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

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ABSTRACT
The current study set out to create a herbal cream with antibacterial properties. *Jatropha variegata* is one species of Euphorbiaceae family, it has antimicrobial agent for wound healing activity against many species of bacteria. Wounds affect a large number of patients and can seriously reduce the quality of life. The purpose of this study was designed to formulate the most active extract as oil in water cream (O/W) and evaluate its wound healing activity of *Jatropha variegata* extract. The selection of ingredients is done based on the different medicinal and therapeutic properties of the agents. The herbal creams (O/W) were formulated from methanolic *Jatropha variegata* extract. Four formulations were prepared with various concentration of excipients. In quality evaluation of formulation (F1-F4) were performed on different parameters like pH, viscosity, spreadability, washability, irritancy and phase separation. There were no changes observed in physical properties of herbal cream. The formulation showed good spreadability, no phase separation and good consistency. F4 is better formulation than F1, F2 and F3 of formulation of multipurpose herbal cream. The pH of prepared cream was near to skin pH, and cream was homogeneous, non-greasy and easily removed after application. It was concluded that the best formulation selected F4 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 *Jatropha variegata* could be used as an alternative for wound healing activity as Formulation cream NDDS.

KEYWORDS: *Jatropha variegata*, Cream Formulation, Antimicrobial agent, Wound Healing Activity, Nutraceutical Drug Delivery Systems.

INTRODUCTION

Background of The Genus *Jatropha Variegata*[1,30]

Jatropha variegata is a large genus (family Euphorbiaceae) with an interesting geographical distribution, it is a very diverse subtropical and tropica genus including succulents, caudicifonn species Herbaceous perennial and woody trees. A few species is found in Arabia and India. In Yemen, there are about seven species including J.curcus, J. ldhfarica, J. glauca, J. Pelargoniifolia, J. spinosa and two are endemic including including J. unicostara and J. variegata. Some species including J. curcas, J. podagrica., J. multifida, and J. gossypiifoIia, are employed as traditional herbal medicines due to their anti-platelet-aggregative, antioxidating, diuretic, antifungal, and anti-inflammatory activities. A number of bioactive natural products, mainly diterpenes and triterpenes, have been reported in the extract of Jatropha species, and these are a promising bio-resource for the development of potential drugs and other products. The chemical constituents reported so far from genus Jatropha total to ca. 143 including diterpenes, triterpenes, lignans and coumarins flavonoids, alkaloids, phytosterols, and some others (. A number of Jatropha species especially J. curcas yield oils of biofuel value which has brought much attention to the genus especially in India. The oil is also used for manufacture of candles and in cosmetics industry.

Botanical Character of *Jatropha Variegata*

It is a shrub with round branches, the bark at bottom brown and chunky, glaucous above. leaves sub petioles, alternate, blunt, ending in a stiff dagger-point, very smooth, sometimes variegated on the upper surface, two inches long (Figure 1). Stipules in pairs on each side by

the sole of the petiole, placed on a callus awl-shaped, stiff, often longer than the petiole, divaricating, permanent, the lower like prickles. Corymbs axillary, shorter than the leaf. Bracts lanceolate, keeled. Calyx five-parred, with oblong segments. Corolla five paired; petals oblong. Anthers eight. capsule smooth, oblong (Figure 2). it varies with broader and narrower leaves.



Fig. 1: Leaves and Fruits of *Jatropha Variegata*



Fig. 2: Leaves and Fruits of *Jatropha Variegata*.

Traditional uses of Jatropha Species

Jatropha species have been use as medicinal plants by native people in the tropical and subtropical countries. Species from this genus have been popular to cure stomachache, toothache, swelling, inflammation, leprosy, dysentery, dyscrasia, vertigo, anemia, diabetes, as well as to treat HIV and tumor, ophthalmia, ringworm, ulcers, malaria, skin diseases, bronchitis, asthma and as an aphrodisiac. They are also employed as ornamental plants and energy crops.

Phytochemistry *Jatropha variegata* previous study has reported the steroids and flavonoids from the leaves of *Jatropha variegata*.[3] An old study has reported five components from the stem of *Jatropha variegata* that is kaempferol 3-O-α- L-rhamnopyranoside, 3-0- α - Larabinopyranoside, 3-O-P-D-glucopyranoside, 3-O-P-D-galactopyranoside and kaempferol.

Wound Healing[1,57]

Wound is defined as disruption of cellular, anatomical, and functional continuity of a living tissue. It may be produced by physical, chemical, thermal, microbial, or immunological insult to the tissue. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis (the deepest skin layer) begin to increase collagen (connective tissue) production. Later, the epithelial tissue (the outer skin) is regenerated. Wound healing is the interaction of a complex cascade of cellular and biochemical actions leading to the restoration of structural and functional integrity with regain of strength of injured tissues. It involves continuous cell-cell interaction and cell-matrix interactions that allow the process to proceed in different overlapping phases and processes including inflammation, wound contraction, re-epithelialization, tissue remodeling, and formation of granulation tissue with angiogenesis. The phases of wound healing normally progress in a predictable, timely manner, and if they do not, healing may progress inappropriately to either a chronic wound such as a venous ulcer or pathological scarring such as a keloid scar.

The Four Phases of Wound Healing

It is believed that chronic wounds also undergo 4 basic phases of healing (although some authors combine the first 2 phases). first, Hemostasis; In wound healing, the platelets are the cells that act as utility workers sealing off the damaged blood vessels. The blood vessels themselves constrict in response to injury, but this spasm ultimately relaxes. The platelets secrete vasoconstrictive substances to aid this process, but their prime role is to form a stable clot sealing the damaged vessel. platelets also secrete growth factors such as platelet-derived growth factor, which is recognized as one of the first factors in initiating the subsequent healing steps. These growth factors recruit neutrophils and monocytes (initiating the next phase of wound healing), stimulate epithelial cells and recruit fibroblasts. Second, Inflammation, this stage usually lasts up to 4 days post injury. The inflammatory response causes the blood vessels to become leaky, releasing plasma and neutrophils into the surrounding tissue. The neutrophils phagocytose debris and microorganisms and provide the first line of defense against infection. As they digest bacteria and debris, neutrophils die and release intracellular enzymes into the surrounding matrix, which further digest tissue. As fibrin is broken down as part of this clean-up, the degradation products attract the next cells involved such as fibroblasts and epithelial cells. They are aided by local mast cells. Third, Proliferation (also known as granulation and contraction) approximately 4 days after wounding and usually lasts until day 21 in acute wounds, depending on the size of the wound and the health of the patient. It is characterized by angiogenesis, collagen deposition, granulation tissue formation, wound contraction and epithelialization. Clinically, proliferation is observed by the presence of pebbled red tissue or collagen in the wound base and involves replacement of dermal tissues and sometimes subdermal tissues in deeper wounds, as well as contraction of the wound. Fourth, Remodeling (also known as maturation) in wound repair, the healing process.

involves remodeling and realignment of the collagen tissue to produce greater tensile strength. In addition, cell and capillary density decrease. The main cells involved in this process are the fibroblasts. Remodeling can take up to 2 years after wounding. This explains why closed wounds can quickly breakdown if attention is not paid to the initial causative factors.

Semisolid Drug Delivery Systems[58,150]

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 semi-solid 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. Herbal ingredients like Neem have antifungal and anti - inflammatory properties, and it is used to reduce scar, pigmentation, redness and itching of the skin.

Research Paths[86,166]

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 *Jatropha variegata* 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 *Jatropha variegata* 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 *Jatropha variegata* extract as shown in Table 1.

Wound Healing Activity

The antibacterial and wound healing activity was studied in a previous study.[3]

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 path (Scichemtech, USA), Autoclave (Shanghai.5117.Co, China.

Table 1: Materials Used in Cream Formulation with *Jatropha Variegata* Extract.

Materials	Company	Country
White Soft Paraffin	Merk	Germany
Ceto Stearyl Alcohol	Yuyu	China
Sodium Lauryl Sulfate	Merk	Germany
Citric Acid Anhydrous	Merk	Germany
Dibasic Sodium Phosphate	Merk	Germany
Chlorocresol	Benzo chemind	China
Liquid Paraffin	Merk	Germany
Titanium Dioxide	Zhongbac	China
Tween	Merk	Germany

Formulation and Evaluation of *Jatropha Variegata* Cream Novel Drug Delivery Systems[58,169]

Formulation of *Jatropha Vvariegata* Cream Novel Drug Delivery Systems

Begin the process by heating liquid paraffin, white soft paraffin and sodium lauryl sulfate in a borosilicate glass beaker at 75 ºC, maintaining this temperature (Oil phase). In a separate beaker, dissolve dibasic sodium phosphite, citric acid anhydrous, and chlorocresol in distilled water at 75ºC 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 *Jatropha variegata* 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 ± 2ºC) in tubes. As shown in Table 2.

Table 2: Composition of *Jatropha Variegata* Cream Formulations.

NO	Ingredients		Formulation Code			
			F1	F2	F3	F4
1		***Jatropha variegata* Extract**	1	1	1	1
2	**Oil Phase**	**Sodium Lauryl Sulfate**	0.7	0.15	0.15	0.15
3		**White Soft Paraffin**	3.692	3.742	2.560	2.482

4		**Ceto Stearyl Alcohol**	---	---	---	1.5
5		**Liquid Paraffin**	1.2	1.9	1.9	1.2
6		**Tween**	---	---	0.9	---
7	**Aqueous Phase**	**Purified Water**	Qs	Qs	Qs	Qs
8		**Dibasic Sodium Phosphite**	0.275	0.955	0.275	0.275
9		**Citric Acid Anhydrous**	0.069	0.069	0.069	0.069
10		**Chlorocresol**	0.015	0.015	0.015	0.015
11		**Lavender Oil**	---	0.12	---	0.012
12		**Titanium Dioxide**	---	---	0.42	0.12

Evaluation of *Jatropha Variegata* 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 cream was measured by using Viscometer.

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 ($1cm^2$) 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℃ 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 four 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	Brown	Pleasant	Smooth	Semisolid
3	F3	---	---	---	---
4	F4	Light Green	Pleasant	Smooth	Semisolid

Phase separation: Prepared cream was kept in a closed container at a temperature of 25-100℃ 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 F3. While the F2 and F4 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	Phase Separation
4	F4	No Phase Separation

pH: According to the results, the pH of the three formulations that is F2, F3 and F4 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	6.2
3	F3	6.4
4	F4	6.6

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 were easily washable. As shown in Table 6.

Table 6: Washability Observations.

NO	Formulation Code	Washability
1	F1	---
2	F2	Easily Washable
3	F3	---
4	F4	Good Washable

Irritancy: Mark the area ($1cm^2$) 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 is F2 and F4 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	---	---	---
4	F4	Nil	Nil	Nil

Spreadability: The spreadability of the formulations that is F2 and F4 was carried out and out of that for F4 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 F4 showed better spreadability. As shown in Table.

Table 8: Spreadability Observations.

NO	Formulation Code	Spreadability
1	F1	---
2	F2	Low
3	F3	---
4	F4	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 and F4 formulations were non-greasy. As shown in Table 9. According to the results of viscosity test, we can say that F4 formulation was good viscosity. As shown in Table 10.

Table 9: Greasiness Observations.

NO	Formulation Code	Greasiness
1	F1	---
2	F2	Non-greasy
3	F3	---
4	F4	Non-greasy

Table 10: Viscosity Observations.

NO	Formulation Code	Viscosity
1	F1	---
2	F2	Low
3	F3	---
4	F4	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. One month stability study was carried out at normal room conditions the results were unchanged.

All of the formulated creams were evaluated for pH, viscosity, washability, irritability, color and spread ability in this study, *Jatropha variegata* 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

Jatropha variegata is one species of Euphorbiaceae family, it has antimicrobial agent for wound healing activity against many species of bacteria. Certain concentrations of *Jatropha variegata* extract were used to prepared the four cream formulations NDDS with different excipients. It can used as wound healing activity, The evaluation tests were including pH, Spreadability, homogeneity, physical evaluation, stability and irritancy. The stability of the formulation at room temperature suggests its safe application on the skin. Prepared formulation shows good speadability, washability, no phase separation and good consistency during the study period, based on the results the formulation F4 has shown better characteristics compared to F1, F2 and F3. It was concluded that the best formulation selected F4 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 *Jatropha variegata* could be used as an alternative for wound healing activity as Formulation cream NDDS.

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