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FORMULATION AND EVALUATION OF WOUND HEALING HERBAL GEL OF *BELLIS PERENNIS* FLOWER EXTRACT

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ABSTRACT

In the present study develop herbal wound healing product based on *Bellis perennis* plants was selected for the present work. The Flower of *Bellis perennis* was reported for wound healing activity and formulate herbal gel of *Bellis perennis* flower extract using gelling agents like Carbopol 934, Polyethylene Glycol, Methyl Paraben, Triethanolamine. Herbal Emulgels with *Bellis perennis* extract was successfully prepared and evaluated for assessing their safety,wound healing efficacy and pharmaceutical quality. Safety evaluations of the emulgels were proved to be free from skin irritation, the desirability of a topical product. *In vitro* release performance is in consistence with *in vivo* efficacy. The product has almost of neutral pH indicating compatibility with the skin and the product too had optimum gel consistency, viscosity, spreadability and extrudability.

Keyword: Herbal Emulgels, *Bellis perennis*, Carbopol.

1. INTRODUCTION

1.1 Wound

The skin has been described as the largest organ in the body. Its functions arenumerous and any injury to its layers increases the vulnerability of the organisms to additional biological and physical hazards resulting in wound. The wound is an injury to the body that necessitates the cracking of the skin and thereby harm the underlying tissue.

1.2 Healing of wound

The Healing is a complex process of restoration of cell structure and layers of tissue, which may occur in two ways:

a) Regeneration – It is the means of renewal of the extinct tissue by a like tissue.

b) Repair – It's the process of replacing the lost tissue by granulation tissue. Thisgranulation tissue fully develops to form the scar tissue. The healing of wound occurs in the following phases: Haemostasis, Inflammatory Phase, Proliferation or Granulation, Remodelling and Scar Formation.

1.3 HAEMOSTASIS

During this first phase of wound healing, the platelets seal off the ruptured blood vessels to heal the wounds.The platelets secrete vasoconstrictive substances to help in the sealing process by forming a stable clot, which sealoff the affected blood vessel. The platelets aggregate in the presence of Adenosine Di Phosphate (ADP) and adhere to the collagen exposed. Formation of fibrin from fibrinogen is initiated by Thrombin.A stable haemostatic plug is formed as the platelet aggregate are strengthened byfibrin mesh. Platelet-derived growth factor (PDGF), which is secreted by platelets, initiates the following steps. Except in case of any clotting disorders, haemostasis occurs immediately after the initial injury

1.4 INFLAMMATION PHASE

It is the second phase of wound healing. It is the body's natural reaction to wound. The blood vessel in the wound area contracts and a thick clot is formed after initial wounding. Dilatation of blood vessels after haemostasis permits the antibodies, growth factors, essential cells, WBC to reach the wounded area. This results inincreased exudate levels. Hence the neighbouring skin should be examined for the signs of softening and breaking down. The typical manifestations of inflammation like abnormal redness of the skin, heat, swelling, pain and functional disorder are seenduring this phase. The Neutrophils and Macrophages are the major phagocytic cells that play an indispensable role in the autolysis of necrotic tissue. The secretion of growth factors like FGF, EGF and IL-1 also occur during this stage.

1.5 PROLIFERATIVE PHASE

During this stage, repair of the wound tissue occurs with the formation of granulation tissue, which is primarily composed of collagen and extracellular matrix. A new network of blood vessels develops into this new granulation tissue. This process is known as angiogenesis. The blood vessels supply sufficient oxygen and nutrients to fibroblast, which in turn results in the proliferation of granulation tissue. Healthy granulation tissue is granular and uneven in appearance and is pink in colour which indicates the wound is healing. If the granulation tissue is dark, it indicates that perfusion is poor and the presence of infection. Finally the epithelial cells reappear over the wound surface and this process is known as epithelialisation.

1.6 REMODELLING OR MATURATION PHASE

During this phase remodelling of the dermal tissues occurs once the wound has closed. The fibroblasts play a key role in this process.Collagen type I get remodelled from type III, cellular activity reduces due to decrease in blood vessels. This helps to carry out the study of the formulated product in the presence of fluids, the blood

of the wound, immune cells etc. It also influences the pharmacological activities of the active drug moiety. Many studies on animals *in vivo* originally examine the security and/or irritation of the product. This primary skin irritation test is inevitable to prove that these products are nontoxic to the tissues. Animal trials for testing the efficacy of the product are performed only after the completion of safety studies.

The different categories of wounds made in test animals for experimental purposes are the following:

a) Open or Excision wound

The *in vivo* animal studies are employed to ascertain the potency of drugs and also to confirm whether they are safe for use in human beings. Excision or open wounds are created on the dorsal side of the animals. The animals mainly employed for the experiment are albino rats, rabbits, or guinea pigs. Depilate the dorsal side of the animal and 2.5 cm^2 area is marked out. Anaesthetize the animal with ether and excise this marked area using sharpened knives and scissors. Categorize animals to two batches; experimental or test batch and standard or control batch. Both groups were caged in different animal cages. The test formulation was applied to the test animals and the control animals receives standard or reference product. The wound contraction in each animal of both the groups is monitored on a daily basis. The number of days required complete regeneration of wound tissues was evidenced from the dropping of flakes resulting in the total disappearance of an unhealed wound. The regeneration of wound tissues was quantified as percentage diminution in the area of the wound. Traced the wounds using a transparent paper and then relocated to 1mm^2 graph sheets. The wound created was of the size 250 mm^2 and was considered as 100% and percentage of wound contraction was determined. Incision or Sutured Skin Wounds

b) Musculoperitoneal Wounds

Based on the type of animals selected, a 2-5cm long abdominal incision is made using a scalpel to completely open up the abdominal region and the wound is closed with interrupted linen stitches. The animal sacrifices after the desired post-operative days and histological studies are performed on the wound tissue.The skin specimens were stained with eosin and haematoxylin and were inspected microscopically for keratinization, epithelialization, fibrosis and angiogenesis.

c) Burn wounds

Suitable animal is selected and depilate the dorsal side of the animal. An iron or copper disk of 4cm diameter with a woody handle, is warmed up to red hot, and is applied to the desired area of the anaesthetized animal up to 10 seconds. The test formulation is applied and the wound contraction in each animal is monitored on a daily basis. Time taken for complete wound closure is determine.

2. MATERIAL AND METHOD

2.1 Collection of plant material

Flowers of *Bellis perennis* Plant was collected from Vindhya Herbal Nursery Bhopal (M.P.) in the month of September, 2021.

2.2 Percentage loss

The weight of the fresh sample and dried powder was determined, and the percentage loss was calculated due to drying and water loss.

The percentage loss was calculated by using following formula:

$$\%\ \textbf{Loss of drying} = \frac{Loss\ in\ weight\ of\ the\ sample}{Weight\ of\ the\ sample} x\ 100$$

2.3 Extraction procedure

100 gram of *Bellis perennis* was exhaustively extracted with ethanol by maceration method. Over their boiling points the extract was evaporated. Finally, the percentage yields for the dried extracts are determined.

2.4 Determination of percentage yield

Following formula was adopted for determination of percentage yield of selected plant materials. The percentage yield of each extract was calculated by using following formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of Extract}}{\text{Weight of Powder drug taken}} \text{x } 100$$

2.5 Phytochemical screening

Medicinal plants are traditional medicinal resources and many of the modern medicinal products are produced indirectly from plants. Phytochemical components consist of two primary bioactive components (chlorophyll, proteins, amino acids, sugar, etc.) and secondary bioactive components (alkaloids, terpenoids , flavonoids, etc.). Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.6 Quantitative estimation of bioactive compounds

2.6.1 Total phenolic content estimation

The extract's total phenolic content was measured using the modified Folin-Ciocalteu method. The absorbance measurement with spectrophotometer at 765 nm.

2.6.2 Total flavonoids content estimation

The method used to determine the total content of flavonoids was using aluminum chloride. 3 ml of this extract and 1ml aluminum chloride was used for calculating flavonoids.

2.7 Antioxidant activity of extracts

2.7.1 DPPH scavenging activity

The DPPH free radical scavenging assay was carried out for the evaluation of the antioxidant activity. This assay measures the free radical scavenging capacity of the investigated extract.

$$\% \textbf{ Reduction} = \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{x100}$$

2.8 Drug –Excipient compatibility study:

The thermograms were registered using a calorimeter for differential scanning.

2.9 Formulation development of Herbal gel

Measured amounts of methyl paraben, glycerin, polyethylene glycol and and Flowers of *Bellis perennis*, was dissolved in about 100 ml of water in a beaker and stirred at high speed using sonicator. Then Carbopol 934 was slowly added to the beaker which contained above liquid while stirring. Neutralized the solution by adding a slow, constantly stirring triethanolamine solution until the gel formed.

Table 2.1: Formulation of Herbal Gel

Ingredients (mg)	**F1**	**F 2**	**F3**	**F4**
Bellis perennis extract	250	250	250	250
Carbopol 934	250	500	1000	2000
Polyethylene Glycol 600	0.2	0.2	0.2	0.2
Methyl Paraben	0.08	0.08	0.08	0.08
Triethanolamine	1.0	1.0	1.0	1.0
Distilled Water	100 ml	100 ml	100 ml	100 ml

2.10. Evaluation of polyherbal gel

A. Appearance and consistency:

The physical appearance was visually checked for the texture of herbal gel formulations and observations reported in table 3.4.

B. Washability

Prepared formulations were added to the skin and then manually tested for ease and degree of washing with water, and findings were recorded in table 3.4.

C. Extrudability

The herbal gel formulations were filled into aluminium collapsible tubes and sealed. The tubes were pressed to extrude the material and the extrudability of the formulation was noted.

D. Determination of Spreadability

The experiment was repeated and the average of 6 such determinations was calculated for each formulation.

$$\boldsymbol{Spreadability} = \frac{m * l}{t}$$

Where, S=Spreadability (gcm/sec)

m = weight tied to the upper slide (20 gram)

l= length of glass slide (6cm).

t = time taken is seconds.

E. Determination of pH

Digital pH meter had calculated the pH of the anti-acne gels. One gram of gel was dissolved in 25 ml of purified water and the electrode was then dipped into gel solution until steady reading was achieved.

F. Drug content

The composition of the medication was measured by taking 1gm of gel mixed with methanol in 10 ml volumetric flask. 3 ml of stock solution has been mixed with 1 ml $AlCl_3$ solution of 2 per cent. The mixture was vortexed for 15s and allowed for the color production to stand at 40°C for 30min, using a spectrophotometer the absorbance was measured at 420 nm.

G. Viscosity

The viscosity of the prepared gel was determined by a Brookfield digital viscometer. The viscosity was assessed using spindle no. 6 at 10 rpm at ambient room temperature of 25-30^{0}C. **H.. *In vitro* diffusion profile**

In vitro diffusion experiments were performed using Franz diffusion cell for all formulations.

I. Skin Irritation Study

1 gm of the herbal gel was used as the test substance was applied to an area of approximately 6 cm^2 of skin and covered with a gauze patch.

Grade: A-No Reaction, B-Slight patchy erythema, C-Slight but confluent or moderate but patchy erythema, D-Moderate erythema, E-Severe Erythema with or without edema.

J. Stability Study

The stability study was performed as per ICH guidelines. The formulated gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz.25^0 C ± 2^{0}C/ 60% ± 5% RH, 30^0 C ± 2^{0}C/ 65% ± 5% RH, 40^{0}C ± 2^{0}C/ 75% ± 5% RH for a period of six months and studied for appearance, pH, viscosity and spreadability.

3. RESULT AND DISCUSSION

3.1 Determination of Percentage Yield

Table 3.1: % Yield of Ethanolic extract of *Bellis perennis*

S.No.	Part	Percentage Yield
1	Flowers	**17.53**

3.2 Phytochemical screening of extract

Table 3.2: Phytochemical screening of Ethanolic extract of *Bellis perennis* Flowers

S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Dragendroff's test	**-ve**
	Hager's test	**-ve**
2.	**Flavonoids**	
	Lead acetate	**+ve**
	Alkaline test	**-ve**
3.	**Phenolics**	
	$Fecl_3$	**+ve**
4.	**Proteins and Amino acids**	
	Xanthoproteic test	**-ve**
5.	**Carbohydrates**	
	Fehling's test	**+ve**
6.	**Saponins**	
	Foam test	**+ve**
7.	**Diterpenes**	
	Copper acetate test	**+ve**

3.3 Results of Estimation of Total Phenolic Contents

Table 3.3: Total Phenolic and Total flavonoid content of *Bellis perennis* Flowers

S. No.	Extract	Total Phenol (GAE) (mg/100mg)	Total flavonoid (QE) (mg/100mg)
1.	**Ethanolic extract**	2.95	0.41

3.4 Results of Antioxidant activity of Flowers extracts of *Bellis perennis* Flowers

The concentration of ethanol extract of Flowers of medicinal plant taken to inhibit 50% of DPPH was indicated.Variable DPPH activity was recorded for ethanolic extract of *Bellis perennis.* The DPPH scavenging activity of Ethanolic extract of *Bellis perennis* was showed IC_{50} value 68.37 µg/ml, as compared to positive control ascorbic acid 21.41µg/ml.

3.5 Results of Drug-Excipient compatibility study:

Herbal extract DSC thermogram showed melting point at 203^0C. The mixture of drug and Carbopol that was held for 30 days in an elevated state of 40^0C/75% RH and was subjected to DSC study. The DSC study shows that extract and carbopol are compatible with each other.

3.6 Results of Herbal gel Formulation

3.6.1 Evaluation of gel formulation of Herbal gel

Table 3.4: Results of Physical Characteristics

Formulation	Colour	Clogging	Homogeneity	Texture	Washability	Extrudability
F1	Greenish	Absent	Good	Smooth	Good	Good
F2	Greenish	Absent	Good	Smooth	Good	Good
F3	Greenish	Absent	Good	Smooth	Good	Good
F4	Greenish	Absent	Good	Smooth	Good	Good

3.6.2 Results of Spreadability

Table 3.5: Results of spreadability of Herbal gel

S.N.	Formulation	Spreadability* (gcm/sec)
1	**F1**	13.82±0.95
2	**F2**	13.21±0.73
3	**F3**	12.46±0.28
4	**F4**	11.78±0.47

*Average of three determinations (n=3 ±SD)

The F-3 Formulation showed Spreadability 12.46±0.28 as optimized formulation was selected.

3.6.3 Results of Viscosity

Table 3.6: Results of Viscosity of Herbal gel

S.N.	Formulation	Viscosity* (cp)
1	**F1**	3585±74
2	**F2**	3417±45
3	**F3**	3269±34
4	**F4**	3093±15

*Average of three determinations (n=3 ±SD)

The viscosity of gels was determined by using a Brookfield viscometer DV-II model. In the above formulations the viscosity of different samples of gel was determined. The formulation F3 has good viscosity.

3.6.4 Results of flavonoid Content

Table 3.7: Results of flavonoid content in Herbal gel using $AlCl_3$ method

S.N.	Formulation	Flavonoid Content(mg/100mg)
1	F1	1.06±0.38
2	F2	1.03±0.61
3	F3	1.09±0.35
4	F4	0.98±0.91

*Average of three determinations (n=3 ±SD)

In the above formulations of gel the percentage of flavonoid content in formulation F3 was found maximum (1.09±0.35/100mg).

3.6.5 Results of *In Vitro* Drug Release Study

Table 3.8: Release Kinetics Regression values of formulation F3

Formulation code	Higuchi	Korsmeyer-Peppas
F3	**0.987**	**0.998**

The Optimized Herbal gel formulation F3 release approx 7.89 percent drug within 15 minutes and approx 99.97 percent of drug release in 4 hours. When the regression coefficient values were compared, it was observed that 'r^2' values of Korsmeyer-peppas were maximum i.e. 0.998 hence indicating drug releases from formulation follow peppas release kinetics.

3.7 Results of Skin irritation study results

Table No. 3.9: Results of Skin irritation study results

Treatment	Day I	Day II	Day III	Day IV	Day V	Day VI	Day VII
Control	A	A	A	A	A	A	A
Herbal Formulation (F3)	A	A	A	A	A	A	A

Grade: A-No Reaction, B-Slight patchy erythema, C-Slight but confluent or moderate but patchy erythema, D-Moderate erythema, E-Severe Erythema with or without edema.

3.8 Results of Stability study of optimized formulation

Table 3.10: Results of Stability study of optimized Herbal formulation

Formulation Code	Months	Spreadability	pH
Herbal Formulation (F3)	**I**	12.46±0.68	7.1 ±0.1
	II	12.41±0.55	7.0±0.2
	III	12.39±0.83	7.0±0.1

CONCLUSION

Study showed that our traditional context masks a range of amazing herbs that could be healthy and effective trade to pain killer. Pharmaceuticals are searching for a viable solution to come free from analgesic gel for dedicated all age group communities. This research work is intended to inspire and motivate valuable tool practitioners, scientists, pharmacists, industrialist to use these herbs more specifically in the topical herbal gel formulation system so that customers can benefit more from natural substances. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. It is a very good attempt has made to establish the herbal gel containing *Bellis perennis* flowers extract. Indian history of medicinal plants has proven that herbs have been used to topical . From the long list of herbs in my research *Bellis perennis,* some of the common herbs that are found in abundant. The chosen plants develop an effective anti-arthritic gel which is environmentally safe. This herbs' encapsulation into gel base demonstrated the stronger anti arthritic properties.

The whole plant or its specific parts are known to have medicinal properties and have a long history of use by indigenous communities in India. The medicinal value of this plant for the treatment of a large number of human ailments is mentioned in Ayurveda, Siddha, Unani and folk medicine In the last more than three decades, several studies have been carried out on this medicinal plant species to facilitate evidence in favor of its conventional uses. The extracts were subjected to qualitative phytochemical screening using standard procedure. Phytochemical screening reveals the presences of Flavonoids, Phenolics, Saponins. Total phenolic and total flavonoid content was found 2.95 and 0.41 mg/100mg in *Bellis perennis* extract respectively.

From the results, it may be concluded that herbal Hydroalcoholic extract of flowers of *Bellis perennis* possess significant anti-arthritic effect may be due to the effect of antioxidants like Flavonoids, Phenols and Saponins present in the plant. All these biological activities may be said to be a promising findings brought out by the present study. These contributions can be used as parameters for the authentication of plant as well as for developing newer drugs based on their activity. Herbal gel had no toxicity; such products could therefore be produced and used effectively. The side effect associated with the topical synthetic drug can be resolved with the use of existing herbal gel. The formulations produced can be translated to large-scale development as technology transfer to the herbal industries. Established basic technological method for plant extract analysis will satisfy global scientific organizations' criteria in order to be able to quantify and compare the outcomes of that work in this area.

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