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Formulation and Evaluation of Tridax procumbensHydrogel Film for Wound Healing

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ABSTRACT: This study focuses on addressing the specific needs of diabetic patients by introducing a novel approach to wound healing. The objective is to develop and evaluate a herbal hydrogel film derived from Tridax procumbens. The aim is to assess its efficacy in accelerating the healing process of open wounds, as thehealing process is reported to be slow in diabetic patients. Wounds are common in diabetics andnecessitate particular wound care measures to avoid consequences includingulceration and infection. With benefits including moisture retention, simplicity ofadministration, and the capacity to foster the ideal environment for wound healing,hydrogel-based films have become a viable option for wound care.

Hydrogels, which are composed of cross-linked hydrophilic polymers have special qualities including high water content and biocompatibility, whichmake them appropriate for use as wound films. Their capacity to sustain a moistwound environment promotes wound healing by facilitating angiogenesis, cellmigration, and the deposition of extracellular matrix components. This study explores the possibility of using the herb Tridax procumbens, whichhas been used traditionally for medical purposes in the form of a hydrogel to speed up the healing process ofwounds. As part of theevaluation method, factors like pH, swelling index, irritancy, etc. are evaluated tomake sure the formulation is safe and effective.The goal of this research is to accelerate wound healing by using a new herbal hydrogel film of Tridax procumbens. The formulation has the potential to expedite wound care, particularly for individuals with diabetes.

KEYWORDS: Tridax procumbens, hydrogel-based films, wound healing, anti-inflammatory

I. INTRODUCTION

Tridax procumbens

T. procumbens is a member of the Asteraceae family of weeds. Of the thirty speciesof the genus Tridax, it is one of the strongest. T. procumbens is used as a naturalremedy for wound healing in Ayurveda. In Maharashtra, Madhya Pradesh, Gujrat,Odisha, and other Indian states it grows inlawns, meadows, croplands, roadsides, and open areas.Other names for T. procumbens include "Jayanti Veda" (Sanskrit), "Gharma"(Hindi), "Dagadipala" (Marathi), "Coat button" and "Tridax daisy" (English),"Vettukkaaya-thalai" (Tamil/Siddha), and "Akala kohadi."

Phytochemical and pharmacological investigations have been conducted on T. procumbens. Various compounds are isolated and identified as flavonoids, essentialoils, saponins, tannins, steroids, alkaloids, carbohydrates, carotenoids, and terpenoids. Numerous pharmacological properties are reported for this plant namely antibacterial, antioxidant, anticancer, wound healing,antileishmanial, anti-inflammatory, vasorelaxant, and larvicidal.

The wildly growing herb T. procumbens, available throughout India, Nepal, and Nigeria,is used to cure inflammation, dysentery, diarrhea, and bronchial catarrh. Ayurvedareports usage of the plant alone or in combination with other preparations to treatwounds. The leaf is used to stop bleeding from cuts, bruises, and wounds. It alsohas insecticidal and anti-parasitic effects. Since ancient times, people have used thejuice of the fresh leaf of T. procumbens to cure wounds, skin conditions, typhoid fever,fever, cough, and to prevent blood clotting.[1,2]

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Fig. 1:Tridax procumbens

Open wounds:
Open wounds are injuries that involve a break in the skin and leave the internal tissue exposed.

Types of open wounds:
I. Laceration: It refers to a skin tear that can extend to underlying tissues, caused typically by blunt force trauma. Unlike abrasions, there is no loss of skin. For deep or extensive lacerations, medical attention may be necessary especially if a cut shows persistent bleeding.
II.Puncture Wound: A puncture wound involves the creation of a hole through all layers of the skin due to accidental contact with sharp objects like needles or nails. Animal bites can also lead to puncture wounds. Immediate medical attention is crucial in such cases since exposure to metal and animal bites can be risky. Metals are often contaminated, and animal bites can introduce bacteria or viruses from the animal's mouth, potentially causing rapid and severe infections if not promptly treated by a medical professional.
III.Burn Wound: Burns occur due to excessive exposure to various agents such as heat, electricity, radiation, chemicals, or lasers. While minor burns can be managed at home, serious burns necessitate immediate medical intervention.

Burn injuries can be categorized by their severity:
● First-degree burns are those that only affect the outer layer of skin (epidermis), leading to redness and discomfort.
● Second-degree burns extend into the dermis resulting in blistering.
● Third-degree burns penetrate deep into the fatty layer beneath the skin, often damaging nerves.

IV.Avulsion: An avulsion, or skin tear, involves a deep rupture of the skin, separating it from the underlying tissue. Such wounds are usually more severe and extensive. Common causes include accidents involving traffic or machinery. In cases where avulsions affect a large area of skin, doctors often consider skin grafting as part of the treatment and reconstructive process.[3]

Wound Healing

Wound healing is a natural physiological reaction to tissue injury involving intricate interaction of the vascular system, cytokines, mediators, and a variety of cell types. The initial cascade of blood vessel constriction and platelet aggregation is intended to halt bleeding. A variety of inflammatory cells begin to infiltrate after this, beginning with neutrophils. In response, these inflammatory cells release a range of cytokines and mediators that encourage thrombosis, angiogenesis, and reepithelialisations. In response, the fibroblasts deposit extracellular elements that will act as scaffolding.[4] The process of wound healing occurs in three phases as depicted in **Fig. 2**.

- Hemostasis, chemotaxis, and enhanced vascular permeability are features of the inflammatory phase that help to stop more damage, seal the wound, eliminate germs and cellular debris, and promote cellular migration. The inflammatory stage typically lasts for a few days.
- The development of granulation tissue, reepithelialisation, and neovascularization are features of the proliferative phase. This stage may extend for a few weeks.
- The wound reaches its maximum strength during the remodeling and maturation stages

Natural remedies that are used for wound healing Aloe Vera: Aloe vera promotes wound healing by enhancing collagen production, reducing inflammation, and maintaining skin moisture, especially effective for first and second-degree burns.
Some natural substances that aid in wound healing include:

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 3 May-June 2025, pp: 2000-2010 www.ijprajournal.com ISSN: 2456-4494

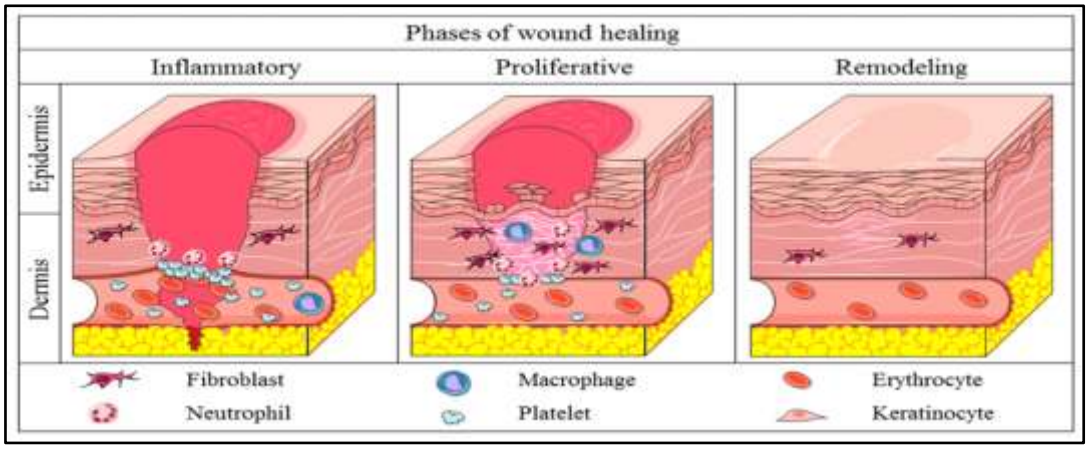

Fig. 2:Phases of wound healing

1. Honey: Honey accelerates wound healing, reduces scarring, and prevents infection due to its antibacterial and anti-inflammatory properties, particularly helpful for burns and partial-thickness wounds[5.]
2. Turmeric Paste: Turmeric, through its active compound curcumin, speeds up wound healing by boosting growth factors and collagen production, best used on minor closed wounds[6] .
3. Garlic: Garlic, containing allicin, exhibits antimicrobial and anti-inflammatory effects that aid wound healing, though more human studies are needed to confirm clinical efficacy[7].
4. Coconut oil is hydrolysed in wounds into lauric acid and monolaurin, which have broad-spectrum antimicrobial effects in vitro[8].

Hydrogels

Hydrogels are three-dimensional networks composed of hydrophilic polymers that are cross-linked either chemically or physically, allowing them to swell without dissolving. These structures absorb polar wound exudates and facilitate oxygen diffusion at the wound site, thereby accelerating the healing process. Their high water content helps retain moisture, enabling painless autolytic debridement of necrotic tissue and promotes granulation and re-epithelialization. Because of their semi-occlusive nature, hydrogels are most effective for wounds with light to moderate exudation. Engineered hydrogel films can be designed to be biocompatible, adhere well to tissue, prevent bacterial infections, and maintain the moist environment essential for healing. They can also adapt to changes in the wound's microenvironment, supporting processes like angiogenesis, fibroblast recruitment, and cellular proliferation. Hydrogels exhibit an elastic response to stress; for example, collagen-based gels demonstrate toughness and low friction, which helps reduce mechanical stress-induced damage. Their mechanical and physical properties are designed to mimic the three-dimensional extracellular matrix of human skin, improving integration and function. Additionally, application and removal techniques are optimized to minimize tissue trauma and pain. Hydrogels are broadly categorized into natural (derived from polysaccharides like alginates or proteins like collagen), synthetic (such as PVA or PEG), and hybrid types. Hybrid hydrogels combine elements of both natural and synthetic materials and may include nanoparticles or growth factors to enhance therapeutic outcomes[9-11].

Advantages of hydrogel films in wound healing

- Hydrogel films are the best option for wound films in many ways. By encouraging the clearance of infected or necrotic tissue by autolysis, they can be used to both dry wounds and for sloughing of necrotic wounds, making and maintaining them clean.
- Hydrogel bandages maintain the wound's warmth, wetness, and closure. Furthermore, they don't irritate or react with tissue. When applied, they don't stick to the surfaces of wounds and let metabolites flow through. Patients find these films to be quite pleasant since they help to create a cooling effect on the wound.
- Since they somewhat mimic skin structure and stimulate the formation of skin components, they aid in wound re-epithelialization. They can also be used to include medications that speed up the healing of wounds. Finally, they can be used to treat any kind of wound, at any stage, including

International Journal of Pharmaceutical Research and Applications
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painful wounds, full-thickness and partial-thickness wounds, radiation wounds, small burns, and dry wounds, with the exception of those that have a lot of exudate[12].

II.MATERIALS AND METHODS

Materials

Drug (Tridax procumbens) hydroethanolic extract, Di- sodium tetraboratedecahydrate LR (Borax), Guar Gum, Glycerine LR, Distilled Water Equipment used: Weighing Balance (Eureka), Ultraviolet-Visible Spectroscopy (Peak Instruments (C-200S)), pH Meter (Elico), Magnetic Stirrer (Remi), Franz Diffusion Apparatus, Cyclone Mixer (Remi)

Methodology

1.Collection & authentication of the plant: The aerial part of Tridax procumbenswere collected from local area of Mumbai (Maharashtra).Herbarium was maintained followed by macroscopical & microscopical identification for the authentication of the plant.

2.Extraction: Plant materialwas rinsed thoroughly with waterand subsequently dried completely in an oven at 60℃. Powdered plant material was subjected to maceration using 70:30: :ethanol:water for two days. Hydroethanolic extract was distilled to get crude extract. (Percent Yield (%) = 26.6%)

3.Phytochemical screening: Extract obtained was subjected to phytochemical analysis[13].

Fig.3: Phytochemical screening

4.Preparation of the hydrogel film

The desired weight of glycerin and guar gum were combined together in a beaker,50 ml of distilled water was added to the mixture gradually withconstant swirling to prevent lumps. 1% borax solution was preparedand added to Guar gum solution in glycerinuntil the cross-linking occurs. The crosslinked solution was poured into petri plate anddried at 60°C to make a hydrogel film.Various combinations of ingredients in different proportions were used to get the best hydrogel film(Table1).

Table 1: Formulation development

Name of Ingredient	Formula code (in %w/v)						
	F1	F2	F3	F4	F5	F6	F7
Guar gum	8	4	8	8	5	5	5
Glycerine	30	50	50	50	25	35	35
Borax	4 (10ml)	4 (10ml)	4 (10ml)	4 (10ml)	1 (10ml)	1(10ml)	1(10ml)
Distilled water	10 ml	10 ml	10 ml	10 ml	50 ml	50 ml	50 ml
Drug (Tridax)	-	-		3	-	-	15

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 3 May-June 2025, pp: 2000-2010 www.ijprajournal.com ISSN: 2456-4494

F1 F2 F3 F4

F5 F6 F7

Fig.4: Hydrogel films (trial formulations)

5.Evaluation of Hydrogel film[14]

a. Physical Appearance

b. Transparency -Through visual inspection transparency is checked

c. Diameter - The patch was measured from all sides.

d. Content Uniformity - In this assessment, three patches of identical size were dissolved in 10 ml of distilled water and exposed to UV to measure absorbance and concentration was calculated.

e. Thickness - The patch was folded six times, and its thickness was measured using a vernier calliper at three different sides of the patch.

f. Folding Endurance - The hydrogel withstood 100 folds without developing creases or breaking.

g. Swelling Index - A small 1x1cm patch was cut, weighed and soaked in distilled water for fifteen minutes and after that the weight of film was measured and the process was repeated till a constant weight was achieved.

(Ws -Wi / Wi)100 = (0.15-0.04 / 0.04)*100 = 275% *Ws = swelling weight ; *Wi= initial weight

h. pH

i. Weight variation - Five patches of same dimensions were taken and weighed individually and then together on weighing balance.

6.Standard curve of Quercetin & Tridax procumbens

A quercetin standard curve was plotted to calculate the release of quercetin, as it as a Marker compound present in Tridax procumbens, a flavonoid responsible for wound healing[15].

To find out the λ_{max} of Tridax procumbens extract various concentrations of the extract from 300 to 600 nm were scannedto find maximum absorbance. The information obtained was used to proceed for theconstruction of the standard curve.

7.In-vitro Drug Release Studies[16]:

Diffusion was performed using a Franz Diffusion cell in order to determine the percent release of a drug from the patch into the receptor medium. The studies ran for 6/9 hours to determine the percent release of drug from the patch. It was carried out using a cellulose parchment paper, which resembles the skin, in contact with the patch; a buffer of pH 7.4 and the contents were kept on stirring using a magnetic stirrer to provide the sink condition for the release of the drug. A 1x1 hydrogel film was positioned on the membrane and placed between the donor and receptor compartments, clamped. The absorbance of the samples was measured at 404 and 415 nm.

8.Protein Denaturation Test[17]

Protein denaturation, which can trigger inflammation, is a crucial process to study for its implications in various biological and medical fields. Using heat to denature egg albumin is a common model for testing anti-inflammatory activity.The ability of Tridax procumbens to inhibit the heat-induced denaturation of egg albumin, provides insights into its potential anti-

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inflammatory properties[14].This simple and effective method is used to quantify and compare the efficacy of various compounds in preventing protein denaturation and subsequent inflammation.

9.Blood Clotting Test:

Tridax procumbens leaf extract accelerates blood clotting,[18]a crucial aspect in diabetic patients for wound healing. A drop of human blood was collected in the cavity slide containing the drug extract. The blood and extract were mixed and the time taken for clot formation was recorded using a stopwatch.

10. HET CAM Assay (Anti-irritancy testing)[19]:

For the test fertilized eggs of hen were used and the upper part of outer shell was broken to take out the membrane. 1cm x1cm patch of the formulation was used to check the irritancy score using the following formula:

Irritation Score = (301 - Ht)/300 * 5 + (301 - Ct)/300 * 7 + (301 - Lt)/300 * 9, where

Ht = Haemolysis time

Ct = Coagulation time

Lt = Vessel Lysis time

Table 2:Irritancy Score

Category	Irritation Score
Non-Irritative	0-0.9
Slightly Irritative	1-4.9
Moderately Irritative	5-8.9
Severely Irritative	9-21

III.RESULTS

The results obtained from the study on Formulation & Evaluation of Wound Healing Hydrogel Film of Tridax Procumbens are presented below:

1.Herbarium collected & microscopical studies were conducted to authenticate the plant material.

2.Hydroethanolic macerate of the the drug was collected and yield was found to be 26.6% w/w.

3. Phytochemical screening tests conducted, the results for which are summarised in**Table 3**

Table 3: Phytochemical screening test results

Test	**Observation**
Test for Flavonoids	
1. Shinoda test	**Reddish brown**color was observed
2. 10% NaOH test	**Yellow color**was observed
Test for Tannins	
1. FeCl3 test	**Greenish color** was observed
Test for Saponin	
1. Foam test	Foam should **not** stable for more than 2 minutes
Test for Alkaloid	
1. Dragendorff test	**Red color** was observed

4.Optimised hydrogel film

An optimized hydrogel film (**F7**) having the following formula (Table 4) was successfully developed, exhibiting desirable characteristics. The formulation selected was subjected to various evaluation parameters to ascertain the viability of the product.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 3 May-June 2025, pp: 2000-2010 www.ijprajournal.com ISSN: 2456-4494

Table 4: Optimised formula for hydrogel film

Name of the ingredients	F7 Concentration (% w/v)	Fig.4: Hydrogel films (final formulation)
Guar gum	5	
Glycerine	35	
Borax	1(10ml)	
Distilled water	50 ml	
Drug (Tridax)	15	

5. **Evaluation of Hydrogel film:** The final hydrogel film was evaluated against the predefined performance criteria (**Table 5**), and it was observed that all measured results were aligned with the expected benchmarks for an optimal formulation.

Table 5:Evaluation of Hydrogel film

Sr.No.	Tests	Observations	Inference
a	Physical Appearance	Hydrogel is green with a soft film texture, adheres to the skin, smooth appearance and touch.	Suitable for topical application due to its smoothness and adhesion.
b	Transparency	Translucent with a slight green tint.	Partially transparent, indicating a uniform dispersion of contents.
c	Diameter	Measured diameter: 7.5 cm.	Uniform size; suitable for standardized application.
d	Content Uniformity	Absorbance = 0.459; concentration per patch calculated as 3,697.29 mcg (3.697 mg).	Content is evenly distributed across patches, indicating good uniformity.
e	Thickness	Measurements: 0.225 mm, 0.217 mm, 0.217 mm.	Consistent thickness, showing good manufacturing precision.
f	Folding Endurance	Withstood 100 folds without creasing or breaking.	Excellent flexibility and mechanical strength.
g	Swelling Index	Initial weight = 0.04 g; final = 0.15 g; swelling index = 275%.	It is observed that hydrogels made of guar gum can increase in size by anywhere from 100% to 600% when they absorb water[20]
h	pH	Measured pH = 6.8.	Within acceptable range (4.5–7.5); safe for skin application.
i	Weight Variation	Mean of weight/No of patch = 0.230/5 = 0.046 gm	

6. **Standard curve of Quercetin and UV spectrum of Tridax procumbens**

A quercetin standard curve was plotted to calculate the release of quercetin, as it as a marker compound present in Tridax procumbens, a flavonoid responsible for wound healing[13]. UV scanning of the extract suggested the peak at 404 nm which is in the range of flavonoids that is from 400-450 nm.

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 3 May-June 2025, pp: 2000-2010 www.ijprajournal.com ISSN: 2456-4494

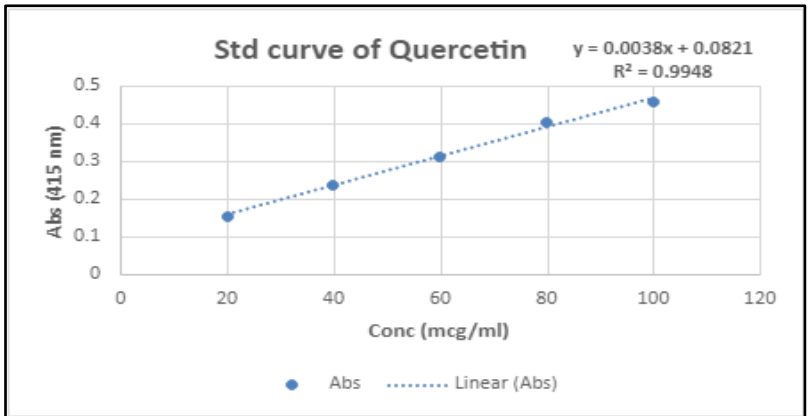

Fig.5: Standard curve of quercetin

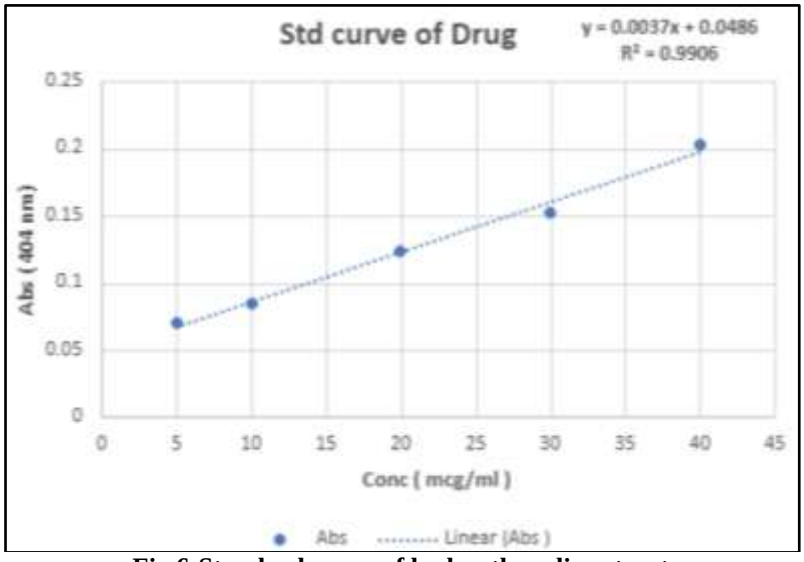

Fig.6:Standard curve of hydroethanolic extract

7. **In-vitro Drug Release Studies:** The graph shows good percent release of the drug (Fig.7)

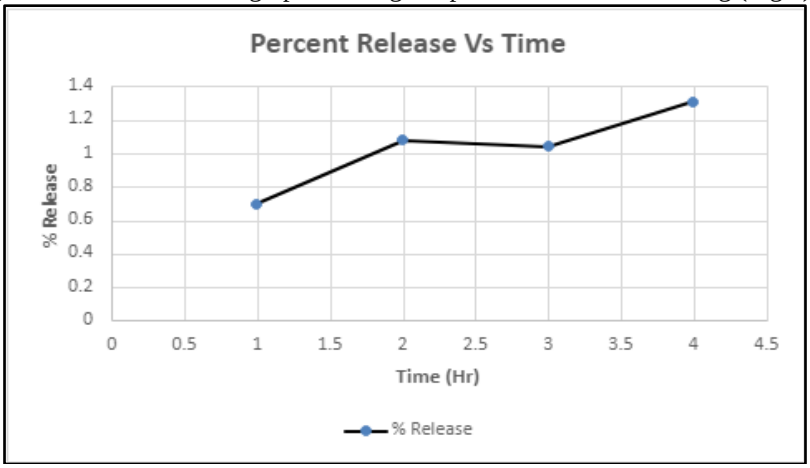

Fig.7: % Release of drug

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 3 May-June 2025, pp: 2000-2010 www.ijprajournal.com ISSN: 2456-4494

8. Protein denaturation test: Results indicate wound healing potential of the hydrogel filmas activity was confirmed by the formulation'scapacity to significantly inhibit heat-induced denaturation of albumin in vitro. Percentage inhibition is 93.44 (**Fig. 8**)whichindicates potent protein-stabilizing (anti-denaturation) effects, suggesting effective anti-

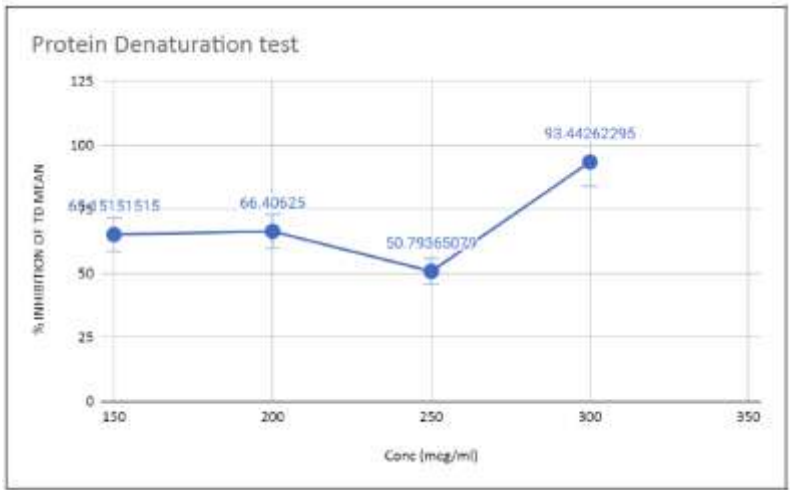

Fig.8:Protein denaturation test

9. Blood Clotting Test

Results indicate that an increase in the percentage of the drug leads to a reduction in clotting time which clearly indicates potential wound healing property of the drug (Table 6).

Table 6:Effect of varying doses of Tridax procumbens extract on clotting time

Drug (in %)	Clotting time
Without drug	7.34 min
0.1	4.37 min
0.2	3.25 min

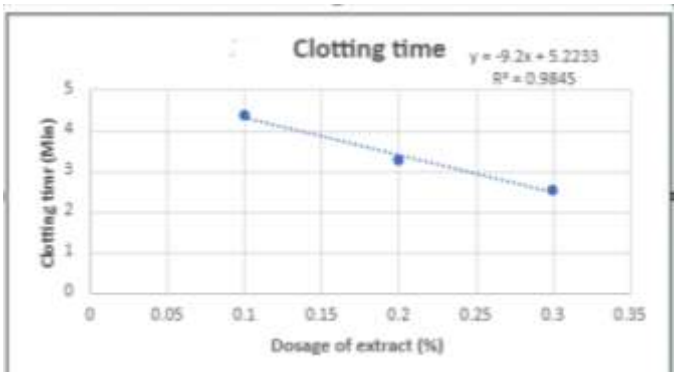

Fig.9: Effect of varying doses of Tridax procumbens extract on clotting time

International Journal of Pharmaceutical Research and Applications
Volume 10, Issue 3 May-June 2025, pp: 2000-2010 www.ijprajournal.com ISSN: 2456-4494

10. HET CAM Assay (Anti-irritancy testing)

No change in blood vessels of the egg was observed hence it was proved that the formulation was non irritant

Calculated irritancy score of the patch is found to be **0.5446** which falls in the range of**non-irritant**(**Table 2**).

IV CONCLUSION

Aim of the research to develop a wound healing hydrogel film ofTridax procumbenswith promising healing properties and good patient compliance. All the studies conducted in order to prove the viability and efficacy of the hydrogel film supported the concept. A herbal hydrogel film of Tridax procumbens is an attempt to have a safer alternative in the segment of wound healing products available in the market. Selection of herb was made on the basis of availability and therapeutic potential of the drug. Its clotting time decreasing potential makes it a more eligible candidate as a wound healing agent in cases of diabetic patients.

Incorporating herbal extracts into hydrogel films offers a multifunctional breakthrough compared to existing synthetic dressings. The formulation not only maintains the crucial moist environment and mechanical support characteristics of traditional hydrogels but also deliversbiocompatible, biodegradable, antimicrobial, and anti-inflammatory properties derived from plant bioactives without introducing synthetic additives. As a result, herbal hydrogel films promote faster, cleaner healing, reduce the risk of infection, and enhance patient comfortproviding a next-gen wound dressing that aligns with both efficacy and holistic care.

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