DEVELOPMENT AND *IN-VITRO* EVALUATION OF *IXORA POLYANTHA* WIGHT. HYDROGEL FILM FOR TOPICAL APPLICATION IN THE TREATMENT OF DIABETIC WOUND HEALING.

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ABSTRACT

Ixora polyantha Wight, recognized for its traditional medicinal uses, has been evaluated in this study for its potential to treat diabetes mellitus-related wound healing complications via a hydrogel film. Various formulations were synthesized and assessed for key parameters including thickness, weight variation, folding endurance, moisture content, moisture uptake, swelling index, and in vitro drug release. The hydrogel films exhibited consistent thickness and weight, with formulation F5 showing the highest weight (0.896 g) and notable folding endurance (77). Moisture content and uptake analyses indicated significant moisture retention, crucial for effective wound healing. The swelling index demonstrated an impressive increase over time, with F5 reaching a maximum of 135% at 7 hours, indicating substantial hydration capacity. In vitro drug release studies revealed a sustained release profile, essential for prolonged therapeutic action. These results suggest that *Ixora polyantha*-derived hydrogel films possess favorable characteristics for topical applications. The findings support the potential of this natural hydrogel as an effective treatment for enhancing wound healing in diabetic patients, highlighting the need for further *in-vivo* studies to elucidate its efficacy and mechanisms in wound care management.

KEY WORDS: *Ixora polyantha* Wight, Hydrogel film, diabetic wound healing.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. From the very beginning of human existence, man has familiarized himself with plants and used them in a variety of ways throughout the ages. Medicinal plants are considered

as rich resources of ingredients which can be used in drug development pharmacopoeial, nonpharmacopoeial or synthetic drugs. A part from that, these plants play a critical role in the development of human cultures around the whole world. Plant is an important source of medicine and plays a key role in world health. Impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Herbal medicines had recently gained a sky rocketingly increased popularity in the western societies as a suitable complementary source of nutrients alongside the orthodox medicine.

Figure 01: *Ixora polyantha* Wight

Ixora polyantha Wight., a member of the *Rubiaceae* family, is a plant recognized for its diverse medicinal properties. The *Rubiaceae* family, commonly referred to as the coffee family, is a diverse group of flowering plants encompassing over 13,000 species. This family is notable for its ecological and economic significance, including key plants such as coffee (Coffea), quinine (Cinchona), and various ornamental species. Among the many genera within the *Rubiaceae* family, Ixora is particularly prominent for its ornamental and medicinal uses. Ixora species are widely cultivated for their striking appearance, featuring dense clusters of small, colorful flowers that range in shades from red and pink to yellow and white. In addition to their aesthetic appeal, Ixora plants have been used in traditional medicine for their therapeutic benefits. Various species within this genus are reported to possess anti-inflammatory, antimicrobial, and antioxidant properties. The promising results from the literature review of wound healing assays indicate that the ethanolic extract can enhance tissue repair processes, making it a valuable candidate for developing natural wound care products. In vitro studies further proved the extract's efficacy in inhibiting alpha-amylase activity, supporting its traditional use in

managing diabetes.

Hydrogel

Hydrogels are among the most used dressing materials that have confirmed their effectiveness in wet wound therapy. The three-dimensional polymer networks formed in hydrogels have the capacity to incorporate large quantities of water, ensuring not only the humid environment necessary for wound healing, but also an excellent biocompatibility. The water retaining properties of the hydrogel dressings are induced by the presence of hydrophilic groups in the polymer chains, with the higher water content assuring a porous, soft, and elastic structure, thus enhancing the compatibility with biological tissues. Hydrogels can be loaded with plant extracts to deliver a range of plant-derived ingredients and their mixtures. It enhances water retention, absorb exudate around the wound site, increase pliability and biocompatibility, and resemble extracellular matrix in a wound dressing material.

Hydrogel film

Hydrogel film dressings are known to be one of the most favorite choices. Films as wound dressing are thin and easy to use. These materials are semipermeable for efficient oxygen and water vapor exchange. The film transforms into a gel when contacts with wound exudates and creates a humid environment around the wound area. The thin hydrogel films as three-dimensional, water-swollen polymer networks, mimic the physicochemical and biological properties of tissue microenvironment due to their excellent stimuli-responsive behavior, macroporous structure, exhibiting an intermediate behavior between solid and liquid materials and present soft tissue-like mechanical properties. So, it can potentially be used for special biomedical applications such as drug carrier, tissue scaffold, outstandingly in wound dressing application.

Recently, both synthetic (such as PVA and PVP) and natural polymers have been successfully employed for designing film hydrogel dressing. However, natural origin polymers (such as polysaccharides, polypeptides, and proteins) derived from plants or different tissues of aquatic organisms are preferred over synthetic ones, because of their ability to degrade in the body over time, great structural versatility and their similarity to the extracellular matrix, which is usually considered as the ideal case for controlled drug delivery vehicles. Among these sources, hydrogels based on polysaccharides have

attracted considerable attention in wound dressing applications due to their biocompatibility, biodegradability, easy availability, low production cost, non-toxicity along with their easy synthesis and processability. Thereby, the addition of polysaccharide in the polymer matrix also improves the equilibrium swelling and drug release properties.

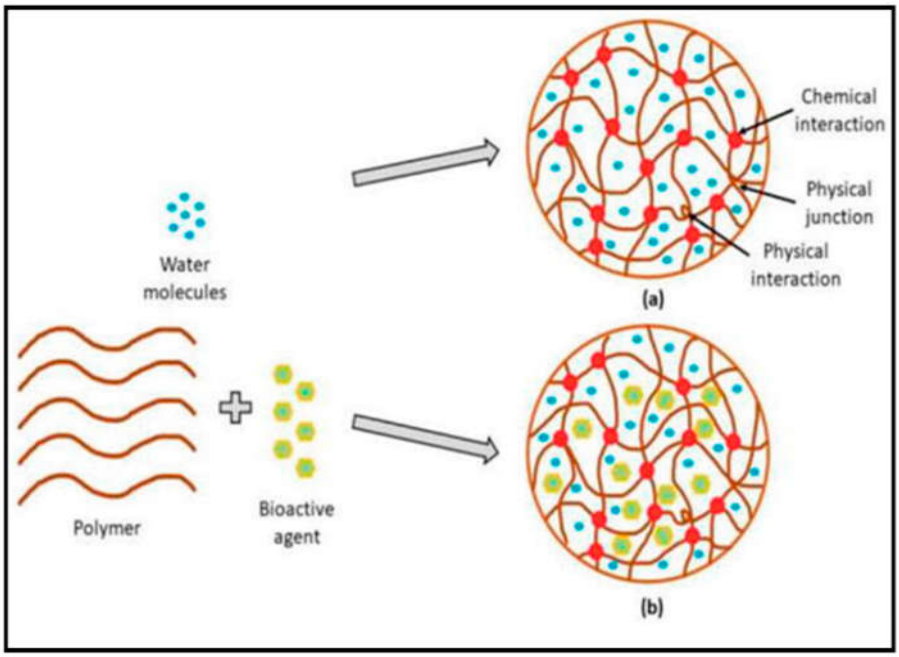


Figure 02: Schematic representation of hydrogel film

Mechanism of drug release from hydrogel film

Herbal extract-loaded hydrogel films are designed to provide controlled and sustained release of active compounds. The mechanism of drug release from these films primarily involves a combination of diffusion and swelling processes. When the hydrogel film comes into contact with moisture or bodily fluids, it absorbs water due to its hydrophilic polymer network, causing the gel to swell. This swelling creates an expanded network with larger pathways for the herbal extract to diffuse through. As the hydrogel swells, the encapsulated herbal extract, which was initially trapped within the polymer matrix, begins to diffuse from areas of high concentration inside the hydrogel to lower concentrations outside. This diffusion process is influenced by the rate of swelling and the nature of the polymer network. In addition to diffusion, the release rate can also be modulated by the degradation of the hydrogel matrix, which gradually breaks down over time, further facilitating the release of the herbal compounds. This controlled release mechanism ensures that the herbal extract is delivered steadily over an extended period, potentially enhancing therapeutic efficacy

and reducing the frequency of application.

Advantages of hydrogel films in antidiabetic wound healing

1. **Moisture Retention:**
    - Maintains a moist wound environment, promoting faster healing and reducing dehydration and eschar formation.
2. **Enhanced Wound Healing:**
    - Supports all phases of wound healing (inflammation, proliferation, remodeling) and aids in the formation of granulation tissue.
3. **Reduced Pain and Discomfort:**
    - Provides cushioning and reduces friction, which alleviates pain and discomfort at the wound site.
4. **Controlled Drug Release:**
    - Allows for the sustained release of antidiabetic agents or herbal extracts, improving therapeutic efficacy and reducing dressing change frequency.
5. **Protection Against Infection:**
    - Acts as a barrier against contaminants and microorganisms, lowering the risk of infection; some can be infused with antimicrobial agents for added protection.
6. **Biocompatibility:**
    - Generally well-tolerated by the body, minimizing adverse reactions and making them suitable for long-term use in chronic wounds.
7. **Ease of Application and Removal:**
    - Simple to apply and remove, minimizing trauma during dressing changes, which is beneficial for patients with delicate or compromised skin.
8. **Customization and Versatility:**

- Can be tailored in composition, thickness, and drug-loading capacity to meet specific treatment needs and accommodate different wound types.

9. **Promotes Autolytic Debridement:**

- Facilitates the natural removal of necrotic tissue by supporting autolytic debridement, which prepares the wound bed for further healing.

Preparation of plant extract

Extraction

The leaves of *Ixora polyantha* Wight. was shade dried and coarsely powdered and 90 g of dried coarse leaves powder was extracted with Soxhlet apparatus by using ethanol as a solvent (approx. 2 days). After completion of the extraction process extract was dried by solvent evaporationand stored in well closed air tight container.

Preparation of hydrogel film

The hydrogel films were prepared by a solvent-casting method. Chitosan solutions were prepared by dissolving chitosan in acetic acid solution (3% *v*/*v*) with constant stirring for 2 h. PVA solution (5% *w*/*v*) was prepared by dissolving PVA in distilled water with constant stirring at 50 °C for 4 h. The chitosan and PVA solutions were combined with plant extract in variable proportions, with mechanical blending at 1000 rpm for 10 min. The mixture (0.5 g) was transferred into Pyrex petri plates (5-inch diameter) and allowed to air dry at normal room conditions for 2 days. Dried films were then peeled from the petri plates and stored in a desiccator for further use.

Evaluation of Hydrogel Film

1. **In vitro drug release study**

In vitro drug release of the hydrogel sheets was carried out using a modified dissolution test instrument. The method followed was total immersion method. Hydrogel sheets were immersed with the help of a support in a beaker containing 20 ml of simulated wound fluid (142 mM sodium chloride and 2.5 mM calcium chloride in water). The beaker was placed on a magnetic stirrer and was magnetically agitated at 75 rpm. Samples of 1 ml were withdrawn from the beaker at specified time intervals over a period of 24 h and were replaced by equal volume of fresh SWF. The amount of drug released with respectto time was determined by UV-

Vis spectrophotometer at 290 nm against a standard curve of embelin in simulated wound fluid in the concentration range of 1 to 10 µg/ml. The study was performed in six replicates.

2. Thickness and Weight Variation

The thickness of hydrogel films was recorded using a digital calibrated micrometer (Mitutoyo, Japan). The average and standard deviation of the three readings were recorded. For the weight variation test, the films were weighed individually, and results were determined using the average ± SD. The evaluations were performed in triplicate.

3. Folding Endurance

The folding endurance was evaluated to verify the number of times the film can be folded. The number of times a film sample could be folded at the same place without breaking indicated the folding endurance value. The experiment was performed in triplicate.

4. Moisture Content

The hydrogel films were initially weighed (Wi) and were placed in a desiccator containing activated silica gel for 24 h. The films were weighed repeatedly until a constant weight (Wd) was observed. The moisture content was determined as per the following equation:

$$\text{Moisture Content (\%)} = (Wi - Wd)/Wd \times 100$$

The moisture content determination experiment was performed in triplicate.

5. Moisture Uptake

The hydrogel films were initially weighed (Wi) and were placed in a desiccator containing activated silica gel for 24 h. The films were transferred to another desiccator for 72 h containing saturated sodium chloride solution with relative humidity maintained at 75%. The final weight of the films (Wm) was recorded and the moisture uptake capacity was determined according to the equation given below:

$$\text{Moisture uptake (\%)} = (Wm - Wi)/Wi \times 100$$

6. Swelling index

The pieces of hydrogel sheets (1 cm × 1 cm) were dried at 60 °C in oven for 12 h (Wd). They were then soaked in a simulated wound fluid (SWF) pH 7.7 at 37 °C (Ws). The swelling index percentage was calculated using the following equation.

$$\text{Swelling index} = \frac{Ws - Wd}{\text{Ws}} \times 100$$

where Wd and Ws were the weights of hydrogel samples dried for 12 h at 60 °C and soaked in PBS at 37 °C, respectively.

RESULT

The in vitro drug release study demonstrated a sustained release profile across all formulations, essential for prolonged therapeutic action. The rate of drug release was influenced by the composition and properties of each hydrogel formulation, suggesting that Ixora polyantha can effectively deliver therapeutic agents over an extended period, enhancing its application in wound healing.

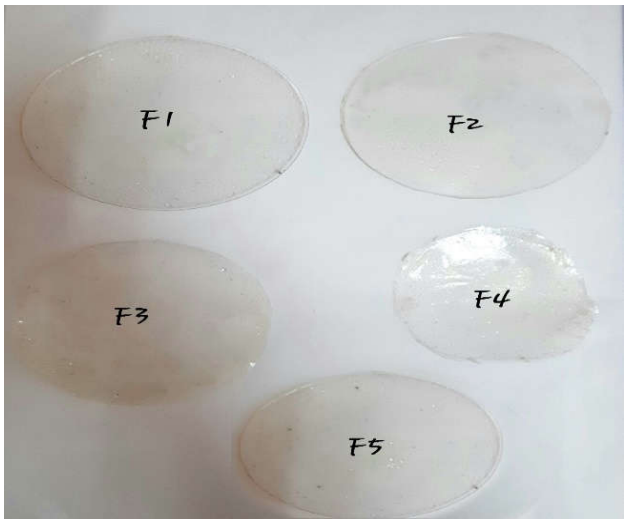


Figure 03: Hydrogel films in different concentration

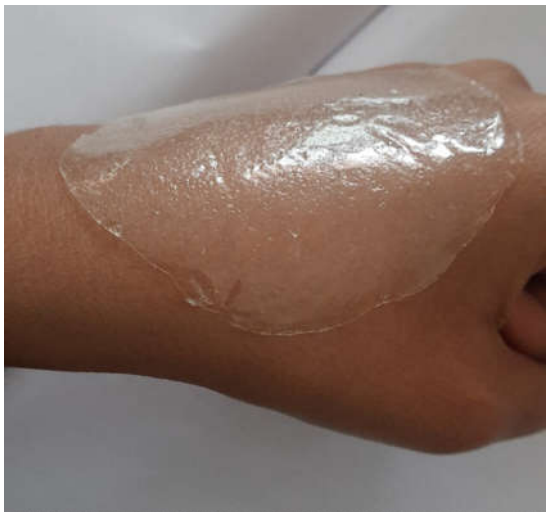


Figure 04: Hydrogel film

Evaluation of hydrogel film

1. In vitro drug release study

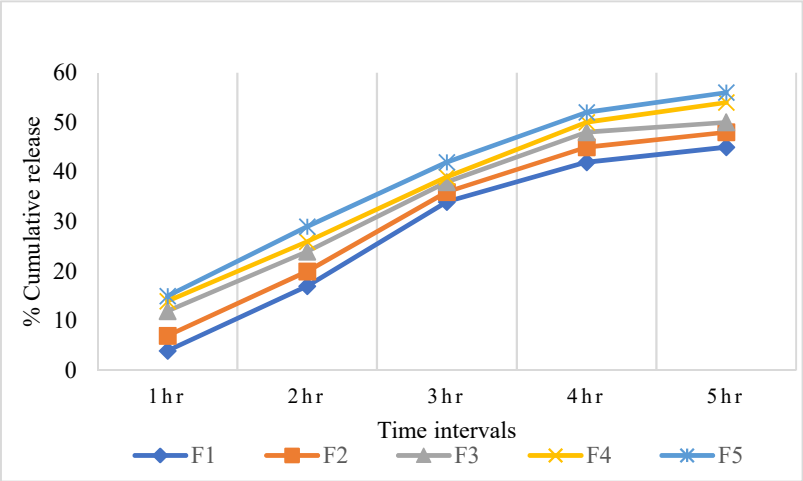
The cumulative drug release from the hydrogel films after various time intervals (1hr to 5hr) was measured and is presented in the following table:

Formulations	% Cumulative release				
	1hr	2hr	3hr	4hr	5hr
F1	4	17	34	42	45
F2	7	20	36	45	48
F3	12	24	38	48	50
F4	14	26	39	50	54

F5	15	29	42	52	56

Table 01: % Cumulative release of drug from hydrogel film

The data reveals that all formulations exhibited an increasing trend in cumulative drug release over time. Notably, formulation F5 demonstrated the highest cumulative release, reaching 56% at 5 hours, while formulation F1 had the lowest at 45%. Formulation F3 showed a significant improvement in release compared to F1 and F2, suggesting that the composition of the hydrogel film can significantly influence drug release kinetics.



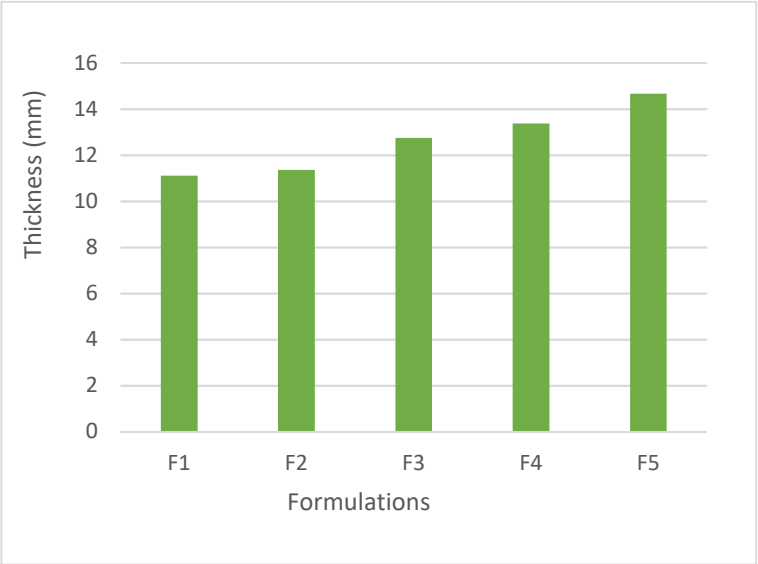
Graph 01: I*n-Vitro* drug release from hydrogel film.

2. Thickness

The thickness of the hydrogel films ranged from 0.053 mm (F1) to 0.091 mm (F5). Formulation F5 exhibited the highest thickness, which can contribute to better mechanical strength and moisture retention. The uniformity in thickness across formulations indicates consistent manufacturing processes, crucial for ensuring reliable performance in clinical applications.

Sl. No	**Formulation**	**Thickness (mm)**			**Mean**	**Mean± standard deviation**
1	F1	0.053	0.058	0.059	0.056667	0.056±0.003
2	F2	0.064	0.068	0.066	0.066	0.066±0.002
3	F3	0.071	0.076	0.073	0.073333	0.073±0.002
4	F4	0.079	0.081	0.08	0.08	0.08±0.001
5	F5	0.091	0.093	0.097	0.093667	0.093±0.003

Table 02: Thickness of hydrogel film



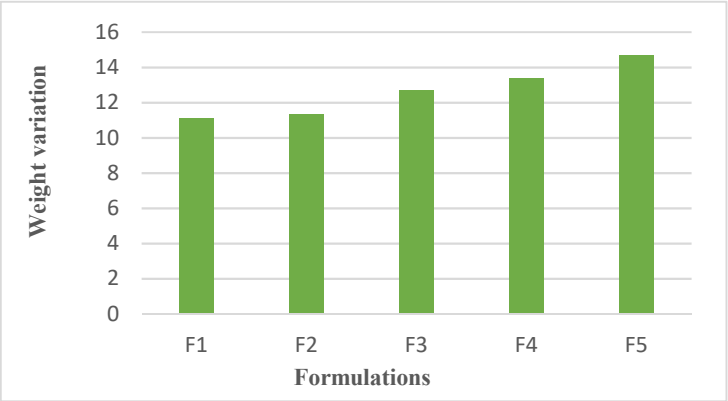
Graph 02: Thickness of hydrogel film

3. Weight variation

Weight variation results showed that the formulations had consistent weights, with F5 showing the highest weight (0.896 g). This consistency is vital for dosage accuracy and ensures the therapeutic effects are predictable when applied. The low standard deviation values indicate minimal variability, enhancing the reliability of the formulations.

Sl. No	Formulation	Weight of the formulation			Mean	Mean± standard deviation
1	F1	0.432	0.419	0.494	0.448	0.448±0.04
2	F2	0.512	0.548	0.58	0.546	0.546±0.034
3	F3	0.677	0.631	0.601	0.636	0.636±0.038
4	F4	0.798	0.767	0.791	0.785	0.785±0.016
5	F5	0.893	0.895	0.901	0.896	0.896±0.004

Table 03: Weight variation of Hydrogel film



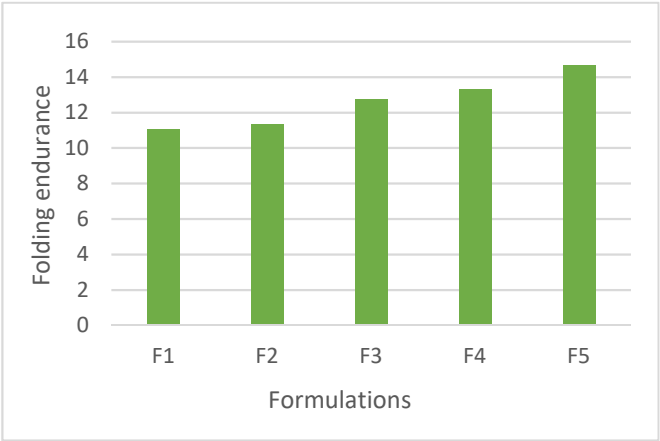
Graph 03: Weight variation of Hydrogel film

4. Folding endurance

Folding endurance ranged from 35 (F1) to 77 (F5), indicating good flexibility of the hydrogel films. Higher folding endurance in F5 suggests that this formulation is better suited for application on dynamic or contoured areas of the skin, enhancing comfort and wearability for patients.

Sl. No	Formulation	Folding endurance			Mean	Mean± standard deviation
1	F1	37	31	37	35	35±3.464102
2	F2	42	43	40	41	41±1.527525
3	F3	55	50	59	54	54±4.50925
4	F4	60	63	68	63	63±4.041452
5	F5	78	75	80	77	77±2.516611

Table 04: Folding endurance of hydrogel film



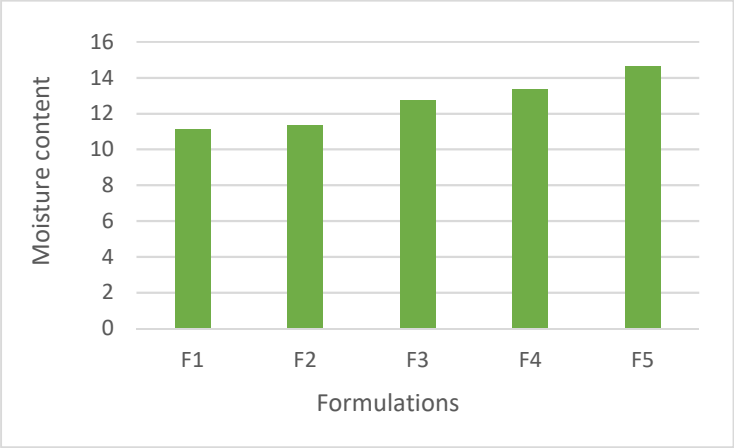
Graph 04: Folding endurance of hydrogel film

5. Moisture content

Moisture content varied significantly, with F5 having the highest content (26.028%). High moisture content is beneficial as it can prevent the dressing from drying out, maintaining an optimal environment for wound healing and reducing scab formation.

Sl. No	Formulation	Moisture content			Mean	Mean± standard deviation
1	F1	12.53	12.81	11.84	12.393	12.393±0.499
2	F2	14.51	13.02	17.01	14.846	14.846±2.016
3	F3	18.081	19.981	17.61	18.557	18.557±1.255
4	F4	20.932	23.075	24.87	22.959	22.959±1.971
5	F5	28.89	23.081	26.115	26.028	26.028±2.905

Table 05: Moisture content of hydrogel film



Graph 05: Moisture content of hydrogel film

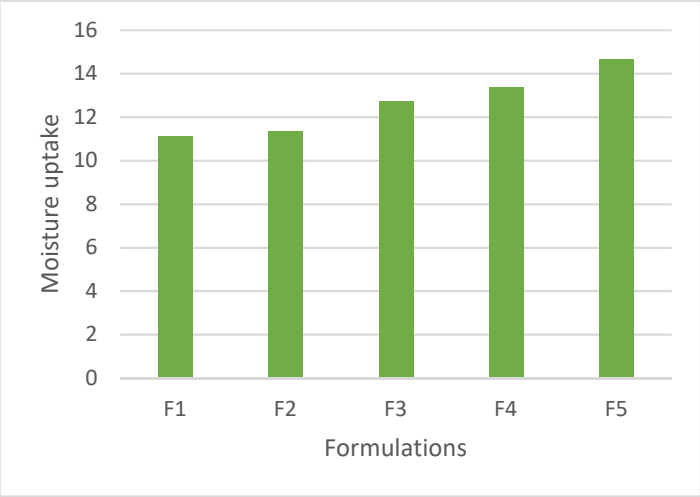
6. Moisture uptake

Moisture uptake ranged from 11.103% (F1) to 14.666% (F5). The ability to absorb moisture indicates that these hydrogels can maintain hydration at the wound site, which is critical for promoting healing and preventing infections.

Sl. No	Formulation	Moisture uptake			Mean	Mean± standard deviation
1	F1	10.35	11.98	10.98	11.103	11.103±0.821
2	F2	11.011	11.09	11.98	11.36	11.36±0.538

3	F3	12.908	12.324	12.98	12.737	12.737±0.359
4	F4	13.012	13.987	13.098	13.365	13.365±0.539
5	F5	14.98	14.871	14.148	14.666	14.666±0.452

Table 06: Moisture uptake of hydrogel film



Graph 06: Moisture uptacke of hydrogel film

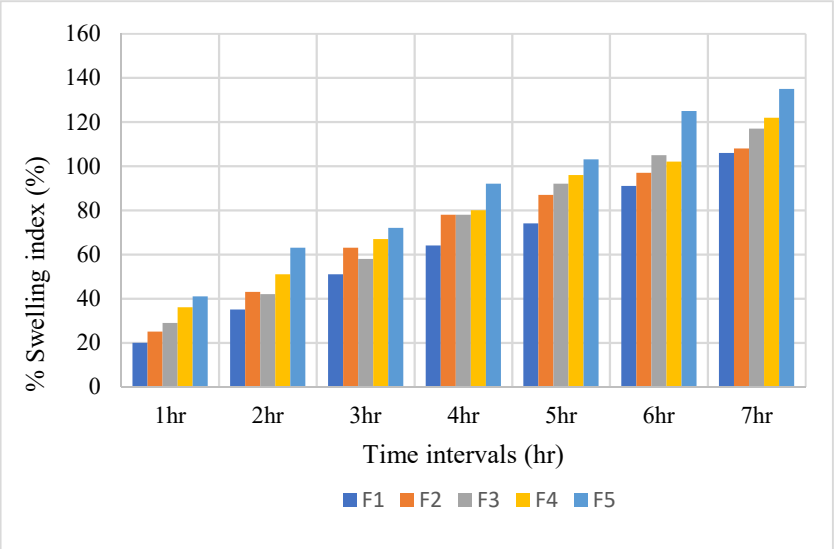
7. Swelling index

The swelling index increased significantly over time, with F5 reaching a maximum of 135% at 7 hours. This swelling capability enhances the hydrogel's ability to retain moisture and potentially deliver embedded therapeutic agents effectively. The swelling index is a crucial factor in ensuring that the hydrogel can conform to the wound bed, facilitating better healing outcomes.

Sl. No	**Formulation**	**Wd**		**Time intervals (hr)**						
				1	**2**	**3**	**4**	**5**	**6**	**7**
1	F1	0.297	Ws	0.359	0.401	0.45	0.49	0.519	0.569	0.612
			% Swelling index (%)	20	35	51	64	74	91	106
2	F2	0.48	Ws	0.601	0.691	0.785	0.856	0.901	0.95	1
			% Swelling index	25	43	63	78	87	97	108

3	F3	0.561	Ws	0.729	0.799	0.89	1	1.081	1.181	1.221
			% Swelling index	29	42	58	78	92	105	117
4	F4	0.618	Ws	0.844	0.934	1.034	1.114	1.214	1.254	1.374
			% Swelling index	36	51	67	80	96	102	122
5	F5	0.753	Ws	1.064	1.234	1.30	1.451	1.531	1.701	1.775
			% Swelling index	41	63	72	92	103	125	135

Table 07: Swelling index of Hydrogel film



Graph 07: Percentage swelling index of hydrogel film

DISCUSSION

The swelling index is a critical parameter in evaluating hydrogel films, as it directly correlates with the material's ability to absorb fluids and maintain a hydrated environment conducive to wound healing. In this study, formulation F5 exhibited a swelling index of 135% at 7 hours, indicating excellent moisture retention capabilities. This high degree of swelling suggests that the hydrogel can absorb and hold significant amounts of exudate from the wound site, which is essential for preventing desiccation and promoting an optimal healing environment. The

ability to swell also enhances the hydrogel's contact with the wound bed, facilitating intimate interaction between the dressing and the tissue.

Moreover, the swelling behavior may play a crucial role in the sustained release of therapeutic agents embedded within the hydrogel matrix. As the hydrogel absorbs moisture, it expands, potentially leading to the gradual release of these agents over time. This mechanism is particularly beneficial in wound care, as it allows for a continuous therapeutic effect without the need for frequent dressing changes. Additionally, the swelling index contributes to the mechanical properties of the hydrogel, enabling it to conform to various wound shapes and sizes, which is important for ensuring adequate coverage and protection. The favorable swelling characteristics of F5 position it as a promising candidate for clinical applications, especially in the context of managing chronic wounds often associated with diabetes, where maintaining moisture and delivering active compounds are paramount for healing.

CONCLUSION

The findings from this study highlight the potential of Ixora polyantha-derived hydrogel films for treating wound healing complications associated with diabetes mellitus. The comprehensive evaluation of physical properties, including swelling index, moisture content, and drug release characteristics, demonstrates that these hydrogels are well-suited for creating a conducive environment for wound healing. The significant swelling index observed in formulation F5 suggests that it can effectively retain moisture, which is vital for preventing infections and facilitating cellular migration. Additionally, its ability to deliver embedded therapeutic agents over an extended period enhances its utility in clinical settings. Overall, the promising results warrant further investigation through in vivo studies to better understand the mechanisms of action and optimize formulations for specific wound care applications. The integration of natural plant extracts like *Ixora polyantha* into hydrogel systems could lead to the development of innovative treatments that address both metabolic and tissue repair issues in diabetic patients, offering a multifaceted approach to wound management.

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