International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 21s, 2025
https://theaspd.com/index.php

Formulation And Evaluation Of Wound Healing Herbal Gel From Achyranthusaspera Extract

Anju Kalyankar[1], Poonam R.Mulay[1], Kiran Makhija[1], Sarita Sasane[1], Tejashree Deokule[1], Kalyani Sahare[1], Aboli Marale[1], Kajal Bhagat[1]
[1]Dr. D.Y. Patil College of Pharmacy, Akurdi, Pune: 411044, M.S., India.
Corresponding Author: Anju Kalyankar, Dr. D.Y. Patil College of Pharmacy, Akurdi, Pune: 411044, M.S., India. academicanju@gmail.com

ABSTRACT:
The leaves of Achyranthesaspera L. (Amaranthaceae) have been used traditionally for the treatment of wound in various parts of Ethiopia.AchyranthesasperaLinn (Amaranthaceae), commonly Known as apamarga, is a commonly available plant in India. This plant has been used in the treatment of cuts by the people living in Tamil Nadu. The various fractions of leaves of Achyranthesaspera L. have not yet been explored scientifically for in-vivo wound healing and anti-inflammatory activities.The extracts of these plants can further be developed into phytomedicines for the management of septic woundsthe enhanced wound healing may be due to the free radical scavenging action and immune-enhancing property.
Keywords: *wound healing, apamarga, gel, phenolic content.*

INTRODUCTION:

Wounds are physical injuries that result in an opening or break of skin.They have significant impact on public health care resources.[1]Decoction of whole plant of Achyranthesasperahas diuretic properties and it used in pneumonia.[2]Achyranthesaspera Linn. is a species of plant in the Amaranthaceae family, commonly known as Apamarga or Chirchita in Hindi. It is a popular medicinal plant which has occupied a pivotal position in folk medicine throughout the tropical Asian and African countries.[5]Plant is pungent, purgative, diuretic and is used in dropsy, for piles, boils, and skin eruptions, colic and externally for snake-bite.[12]

Herbal gel:

Gel is a solid / semisolid system of at least 2 constituents, constiting of condensed massenclosing & interpenetratedby liquid. Gel & jellies are composed of small amounts of solids dispersed in large amount of liquid. [26] Herbal gel is solid, jelly like substance that can have properties ranging from soft and weak to hard and tough preparation. It is used topically for a variety of purposes, such as protectants, antiseptics, and antimicrobials.[27]

Properties of gel:

1. The gelling agent must be inert, safe and cannot react with other constituents.
2. The gelling agent should produce a functional solid-like nature at the time of storage which is easily broken when exposed to shear forces produced by squeezing the tube, trembling the bottle or at the time of topical application.
3. The topical gel must not be gluey.
4. The ophthalmic gel must be sterilized.
5. The apparent viscosity or gel strength increases with an increase in the effective crosslink density of the gel.
6. They exhibit the mechanical features of the solid state.
7. Each constituent is continuous throughout the system.
8. There is high degree of attraction amongst the dispersed phase and water medium so the gels remain equally distributed upon standing and doesn't freely settle. [29]

Topical Drug Delivery System:

1. Advantages of Topical Drug Delivery System:

- Avoidance of first pass metabolism.
- Convenient and easy to apply.

- Avoidance of the risks and inconveniences of intravenous therapy and of the varied conditions of absorption, like pH changes, presence of enzymes
- Achievement of efficacy with lower total daily dosage of drug by continuous drug input.
- Avoids fluctuation in drug levels, inter- and intrapatient variations.
- Ability to easily terminate the medications, when needed
- A relatively large area of application in comparison with buccal or nasal cavity
- Ability to deliver drug more selectively to a specific site.
- Providing utilization of drugs with short biological half-life
- Improving physiological and pharmacological response.[26]

2. Disadvantages of Topical Drug Delivery System:

- Skin irritation of contact dermatitis may occur due to the drug and/or excipients.
- Poor permeability of some drugs through the skin.
- Possibility of allergenic reactions.
- Can be used only for drugs which require very small plasma concentration for action
- Enzyme in epidermis may denature the drugs
- Drugs of larger particle size not easy to absorb through the skin.[26]

AIM AND OBJECTIVES:

Aim: The formulation and testing of herbal gel for wound healing activity.

Objectives: The primary goal of herbal medicine is to produce long lasting effects with fewer negative effects which is affordable alternativeto synthetic medications that is more effective.

Need of Work:

Topical application of natural AchyranthesAspera extract herbal gel produce highest outcomes with fewer negative side effects. New opportunities in pharmaceutical industry have been made possible by the rising demand for natural products. To enhance customer demand for herbal products.

PLAN OF WORK:

1. Literature Survey.
2. Selection and Collection of plant material and excipients.
3. Phytochemical and Pharmacognostic study of AchyranthesAsperaextract.
4. Preparation of herbal gel
5. Evaluation of herbal gel

- Physical Appearance
- Determination of pH
- Homogeneity
- Spreadability
- Viscosity

PLANT PROFILE:

Morphology:

Achyranthesaspera L. is an erect or procumbent, annual or perennial herb of about 1-2 meter in height, often with a woody base. Stems angular, ribbed, simple or branched from the Base, often with tinged purple colour.[6]

Taxonomic classification:

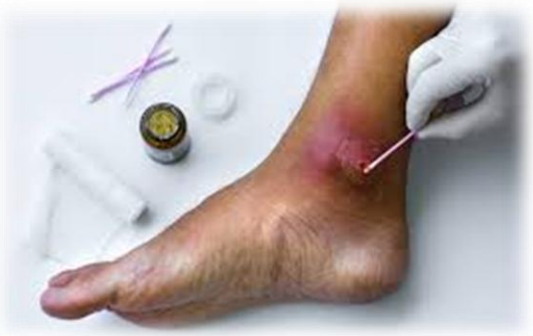
Kingdom: Plantea
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Caryophyllales
Family: Amaranthaceae
Species: Achyranthusaspera[6]
Synonym:
Sanskrit: Aghada
Hindi: Chirchira[6]
Biological Source: Dried whole plant of Achyranthusaspera

Active constituents of Achyranthusaspera are as follows:

Earlier phytochemical studies reported that it contains saponins, alkaloids (betaine, achyranthine), amino acids, steroids (stigmasterol), triterpenoids (oleanolic acid and its glucoside), phenolic content (indole acetic acid oxidase), and flavonoids [21]

A) Alkaloid:

1. Betaine - Betaine/polyhexanide physically removes microbes and the biofilm, along with slough and debris from wounds. It decreases lipid peroxide levels and increases glutathione (GSH) levels, which indicates its anti-oxidant property. [15]



2. Achyranthine - It possessanti - oxidant & anti - inflammatory activity.[16]

B) Amino acid:

1. Glutamine - decreases infectious complications but has not been shown to benefit wound healing directly.

2. Arginine - improves collagen accumulation and improves healing time.[17]

C) Steroids: Stigmasterol-possess anti parasitic activity, antifungal activity, anti-inflammatory activity.[18]

D) Triterpenoids: In surgical wounds, the triterpenes induced a reduction in time to closure, and this effect was reported in virtually all wound types. Triterpenes also modulate the production of ROS in the wound microenvironment, accelerating the process of tissue repair.[19]

E) Flavonoids: Flavonoids, which are prominently known for their wound-healing properties.[20]

Medicinal properties:

1.Anti – inflammatory
2.Anti – allergic
3.Anti – fungal
4.Expectorant
5.Antibacterial.[9]

DRUG ACTIVITY:

Wound healing:

Wound infection is one of most common disease in developing countries because of poor hygienic conditions.[2] Healing is a complex process that involves a series of biochemical and cellular reactions initiated in response to an injury that restores the function and integrity of damaged tissues. Wound healing involves platelet aggregation and blood clotting, formation of fibrin, an inflammatory response to injury, angiogenesis and re-epithelization.[24]

In east Africa the plant is used for treating tonsilitis,head wounds and ringworm.[10] Achyranthusaspera known as apamarga is herb that grows wild & abundantly in India. Folkpractitioner& local people of kanyakumari district use leaves of this plant for healing wound pharmacological properties such as antimicrobial, analgesic,antipyretic,anti-inflammatory, immunostimulant, antioxidant, antifertility, hypoglycemic, diuretic, hypolipidemic, cardiac stimulant, antihypertensive, antinoiceptive, prothyrodic, antispasmodic and hepatoprotective properties.[25]

Mechanism of action:

Phases of Normal Wound Healing- Cellular and molecular events during normal wound healing progress through four major integrated, phases of haemostasis, inflammation, proliferation and remodelling.**Haemostasis Phase**- At the time of injury, the fibrin clot forms the provisional wound matrix and platelets release multiple growth factors initiating the repair process.**Inflammation Phase**- Within a day following injury, the inflammatory phase is initiated by neutrophils that attach to endothelial cells in the vessel walls surrounding the wound (margination), change shape and move through the cell junctions.**Proliferation Phase** - Fixed tissue monocytes activate, move into the site of injury, transform into activated wound macrophages that kill bacteria, release proteases that remove denatured ECM, and secrete growth factors that stimulate fibroblasts.[22]

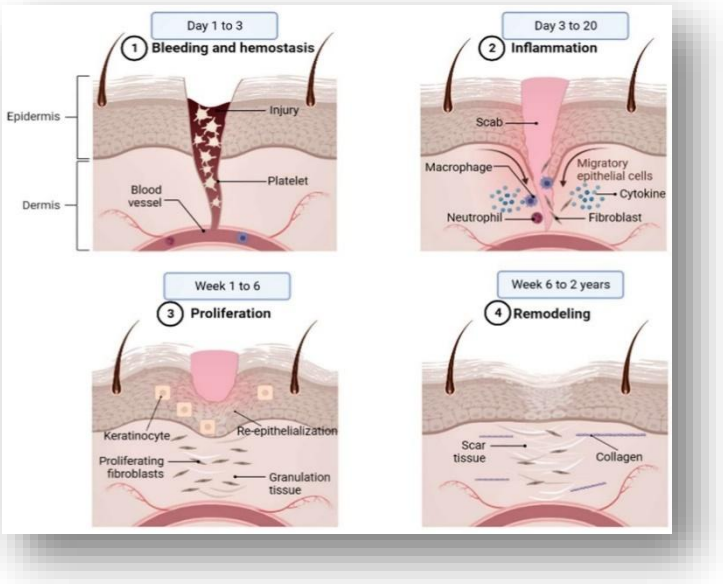
Fibroblasts migrate into the wound in response to multiple soluble mediators released initially by platelets and later by macrophages.The collagen, proteoglycans and other components that comprise granulation tissue are synthesized and deposited primarily by fibroblasts.The process of angiogenesis is stimulated by local factors of the microenvironment including low oxygen tension, low pH, and high lactate levels.Granulation tissue is a transitional replacement for normal dermis, which eventually matures into

a scar during the remodelling phase of healing. Dermal wounds heal by three basic mechanisms: contraction, connective tissue matrix deposition and epithelialization. Wounds that remain open heal by contraction; the interaction between cells and matrix results in movement of tissue toward the center of the wound. Remodelling is the final phase of the healing process in which the granulation.[22]

Summary of acute wound healing

There are four phases of wound healing:

Haemostasis – establishes the fibrin provisional wound matrix and platelets provide initial release of cytokines and growth factors in the wound.



Inflammation - mediated by neutrophils and macrophages which remove bacteria and denatured matrix components that retard healing, and are the second source of growth factors and cytokines. Prolonged, elevated inflammation retards healing due to excessive levels of proteases and reactive oxygen that destroy essential factors.

Proliferation - fibroblasts, supported by new capillaries, proliferate and synthesize disorganized ECM. Basal epithelial cells proliferate and migrate over the granulation tissue to close the wound surface.

Remodelling – fibroblast and capillary density decreases, and initial scar tissue is removed and replaced by ECM that is more similar to normal skin. ECM remodelling is the result of the balanced, regulated activity of proteases.[22]

Mechanism of action of natural contents:

1.Anti- oxidants:

Reactive oxygen species (ROS) are small molecules derived from oxygen that act as oxidizing agents and contribute to cell damage; therefore, they play a crucial role in preparing the normal wound healing response.[23]

2.Anti- inflammatory:

Inflammation is important to to prevent microbial infection, remove dead cells & cellular debris.[23]

3.Antimicrobial:

In normal process of wound repair, infection is prevented by immune system's rapid mechanism to eliminate invading pathogens.[23]

MATERIALS AND METHODS :

Plant material: Dried whole plant of Achyranthusasperaare takenphytochemicals present in Achyranthusasperaare alkaloid, flavonoids, tannins, terpenoid,saponin, glucoside, steroids. Powder of whole plant of AchyranthusAsperaissued from ManikarnikaAyurvedic Medicine shop, Chinchwad.

Preparation of Achyranthesasperaextract -Fresh stems and roots of A. Asperawere collected and dried under shade and then in an oven at a temperature <50°c. Whole Plant were ground into a fine powder. Then, 25 g of powdered stems and roots were boiled in 500 ml of distilled water separately in two flasks for 1/2 hr. 500 milliliters of filtrate was obtained from the whole plant powder, which was then filtered using a filter paper. It was reduced to obtain a solid residue of 3 g by heating it at 60°c. The solid residue obtained was then stored at a low temperature. Dimethyl sulphoxide was used to dissolve the solid residue made from the stems and roots to make different concentrations.[11]

Procedure: below mentioned ingredients are for 75 gm gel formulation

Table 1. Control Batch

Ingredients	Role	Quantity
Aspera Extract	Wound healing	1 gm
Distilled water	Vehicle	40 ml
Triethanolamine	Buffer	2 Drops
Carbapol	Gelling agent	1.5 gm
Methyl paraben	Preservative	1 gm
Glycerine	Humectant	1.5 ml
Propylene glycol	solvent	30 ml

Table 2: Developed herbal gel formulation

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
Extract	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm
Water	40 ml	40 ml	40 ml	40 ml	40 ml	40 ml	40 ml	40 ml
Triethanolamine	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops	2 drops
Carbapol	1 gm	0.8 gm	0.6 gm	1.25 gm	1.7 gm	1.5 gm	1.6 gm	2.6 gm
Methyl paraben	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm	1 gm
Glycerine	2 ml	2.2 ml	2.4` ml	1.7 ml	1.25 ml	1.5 ml	1.4 ml	0.4 ml
Propyl paraben	30 ml	30 ml	30 ml	30 ml	30 ml	3o ml	30 ml	30 ml

Preparation of gel -

1.5 gm carbapol was dissolved slowly with stirring in 30 ml of demineralized water for 1 h to avoid agglomeration. Then 2 drops oftriethanolamine were dissolved in 10 ml of demineralized water separately and stirred for 10 min. Now Mixed1.5 ml of glycerine in abovetriethanolamine mixture with stirring for 10 min. Glycerin and triethanolamine solution were added to gelatin solution and the pH was then adjusted to 7.4 by stirring the solution for 10 min. Stir it for 10 min until a clear consistent gel base was obtained.Add extract in gel base .[7]

EXPERIMENT WORK:

Phytochemical evaluation:

1.Test for Tannins:

About 2 ml of 5% ferric chloride was mixed with 1 ml of the extract. The presence of tanninswas indicated by the observation of a blue-black precipitate.[13]

2.Test for Flavonoids:

Lead acetate test- Extract was taken and few drops of 10% lead acetate solution was added. Appearance of yellow colour precipitate indicates the presence of flavonoids.[14]

3.Test for Alkaloids:

About 1 ml of the extract was mixed with 2 ml of 2N HCL and Mayer's reagent (K2[HgI4] solution). The existence of alkaloids was indicated by the development of slimy white precipitate.[13]

4.Test for Amino Acid

Ninhydrin test - About 0.5 mg of extract was taken and 2 drops of freshly prepared 0.2% ninhydrin reagent were added and heated. The appearance of pink or purple colour indicates the presence of proteins, peptides or amino acids. [14]

5.Test for Phenols:

About 1 mL of the extract was combined with three drops of $FeCl_3$ &1 mL of $K_2Fe(CN)_6$of greenish-blue forms confirmed the presence of phenols.[2]

Table 3: Tests for Phenol

Phytochemical Test	Result
Tannin	+ve
Alkaloid	+ve
Phenols	+ve
Amino Acid	+ve
Flavanoids	+ve

Evaluation Of Extract Powder:

Pharmacogenetic Evaluation:

A)Oraganoleptic characters:

- Colour: greenish yellow
- Odur : Odurless

B)Physicochemical characters :

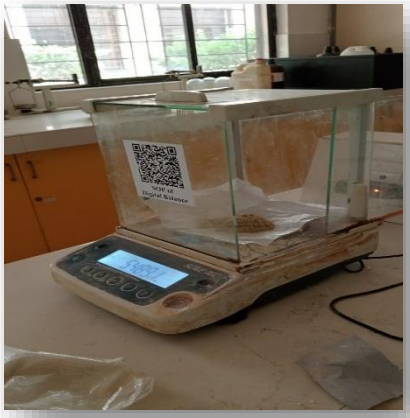
1. Moisture content –

- Empty china dish weight = 69.17 gm
- China dish + 10 gmAspera powder = 79.17gm
- Weight of dish + 10gm powder after heating for ½ hr = 78.52 gm
- Moisture content = 0.67 gm/m^3

2. Tap density = 0.34 g/ml

3.Bulk density = 0.23 g/ml

4.Hausners ratio = Tapped density/ Bulk density
= 0.34 / 0.23
= 1.478



5.Ash value –

- Empty Silica crucible weight = 14.5 gm
- Silica crucible + 2 gm Aspera powder = 16.5 gm
- Ash value = 14.78 gm

Evaluation Of Gel :

1.pH : The pH AchyranthusasperaL. is an erect or procumbent, annual or perennial herb of about 1-2 meter in height, often with a woody base. Stems angular, ribbed, simple or branched from the base, often with tinged purple colour of polyherbal gel formulationWas determined by using pH meter.[8]

2.Appearance and Homogeneity: All developed gels Were tested for physical appearance and homogeneity by visual observation.[8]

3.Viscosity: The measurement of viscosity of the Prepared polyherbal gel was done with Digital Viscometer.The reading was Taken at 100 rpm using spindle no. 4.[8]

4.Spread ability: The spread ability of the gel Formulations was determined by measuring the Spreading diameter of 1 g of gel between two horizontal Plates (20 cm × 20 cm) after one minute.[8]

Table 4: The spreadability of the gel Formulations

ameter	F1	F2	F3	F4	F5	F6	F7	F8
	4-5	4-5	5-6	5-6	6-7	7-8	7-8	8-9
pearance	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brown	Light Brow
mogeneity	Homogenous	Homo		Homogenous	H	ous	Homogenous	Homogen
cosity	10000.3 mPa.s	9032.		7215.4 mPa.s	6	Pa.s	3005.3 mPa.s	2547.4 mI
eadability	7	8		9	1		12	12

5. SOP of Zone of inhibition Test for Antibacterial activity

Principle: Kirby - Bauer Diffusion method is employed for antibiotic sensitivity test based on the fault.

Material require:

- Sterile Petri plate
- Sterile forceps
- Sterile swabs
- Nutrient broth culture
- MHA(Muller Hinton Agar)
- Antibiotic s/Samples
- Autoclave
- Incubators
- Laminar air flow chamber
- Weighing Balance

Preparation of plate:

Sterile MHA was poured in to plates, the depth of the medium should be approximately 4mm. After solidification the plates were dried for 30 minutes in an incubate to remove excess moisture from the surface.

Preparation of inoculum:

Isolates used for sensitivity test 5-6 colonics of S.aureus selected & inoculated the colonies in to. Nutrient both with the help of wire loop.The broth culture incubated at 35-37 degree C for 2-5 hrs.It is using for the test

Inoculation:

A sterile cotton swab dipped in to the diluted inoculum and swab on MHA media Petri plates, isolate the swab inside the way of the tube to remove excess inoculum. The lid of Petri dishes closed and kept at room temperature for 5-10 minutes to dry the inoculum confluent growth is desirable.

Preparation of well:

Prepare well at 7mm diameter in each plates with the help of syringe, keep distance for zone of inhibition. Removed agar should be discarded. This well is used for the application of drug or samples.

Application of sample on well:

Apply disc on each well which is inoculated with culture of S.aureus

Incubation:

Incubate all plates at 37°C 24-48 hrs.

Observation:

After Incubation observed the zone of Inhibition around the each well.

Result:

Measured the zone of inhibition of sample against bacteria.

RESULTS AND CONCLUSION:

Results:

The major findings of study was to demonstrate significance of Achyranthesasperaextract in wound healing. As per pharmacogenetic studies formulation F6 was found to be optimum in gel consistency, spreadability, etc. From the results it can be concluded that Achyranthesasperaextract when formulated as gel shows significant improvement in wound healing.

Parameter	Result
Colour	Green
Moisture Content	0.67 gm/m^3
Ash Value	14.78 gm
Tap Density	0.34 gm/ml
Bulk Density	0.23 gm/ml

CONCLUSION:

In view of above facts, the medicinal plant, Achyranthesaspera L. Traditionally, this plant using since Vedic period to present days using in the treatment of many diseases. It is seen from the literature that AchyranthesAspera is a very important plant for its large number of medicinal properties. The prepared herbal gel was found to have high wound healing & anti-microbial activity against wounds.Study investigated the antimicrobial activity and characterized the functional phenolic content in Achyranthesaspera .

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