

FORMULATION AND EVALUATION OF NATURACEUTICAL ALOE INERMIS EXTRACT FOR WOUND HEALING ACTIVITY AS ANTIBACTERIAL CREAM NOVEL DRUG DELIVERY SYSTEMS

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ABSTRACT

Aloe inermis is a non-African species native to western Yemen and grow in dry stone hills at around 1000-2000 m in elevation. *Aloe inermis* belongs to family Aloeacea and is used traditionally in skin care for burns, rashes, allergic irritation, wounds and infections. According to previous study that reported the wound healing activity of *Aloe inermis* latex. In the current study, the dry latex of *Aloe inermis* was formulated in seven formulations to study the variant characteristics of cream. The present study is aimed to formulate the methanolic extract into a suitable herbal cream and evaluate for its physiochemical, and stability parameters. Among the seven formulations (F1-F7), F7 showed good spreadability, good consistency, homogeneity, appearance, pH; there is no proof of a separation phase and ease of washability. The compositions of formulation F7 were *Aloe inermis* extract, Cetomacrogol, Cetostearyl alcohol, Liquid paraffin, white soft paraffin, Propylene glycol, Cholorocresol, Beeswax, Sodium dihydrogen phosphate, Titanium dioxide, Lavender oil, Methylparaben, and Purified water. It was concluded that the best formulation selected F7 was safe in respect to natural herb offer diverse benefits in cream preparations, as evidenced by this study, highlighting the safety compared to commercial semisolid products. The study showed that the extract of *Aloe inermis* could be used as an alternative for wound healing activity as cream NDDS formulation.

KEYWORDS: *Aloe inermis*, Cream Formulation, Antimicrobial agent, Wound Healing Activity, Novel Drug Delivery Systems.

INTRODUCTION

Background of The Genus *Aloe Inermis*^[1,30]

Aloe inermis is a non-African species native to western Yemen and grow in dry stone hills at around 1000 - 2000 m in elevation. *Aloe inermis* (Figure 1) a suckering small shrubby aloe that forms clumps to 2 feet tall of open rosettes of long narrow decurved pale olive-green leaves on short stems that are smooth to the touch with a deep central channel and toothless margins. In fall into winter

appear the 2- to 3-foot-tall branching inflorescence with salmon red flower buds that are purple green at the tip and open with green stripped cream petal lobes, Plant in full sun in a well-drained soil and irrigate occasionally to very little -quite drought tolerant and hardy to around 25 °F, this is an attractive and unusual aloe as a garden or pot specimen with no teeth or other sharp pointed appendages.



Fig. 1: Leaves and Flowers of Aloe Inermis.

Traditional Uses of Aloe Species

Aloe Vera has been used for over five thousand years. The ancient Egyptians used *Aloe* to heal battle wounds and cure infections. The Greeks used *Aloe* for relieving blisters, burns, leg ulcer and bowel and stomach disorders. In pervious study reviewed the traditional uses of *aloe*; in India the traditional medicine of *Aloe* is used internally as a laxative, antihelminthic, hemorrhoid remedy, and uterine stimulant (menstrual regulator); it is also used topically, often in combination with licorice root, to treat eczema or psoriasis. In Arabian medicine, the fresh gel is rubbed on the forehead as a headache remedy or rubbed on the body to cool it in case of fever, as well as being used for wound healing, conjunctivitis, and as a disinfectant and laxative. The Chinese describe aloe's skin and the inner lining of its leaves as a cold, bitter remedy which is downward and used to clear constipation due and the gel is considered cool and moist.^[6]

Pharmacological Action

Antibacterial, antioxidant, and wound healing activities of *Aloe inermis* was reported previously.^[6]

Active Constituents

phytochemical screening of the latex, methanol extracts of *Aloe inermis* revealed the presence of alkaloids, carbohydrates, phytosteroids, anthraglycosides, phenolic and tannins.^[6]

Wound Healing^[6-64]

Wounds can be major causes of physical disability and may lead to loss of many productive hours. Essentially, wounds are the disruption of functional continuity and anatomical structure of cells and tissues at the sites of injury. They can be caused by insults to the tissue by physical, chemical, microbiological or immunological processes. It has been estimated that 14 million people suffer from wounds and burns annually with over 80 percent of these living in low- and middle-income countries.

Wound healing is a complex and dynamic process in which cellular structure and tissue layer of the damaged tissue are restored to its normal state as closely as possible. Synthetic chemical moieties are the present treatment regimens for the management of under healing of wound but they possess a wide range of side effects. Therefore, research has prompted into herbal and natural products which known to increase the healing of different types of wounds and which can eliminate the side effects that were associated with the synthetic chemical moieties.

WHO encourages, recommends and promotes traditional herbal medicines in national health care programs because these drugs are easily available at low cost, and people have faith in them. Plants and their extracts have immense potential for the management and treatment of wounds. These natural agents induce healing and

regeneration of the lost tissue by multiple mechanisms. Herbal treatment for wounds provides fibro-genetic and concentration of collagen resulting in faster wound healing. *Aloe Vera* is an excellent remedy for minor burns, cuts and sunburns. Both juice and aqueous extract from the leaves shows significant healing properties. It is also reported that it not only speeds up healing but also prevents injured surface from getting infected.

Aloe inermis is one of the non-African species, is native to western Yemen. *Aloe inermis* was evaluated for the potential of latex extract to accelerate the rate of wound healing. Wounds dressed with latex showed remarkably less scar width at wound, less inflammatory cell, and more fibroblast wounds.^[6]

Semisolid Drug Delivery Systems^[15,145]

Semisolid constitute a significant portion of pharmaceutical dosage form. They serve as carriers for drugs that are topically delivered by way of skin, cornea, rectal tissue, nasal mucosa, vagina, buccal tissue, urethral membrane, and external ear lining. Because of their peculiar rheological behavior, semisolid can adhere to the application surface for sufficiently long periods before they are washed off. This property helps prolong drug delivery at the application site.

Semisolids are available as a wide range of dosage forms, each having unique characteristics. Topical semisolid dosage forms are normally presented in the form of creams, gels, ointments, or pastes. They contain one or more active ingredients dissolved or uniformly dispersed in a suitable base and any suitable excipients such as emulsifiers, viscosity increasing agents, anti-microbial agents, antioxidants, or stabilizing agents. The advantages of semisolid dosage form are: It is used external, probability of side effect can be reduced, local action, first pass gut and hepatic metabolism is avoided and patient compliance is increased; the drug termination is problematic cases is facilitated as compared with other routes of drug administration. The disadvantage of semisolid dosage form is; there is no dosage accuracy in this type of dosage form, the base which is used in the semisolid dosage form can be easily oxidized, and if we go out after using semisolid dosage form problems can occur. Semisolid are available as wide range of dosage form, each having unique characteristic.

Creams are the topical preparations which can be applied on the skin. Creams are defined as "viscous liquid or semisolid emulsions of either the oil-in-water or water-in-oil type" dosage forms which consistency varies by oil and water. Creams are used for cosmetic purposes such as cleansing, beautifying, improving appearances, protective or for therapeutic function.

Cream Novel Drug Delivery Systems

Cream is defined as semisolid emulsions which are oil in water (o/w) or water in oil (w/o) type and these semisolid emulsions are intended for external application. It is

applied on outer part or superficial part of the skin and its main ability to remain for a longer period of time at the site of application. The function of a skin cream is to protect the skin against different environmental conditions, weather and gives soothing effect to the skin. There are different creams like cleansing, cold, foundation, night, massage, vanishing creams. Herbal cosmetics are also known as “natural cosmetics”. So, they are oldest products used by mankind. Some common cosmetics include creams, face packs, shampoos, soaps, hair oils etc., The formulation of all these cosmetics products include addition of oils, waxes, natural colors, and parts of leaves, flowers etc. by specific formulation methods. Herbal formulations are receiving more concentration in public because of their high-quality properties and less side effects. The main aim of herbal cream is to give multipurpose effects, like moisturizer, reduces acne and skin irritation, reduce skin diseases like eczema, psoriasis, dry skin, wrinkles, prevents aging additionally adds glow to the face.

Research Paths^[65-145]

Scientific research that is organized in the form of Research Paths is characterized by the fact that, it is the most effective in achieving an idea, innovation, and development. It is linking the inductive plan, steps, goals, research methods, results, conclusion, materials, and equipment required to achievement scientific research. Research Paths are distinguishing that by build on each other and link the common relationship between them.

Pharmaceutical Research Paths

Pharmaceutical research is characterized by having both a natural source and synthetic source for primary active raw materials and excipients, each source is mainly prepared to the effectiveness and safety of the drug.

The Pharmaceutical Research Paths include: Pharmacognosy deals with natural sources of drug, Pharmaceutical Chemistry specializes in synthetic sources of drug, Pharmaceutics specializes in designing of pharmaceutical dosage forms and drug delivery systems from natural and synthetic sources of active pharmaceutical ingredients and excipients that help in developing dosage forms and drug delivery systems.

The Pharmaceutical Research Paths link steps are manufacturing and development of drug according to the standard parameters evaluation such as physiochemical properties, preformulation, formulation, evaluation, drug stability, Pharmaceutical analysis, pre-clinical, post-clinical stages, pre-marketing, post-marketing, Pharmacovigilance, Pharmacoeconomics, Pharmacy Management, Pharmacology, Toxicology, Therapeutics, Pharmaceutical Care, Health Care, Advanced Industrial Pharmacy, Biopharmaceutics and Pharmacokinetics, Advanced Clinical Pharmacokinetics, Pharmaceuticals Cosmetics, Pharmaceutical Biotechnology, Drug Design, Pharmacy Law and Ethics, Pharmacogenomics, Good

Manufacturing Practice, and Good Pharmacy Practice etc.

All of these Pharmaceutical Research Paths are interconnected, and whenever the link between them is made in a scientific relationship and the goal of pharmaceutical care is achieved gradually according to plan of a scientific pharmaceutical research path.

Pharmaceutical Research Paths are the scientific methods through which the scientific relationship between the pharmaceutical team, research, supervisor or specialist researcher, the scientific research materials, equipment's, scientific institution, pharmaceutical companies, reference standards, and the goals of pharmaceutical research improve and development of community services of pharmaceutical care and health care.

Pharmaceutical Scientists are considering natural sources and medicinal herbs in the pharmaceutical industry an important part of drug development because natural sources of drugs have properties that are greater than industrial sources of drugs in NDDS. And the pharmaceutical industry strategies depend on the development of different pharmaceutical dosage forms and recent novel drug delivery systems. Using medicinal herbs and natural sources as important goals of drug development. It is part of the art of innovation in drug development with different of novel drug delivery systems and pharmaceutical care for patients and society, it's the basic of development of the new pharmaceutical industry by developing different novel drug delivery systems from different sources.

The present study has been designed to formulate and evaluate of *Aloe inermis* extract as oil-in-water cream NDDS which has been widely used as topical application for antimicrobial and wound healing activity.

MATERIALS AND METHODS

MATERIALS

The extract of *Aloe inermis* was prepared and gift from (Prof. Dr. Bushra Moharram Professor Dr. of Pharmacognosy, Department of Pharmacognosy, Faculty of Pharmacy, Sana'a University, Sana'a, Yemen). Materials used in the cream formulation from *Aloe inermis* extract as shown in Table 1.

Wound Healing Activity

The antibacterial and wound healing activity of *Aloe inermis* extract was studied in a previous study.^[6]

Equipment's

FT-IR Spectrometer (Scimitar 2000 FT-IR, Varian, USA), Sonicator bath (SCT-SONIC-6, ScichemTech, USA), pH Conductometer 914 (Metrohm, China); Balance (Sartorius, Germany); Mixer (Labtech, Korea), Water bath (Scichemtech, USA), Autoclave (Shanghai.5117.Co, China).

Table 1: Materials Used in Cream Formulation with *Aloe Inermis* Extract.

No.	Materials	Country	Company
1	Stearic Acid	Germany	Merk
2	Di-basic Sodium Phosphate	Germany	Merk
3	Propylparaben	Germany	Merk
4	Chlorocresol	China	Benzo chemind
5	EDTA	Germany	Merk
6	Methylparaben	Germany	Merk
7	Cetostearyl Alcohol	China	Zhongbac
8	Cetomacrogol 1000	China	Zhongbac
9	Beeswax	China	Yuyu
10	Triethanolamine	Germany	Merk
11	Glycerol	Germany	Merk
12	Liquid Paraffin	Germany	Merk

Formulation and Evaluation of *Aloe Inermis* Cream Novel Drug Delivery Systems^[31,151]

Formulation of *Aloe Inermis* Cream Novel Drug Delivery Systems

Begin the process by heating liquid paraffin, white soft paraffin, cetomacrogol in a borosilicate glass beaker at 75 °C, maintaining this temperature (Oil phase). In a separate beaker, dissolve dibasic sodium phosphite, and chlorocresol in distilled water at 75°C using a thermostatic water bath. Stir the solution with a glass rod until solid particles are dissolved (Aqueous phase). Now carefully add the heated aqueous phase to the heated oily

phase by stirring. Once both the phases are mixed, then add measured amount of butterfly *Aloe inermis* extract. Then continue mixing with glass rod until smooth cream formation takes place. Finally add few drops of lavender oil as a fragrance, mix the cream in geometric manner to mix all the ingredients properly. Adjust the consistency of the cream once it reaches smooth texture transfer it into container labelled. The cream was cooled with stirring to room temperature. Prepared creams were storage at the room temperature (25 ± 2°C) in tubes. As shown in Table 2.

Table 2: Composition of *Aloe Inermis* Cream NDDS Formulations.

Ingredients	Formulation Code						
	F1	F2	F3	F4	F5	F6	F7
Aloe Inermis	1	1	1	1	1	1	20
White Soft Paraffin	---	1.5	1.5	1.5	1.48	1.6	30
Cetostearyl Alcohol	0.4	0.7	0.5	0.7	0.7	0.8	14
Cetomacrogol 1000	---	0.5	0.4	0.5	0.5	0.6	10
Cholorocresol	---	0.02	0.02	0.02	0.02	0.02	0.4
Liquid Paraffin	0.3	0.8	0.8	0.8	0.8	0.9	26
Propylene Glycol	0.5	1.2	1.2	1.2	1.2	1.3	24
Sodium Dihydrogen Phosphate	---	0.1	0.1	0.1	0.1	0.2	2
Beeswax	---	---	---	0.2	0.2	0.3	4
Titanium Dioxide	---	---	---	1	1	---	2
Tween	---	---	---	---	0.02	0.3	---
Lavender oil	---	---	---	---	---	---	Drops
Stearic Acid	0.15	---	---	---	---	---	---
Glycerol	0.3	---	---	---	---	---	---
EDTA	0.002	---	---	---	---	---	---
Methylparapen	0.001	0.001	0.001	0.001	0.001	0.001	0.001
Propylparapen	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Triethanolamine	0.2	---	---	---	---	---	---
Purified Water	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation of *Aloe Inermis* Cream Novel Drug Delivery Systems

All the formulated creams were evaluated for following parameters

Physical Evaluation

Physical evaluation: In this test, the cream was observed for color, odor, texture, state.

pH Determination

pH: 0.5g cream was taken and dispersed in 50ml distilled water and then pH was measured by using digital pH meter.

Viscosity Test

Viscosity: Viscosity of the formulation was determined by viscometer at 100 rpm.

Washability Test

Washability: A small amount of cream was applied on the hand and it is then washed with tap water.

Irritancy test

Irritancy: Mark the area (1cm²) on the left-hand dorsal surface. Then the cream was applied to that area and the time was noted. Then it is checked for irritancy, erythema, and edema if any for an interval up to 24 h and reported.

Phase Separation

Phase separation: Prepared cream was kept in a closed container at a temperature of 25 -100°C away from light. Then phase separation was checked for 24h for 30d. Any change in the phase separation was observed/checked.

Spreadability Test

Spreadability: The spreadability test was expressed in terms of time in seconds taken by two slides to slip off from the cream, placed in between the slides, under certain load. Lesser the time taken for Separation of the two slides better the spreadability. Two sets of glass slides of standard dimension were taken. Then one slide of suitable dimension was taken and the cream formulation was placed on that slide. Then other slide was placed on the top of the formulation.

Then a weight or certain load was placed on the upper slide so that the cream between the two slides was pressed uniformly to form a thin layer. Then the weight was removed and excess of formulation adhering to the slides was scrapped off. The upper slide was allowed to slip off freely by the force of weight tied to it. The time taken by the upper slide to slip off was noted.

Determination of Greasiness

The ease of removal of the cream applied was examined by washing the applied part with tap water.

Packaging of Finished Product

The optimized cream formulations were prepared; packed in aluminum collapsible tubes.

Stability Study

The herbal cream was prepared and subjected to various parameters to be evaluated.

RESULTS AND DISCESION

Evaluation results of the seven formulations are gives below.

Physical evaluation: In this test color, odor, texture and state of the formulations were examined. As shown in Table 3.

Table 3: Physical Evaluation Observations.

NO	Formulation Code	Color	Odor	Texture	State
1	F1	---	---	---	---
2	F2	Yellow	Pleasant	Smooth	Semisolid
3	F3	Brown	Pleasant	Smooth	Semisolid
4	F4	Yellow	Pleasant	Smooth	Semisolid
5	F5	---	---	---	---
6	F6	Brown	Pleasant	Smooth	Semisolid
7	F7	Yellow	Pleasant	Smooth	Semisolid

Phase separation: Prepared cream was kept in a closed container at a temperature of 25-100°C away from light. Then phase separation was checked. Any change in the phase separation was observed/checked. According to

the results phase separation was observed in F1 and F5. While the F2, F3, F4, F6 and F7 no phase separation. As shown in Table 4.

Table 4: Phase Separation Observations.

NO	Formulation Code	Phase Separation
1	F1	Phase Separation
2	F2	No Phase Separation
3	F3	No Phase Separation
4	F4	No Phase Separation
5	F5	Phase Separation
6	F6	No Phase Separation
7	F7	No Phase Separation

pH: According to the results, the pH of the six formulations that is F2, F3, F4, F5, F6 and F7 were found

to be nearer to skin pH so it can be safely used on the skin. As shown in Table 5.

Table 5: pH Observations.

NO	Formulation Code	pH
1	F1	---
2	F2	4.14
3	F3	4.7
4	F4	4.68
5	F5	5.23
6	F6	4.9
7	F7	4.5

Washability: Washability test was carried out by applying a small amount of cream on the hand and then

washing it with tap water. The formulations F2, F3, F4, F6 and F7 were easily washable. As shown in Table 6.

Table 6: Washability Observations.

NO	Formulation Code	Washability
1	F1	---
2	F2	Easily Washable
3	F3	Easily Washable
4	F4	Easily Washable
5	F5	---
6	F6	Easily Washable
7	F7	Easily Washable

Irritancy: Mark the area (1cm²) on left hand dorsal surface. Then the cream was applied to that area and the time was noted. Then it is checked for irritancy, erythema, and edema if any for an interval up to 24h.

According to the results the formulations that are F2, F3, F4, F6 and F7 showed no sign of irritancy, erythema and Edema. As shown in Table 7.

Table 7: Irritancy Study Observations.

NO	Formulation Code	Irritant Effect	Erythema	Edema
1	F1	---	---	---
2	F2	Nil	Nil	Nil
3	F3	Nil	Nil	Nil
4	F4	Nil	Nil	Nil
5	F5	---	---	---
6	F6	Nil	Nil	Nil
7	F7	Nil	Nil	Nil

Spreadability: The spreadability of the formulations that are F2, F3, F4, F6 and F7 were carried out the time taken by the 2 slides to separate is less so as said in the description of evaluation test lesser the time taken for

separation of the two slides better the spreadability so according to this results F2, F4, F6 and F7 showed better spreadability. As shown in Table 8.

Table 8: Spreadability Observations.

NO	Formulation Code	Spreadability
1	F1	---
2	F2	Good
3	F3	Low
4	F4	Good
5	F5	---
6	F6	Good
7	F7	Good

Greasiness: Here the cream was applied on the skin surface in the form of smear and checked if the smear was oily or grease-like. According to the results, we can

say that F2, F3, F4, F6 and F7 formulations were non-greasy. As shown in Table 9.

Table 9: Greasiness Observations.

NO	Formulation Code	Greasiness
1	F1	---
2	F2	Non-greasy
3	F3	Non-greasy
4	F4	Non-greasy
5	F5	---
6	F6	Non-greasy
7	F7	Non-greasy

According to the results of viscosity test, we can say that F2, F4, F6 and F7 formulations were good viscosity. As shown in Table 10.

Table 10: Viscosity Observations.

NO	Formulation Code	Viscosity
1	F1	---
2	F2	Good
3	F3	Low
4	F4	Good
5	F5	---
6	F6	Good
7	F7	Good

Stability Study

The herbal cream was prepared and subjected to various parameters to be evaluated. The results shown that the cream maintained the same appearance and good feel on application after the accelerated stability study performed. The initial viscosity of formulations showed constant stability in comparison with after accelerated stability study performed.

The formulated creams were evaluated for pH, viscosity, washability, irritability, color and spreadability in this study, *Aloe inermis* cream was successfully formulated, packaged in aluminum tubes of 20g net weight, and labeled. The type of cream of the formulations used was o/w base. The formulated cream exhibited the desired properties of consistency, spread ability, physicochemical stability and its appearance may make it cosmetically acceptable.

CONCLUSION

In the current study, the dry extract of *Aloe inermis* was formulated in seven formulations to study the variant characteristics of cream. Among the seven formulations (F1-F7), F7 showed good spread ability, good consistency, homogeneity, appearance, pH; there is no proof of a separation phase and ease of washability. The compositions of formulation F7 were *Aloe inermis* extract, Cetomacrogol, Cetostearyl alcohol, Liquid paraffin, white soft paraffin, Propylene glycol, Chlorocresol, Beeswax, Sodium dihydrogen phosphate, Titanium dioxide, Lavender oil, Methylparaben, and Purified water. It was concluded that the best formulation selected F7 was safe in respect to natural herb offer diverse benefits in cream preparations, as evidenced by this study, highlighting the safety compared to commercial semisolid products. The study showed that

the extract of *Aloe inermis* could be used as an alternative for wound healing activity as cream NDDS formulation.

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