the experimented sample (the giver phase) was 0.5 g, where the temperature of the environment was kept at around 37°C by using water. In this experiment, hydro-alcoholic solution10% was used as the receiver phase. As much as 28 ml of hydro-alcoholic solution10% was chosen as the receiver phase, where at each time, 2 ml of it was sampled and 2 ml of hydro-alcoholic solution10% replaced it. Taking samples from the receiver phase was done at 10, 20, 35, 50, 80, 140, 200 min and the concentration of the medication available in the samples was determined using Gallic acid standard curve and through taking dilution coefficient into consideration. To calculate the real amount of diffused drug, a correction factor was employed. This correction factor was calculated according to the following relation and by adding the obtained number to the apparent diffused value, the real value was obtained (13).
Cn= the real concentration of the drug diffused in simple n
C = the apparent concentration of the drug diffused in Sample n
Cn-1= the real concentration of the drug diffused in sample n-1
V= the sample volume
Vt = the total volume of the sample's environment
8. Drug release kinetic studies of gelmask formulations:
drug diffusion from every drug delivery system has its own special kinetics. The diffusion kinetics of a drug suggests explanation of the diffusion trend in terms of a variable called time. In order to investigate drug diffusion kinetics from formulation, the obtained data were examined in zero-order, first-order, and Higuchi models(18). Furthermore, to determine the drug diffusion mechanism, Korsmeyer Peppas equation was employed (19).

Results

3.1. Extraction preparation:

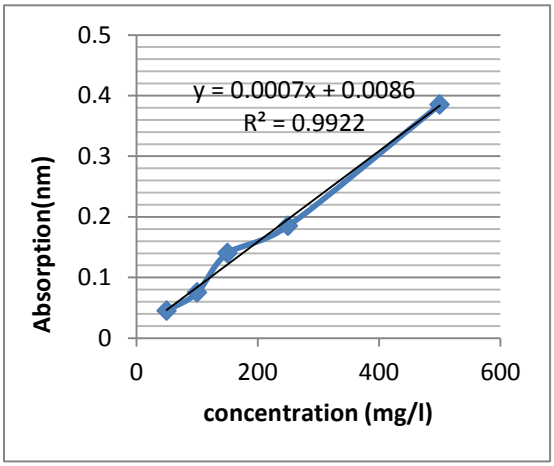
The obtained extract was concentrated using Rotary device at 40°C, then by Bon Mari at the same 40°C for 4-8 h. From every 100 g of the dried and milled plant of Scrophularia striata Boiss, 18 g total concentrated extract and 12.5 g dry substance extract were obtained respectively.

3.2. Determining the amount of the total polyphenols in the plant:

In order to determine the concentration of polyphenols available in the plant, first

standard curve of Gallic acid was plotted. According to this curve and the equation of y=0.0007x+0.0086, the amount of the polyphenol available per gram of the plant was obtained as 106.11 (mg GAC/g).

Diagram 1. The absorption-concentration diagram of calibration (mean ± SD, n = 3)



3.3. The results obtained from preparation of the formulation of gel mask:

In preliminary investigations, 10 base formulation were evaluated in terms of clarity, color, homogeneity, film formation, and drying time. As the initial formulations did not have suitable properties in physiochemical evaluation, some changes were developed in the concentration of the compounds in order to reach the selected formulation. Formulation10 formed a uniform and solid film on the skin within a desirable duration and was suitable in terms of consistency and adhesion. The concentration of PVA in the selected formulation was 7.5%, and the concentration of xantam was 0.5%. Finally, the concentration of ethanol and isopropyl alcohol was 15 and 10%, respectively. In the next stage, the plant's concentrated extract was added by 10% to the base formulation and evaluated physiochemically.

3.4. The results obtained from the physiochemical evaluation experiments of the formulations:

3.4.1. The results of macroscopic examination:

48 hours following synthesis of the selected formulation and achievement of equilibrium, the prepared formulation was investigated in terms of appearance. The formulation lacked tangible and follicle particles and enjoyed the necessary transparency and satisfactory color.

3.5.2. The results of microscopic examination:

The synthesized formulation was investigated using optical microscope with a magnification of 10 and 40 in terms of texture uniformity and air bubbles. It was found that the formulation was uniform and without air bubbles.

3.5.3. The results of pH measurement:

The pH of the selected product 48 hours following the fabrication was 5.65. It had reached this level by adjustment of the amount of triethanol amine. The pH of the product was measured one and three months after the synthesis, which were equal to 5.6 and 5.58, respectively.

3.5.4. The results of centrifugal test:

After reaching equilibrium state, the prepared formulation was transferred to test tubes and centrifuged at 1250 and 2500 rpm for 30 min. Following this duration, it had kept its stability and had no phase separation.

3.5.5. The results of thermal variation test:

Once the formulation reached equilibrium, three 15-g samples of the formulation were kept at 4-6°C, 25°C, and 45-50°C with a relative humidity of 65% for three months. Examination of the formulations in terms of apparent properties was carried out 24 h, one week, one month, and three months later, respectively. Throughout this period, selected

formulation had kept its stability completely and shown no apparent change in terms of color, uniformity, smell, etc.

3.5.6. The results of freezing and thaw test:

Following the fabrication and having reached equilibrium state, the selected formulation was poured into lidded test tubes and exposed to -8°C and 25°C in six consecutive periods for 48 hours at each temperature. By the end of these six periods, the selected formulation had maintained its stability and had no changes in the appearance.

3.5.7. The results of heating and cooling test :

Following the fabrication and having reached equilibrium state, the selected formulation was poured into lidded test tubes and exposed to 45-50°C and 4°C in six consecutive periods for 48 hours at each temperature. By the end of these six periods, the selected formulation had maintained its stability and had no changes in the appearance.

3.5.8. The results of determination of viscosity:

The viscosity of selected formulations was determined 48 hours following the fabrication using a viscometer (Brookfield DV-III) and with spindle No. 74 at 24°C. The value of viscosity was 550 milli pascal/s.

3.5.9. The results of determination of Spreadability:

In this experiment, 0.5 g of the selected formulation was dragged onto a glass plane, on which another glass plane was placed, where it was subject to the effect of the weight force of the glass plane in the form of gel. The difference between the initial and final diameter after placement of the second glass was 20.4 mm.

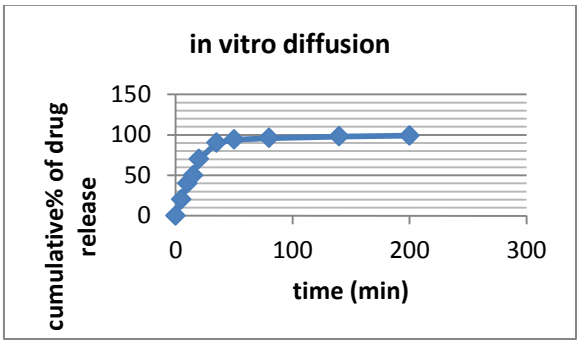
3.5.10. Determination of the drying time:

One gram of the formulation was put on a slide and placed inside an oven at 25°C. The formulation was dried after 10 minutes and formed a film.

3.6. The results related to drug diffusion:

Investigation of the drug diffusion through in vitro method was carried out using Franz diffusion cell and cellulose acetate membrane according to the procedure stated in section 2.6 for the selected formulation. The result was plotted in the form of drug diffusion percentage versus time diagram. As there is 12.5 mg polyphenol in 0.5 g of the product and the volume of the receiver phase is 28 ml, we expect that if the whole drug compound is diffused, it would develop a concentration of 400 mg/l. The collected samples were examined using Folin-ciocalteu colorimetry method. As can be seen in the diagram, the product has diffused 90% of the drug cuisine 35 minutes and 99% of the drug has been diffused within 200 minutes.

Diagram2- In vitro diffusion profile of selected formulation (mean ± SD, n = 3)



3.7. The results of Drug release kinetic studies:

when comparing the correlation coefficients and by taking statistical calculations into account, it was found that there is a significant difference between zero-order, first order, and Higuichi kinetics, with Higuichi having the best line and closest R to 1(Table 2) .

Table 2: drug release kinetics of gelmask formulations (n=3)

Zero-order		First-order		Higuchi	
R^2±SD	K_0	R^2±SD	K_1	R^2±SD	k_h
0.8880	0.142	0.9500	0.142	0.9905	0.006

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