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SUSTAINED MULTI-FLUID RELEASE OF TRIDAX FROM FENUGREEK-BASED BIOPOLYMER FILMS FOR BIODEGRADABLE WOUND DRESSINGS

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Abstract

This research explores the formulation of biodegradable biopolymer films for oral drug delivery using mucilage from fenugreek (Trigonella foenum-graecum). *Tridax procumbens* was used as the model phytodrug, and films were fabricated via solvent casting, incorporating pure glycerol as a plasticizer and incorporated with fresh *Tridax* extract. While these studies highlight FSM's capabilities in specific drug delivery applications, there is a notable gap in research assessing its drug release behaviour in simulated physiological fluids beyond the gastrointestinal tract, like blood and saliva. Comparative analysis revealed that films plasticized with glycerol showed significantly higher *Tridax procumbens* release and faster biodegradability in simulated gastric, intestinal, blood, and saliva fluids than those prepared with mucilage alone or with agar. FTIR confirmed cohesive polymer networks and efficient *Tridax procumbens* integration, while TGA and biodegradability tests supported structural resilience and environmental safety. Drug release studies indicated sustained, pH-responsive behaviour optimized for intestinal absorption. This eco-friendly platform demonstrates potential for plant-based, biodegradable external drug delivery systems tailored for hydrophobic compounds like *Tridax procumbens* that are known for their extreme wound healing capacity.

Introduction

Biopolymers are innately occurring polymers formed by living organisms, plants, bacteria and fungi during the life cycle. Unlike synthetic polymers derived from petroleum, biopolymers are renewable, biodegradable, and generally biocompatible, making them highly attractive for various industrial, medical, and environmental applications. Natural polymers have been extensively explored as the vehicles for encapsulation and delivery of drugs and other bioactive molecules. Their inherent advantages in biocompatibility, biodegradability, renewability, hydrophilicity, mechanical strength, controlled enzyme degradation, specific interactions with some biomolecules, and easy modification endow them with

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versatility in drug delivery (Tiwari et al., 2014).

The Drug Delivery System (DDS) made by illustrative natural polymers, polysaccharides (chitosan and hyaluronic acid) and proteins (silk fibroin and collagen) (Tong et al., 2020). Pay loads mainly contains small molecular weight drug, proteins, and DNA for the applications of tissue engineering, wound healing or anticancer therapy. Besides, the DDS made by their derivatives and with other materials also have been presented focusing on the chemical and morphological modifications, the additions of smart stimuli-triggered or targeted motifs and so on, which clearly promoted the delivery and therapy efficiency. These systems have evolved from traditional methods to advance, targeted, and responsive platforms. They are few recent innovations in drug release technologies: 3D- printed drug delivery systems, microneedle systems, responsive hydrogels, implantable devices with controlled release etc.E.g., Conventional (tablets/capsule, injections, creams), novel/advanced. Kashid *et.al.,* (2025) have researched on buccal drug delivery offers a convenient and safe alternative route, allowing easy termination of drug administration by removing the dosage form if toxicity occurs.

The flowering plant Tridax procumbens, also known as Tridax procumbens L. (T. procumbens), is indigenous to the Americas. The sodium, potassium, and calcium content of T. procumbens is high. T. procumbens leaf extract has been applied to the injured area of the body in order to treat wounds. The PVA nanofibers with T. procumbens leaf extract were made. Inhibition zones of up to 45 mm against S. aureus and up to 36 mm against E. coli were demonstrated by the nanofibers, demonstrating their antibacterial characteristics. (Ganesan and Pradeepa, 2017).

Recent research involved the preparation of diclofenac sodium buccal films using sodium alginate and *Ocimum basilicum* mucilage via solvent casting. Phonrat *et.al.*, (2024) have probed o enhance water stability and mechanical strength of heat-moisture treated starch for wound dressing, natural basil seed mucilage—known for its hydrophilicity and crystallinity—was incorporated. Succinic acid cross-linking improved the material's durability, evidenced by increased gel fraction and mechanical properties. Bhattarai *et.al.*, (2024) have said that Fenugreek seed gum (FSG) is gaining attention for its biocompatibility, biodegradability, and film-forming capabilities in food packaging and biomedical fields. In a recent study of FSG-based Janus films with varying glycerol concentrations were established, showing that 5% glycerol produced the most stable films with increased solubility and structural integrity. The films revealed strong antioxidant activity and substantial antimicrobial effects against S. aureus, E. coli, and Candida albicans, which were further enhanced by adding penicillin. Dhull *et.al.,* (2023) have reported that Fenugreek (*Trigonella foenum-graecum*), a leguminous plant, produces seeds rich in soluble fiber primarily composed of galactomannans. These biopolymers feature a (1,4)-β-D-mannan backbone with D-galactose side chains, giving fenugreek gum a unique 1:1 galactose to mannose ratio, resulting in superior water solubility compared to guar and locust bean gum. Niknam *et.al.,* (2023) have highlighted the growing importance of biodegradable hydrocolloid- based films for sustainable food packaging. A study comparing films made from fenugreek galactomannan and xanthan gum—both

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individually and in combination—found that composite films showed enhanced mechanical, thermal, and barrier properties due to strong biopolymer interactions.

2. Methodology

2.1 Preparation of polymer films

Fenugreek seeds (*Trigonella foenum-graecum*) were procured from the local central market in Chikkaballapur, Karnataka. The seeds were washed and soaked in distilled water for 24 hours at room temperature to allow hydration and mucilage swelling. After the soaking period is over and maximum swelling has been obtained the seeds were boiled for 20 minutes to facilitate enhanced and maximum swelling and mucilage extraction. The softened mixture was then squeezed through a sterile muslin cloth so that the seed residues are separated and only mucilage is extracted. The mucilage separated was separated into three formulation set for film preparation. The Set A consisted of plain fenugreek mucilage; Set B fenugreek mucilage incorporated with glycerol and Set C had fenugreek mucilage incorporated with glycerol and agar agar. Both were added 10% of mucilage volume. The glycerol and Agar-agar served as plasticizer and additives respectively. This enhanced the flexibility, mechanical firmness and structural integrity of the polymer films. The formed Set were incorporated with 5% of *Tridax procumbens* fresh extract and mixed uniformly. These formulations were used for subsequent film casting and characterization.



Fenugreek seed



Fenugreek polymer

2.2. Preparation of synthetic fluids

Standard chemical constituents were used to prepare synthetic body fluids that mimicked the physiological conditions of the human body, namely gastric fluid, intestinal fluid, saliva and blood plasma. Gastric fluid was obtained by adding 0.2 g of NaCl and 0.7 g of concentrated HCl to a distilled water and adjusted volume to 100 mL. An optional enzyme, pepsin (3.2 g) was added to simulate enzymatic action and pH was then set to 1.2. In case of intestinal fluid, KH 2 PO 4 (0.68 g) and 0.2N NaOH (0.064 g) were dissolved in water and measure to 100 mL. Moreover, to mimic the presence of digestive enzymes, 10 g of pancreatin had been added and the pH was held to 6.8. A saliva fluid was prepared by dissolving 0.04 g NaCl, 0.04 g KCl, 0.22 g NaHCO3, 0.08 g CaCl 2 and optionally Na 2 S H 2 O as required and the pH adjusted to a range of 6.8-7.0. Blood plasma was made by adding 0.68 g NaCl, 0.03 g KCl, 0.08 g NaH 2 PO 4, 0.01 g CaCl 2, 0.01 g MgSO 4, 1 g urea,

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0.1 g glucose, and 4 g bovine serum albumin (BSA), followed by adjusting the final pH to 7.4. Distilled water was used in loading all solutions and the 100 mL volume was brought. A calibrated PH meter was used to verify pH value of each fluid. The synthetic fluids were prepared and kept at 4 ^{o} C before further experimentation.



a) b)

Figure 2: Synthetic bodily fluids

2.1. Study of drug release from biopolymer films

In this study, six different types of biopolymer films, each measuring 0.1 x 0.1 cm, were fabricated. The experiment set up included chia polymer, chia glycerol, chia agar-agar, fenugreek polymer, fenugreek glycerol, and fenugreek agar-agar film formulations. Small pieces of these films were immersed in synthetic bodily fluids to simulate the in vivo environment. Observations of *Tridax extract* release from the films were made at three specific time intervals: 15 minutes, 30 minutes, and 45 minutes. At each interval, the amount of *Tridax extract* released was visually assessed, and the release time was carefully recorded. The percentage of *Tridax extract* released from each film was calculated to quantify the release kinetics. Results showed that each film type had a distinct release capacity influenced by its biopolymer composition. Films comprising glycerol exhibited increased plasticity, which appeared to facilitate faster *Tridax extract* diffusion. Agar-agar films, due to their gel-like nature, showed a more controlled and sustained release pattern. Chia polymer films generally released *Tridax extract* at a faster rate compared to fenugreek-based films. The differences in release profiles highlight the impact of polymer type and formulation on drug release behavior. These findings demonstrate the potential to tailor drug release rates by modifying biopolymer compositions. This study provides valuable insights for the design of biopolymer-based drug delivery systems.

2.2. Study of disintegration of biopolymer films

In this study, biopolymer films of 0.1 x 0.1 cm dimensions with variable compositions were set and immersed in synthetic bodily fluids (SBFs) to stimulate physiological conditions such as saliva, blood plasma, gastric fluid and intestinal fluid. The objective was to detect and compare the disintegration behavior of the films over time. At regular intervals, the films were examined for visible breakdown, and the time obligatory for disintegration was recorded. Additionally, the percentage of disintegration was calculated based on mass

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loss measurements. It was detected that different compositions led to varying disintegration rates, with more hydrophilic films disintegrations faster. The disintegration behavior was also influenced by the type of synthetic fluid, its pH, and ionic content. Films that retained their structure longer were noted as potential candidates for applications requiring controlled degradation, such as drug delivery systems. This experiment provided respected insights into the biocompatibility, stability, and degradation profiles of the films under conditions mimicking the human body, offering guidance for their potential biomedical applications.

2.4. Characterization of the polymer films

2.4.1. FTIR spectroscopy

The polymer films developed were categorized using Fourier Transform Infrared (FTIR) spectroscopy, which is a extensively used technique to analyse the functional groups and molecular interactions within polymeric materials. FTIR spectra were verified in the range of 4000 to 450 cm^{-1} using a triglycine sulfate (TGS) detector, which is suitable for detecting mid- infrared absorption bands with good sensitivity and resolution. The samples were analysed in the form of thin films placed directly on the sample holder or prepared using potassium bromide (KBr) pellet or attenuated total reflectance (ATR) techniques, depending on their physical properties. The spectral data provided insights into the chemical structure of the polymer matrices, recognizing the characteristic peaks corresponding to specific functional groups such as -OH, -CH, C=O, C-O-C, and N-H stretching and bending vibrations.

2.4.2. Measurement of thickness of polymer films

The thickness of the developed polymer films was measured using the micrometry method, which provides accurate dimensional analysis of thin materials. Before measurement, the micrometre, ensuring precision and consistency across all readings. This calibration step was vital to eradicate instrumental error and establish a reliable scale for subsequent measurements.
Following calibration, film thicknesses were measured for six different biopolymer formulations: Chia polymer, Chia-glycerol, Chia agar-agar, fenugreek polymer, fenugreek - glycerol, and fenugreek agar-agar. Each film sample was measured at multiple random locations to account for surface uniformity and possible thickness variations across the surface. The average of these readings was taken as the representative thickness of each film. This micrometry-based method offered a non-destructive and repeatable means to compare the influence of various plasticizers (like glycerol) and additives (like agar) on film formation. It was observed that the addition of glycerol generally increased film flexibility and sometimes slightly enhanced thickness due to plasticization, while agar-agar contributed to a denser, more structured network. Accurate thickness measurement is vital, as it directly impacts mechanical, barrier, and optical properties of the films, influencing their suitability for biodegradable packaging or edible film applications.

Calibration of micrometre:

1 division of stage micrometre is 0.01nm = 10 µm Number of divisions of stage micrometre = 10
Value of 10 division of stage micrometre = 10 x 10 = 100 µm Number of divisions of ocular micrometre = 8
Value of 1 division of ocular micrometre = 100/8 = 12.5 µm

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Value of one division = $\frac{\text{number of divisions of stage micrometre}}{\text{number of divisions of ocular micrometre}}$ x 10 µm

3. Results

3.1. Drug release

Table 1. Drug release efficiency from biopolymer film in bodily fluids

Bodily fluids	Fenugreek Polymer	Fenugreek glycerol	Fenugreek agar-agar
Saliva fluid	Slow	Fast	Fast
Blood Plasma	Fast	Fast	Fast
Gastric fluid	Very slow	Medium	Fast
Intestinal fluid+	Slow	Fast	Fast

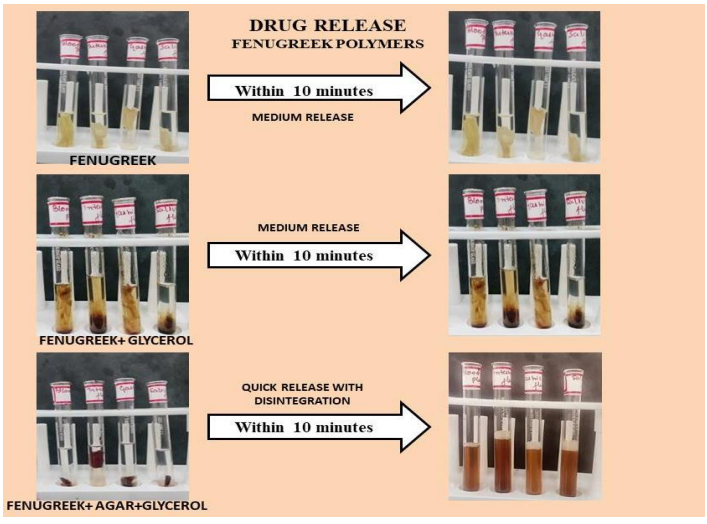

Figure 4: Drug release from different polymers from fenugreek

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3.2. Polymer disintegration:

Table 2 Percentage of disintegration of biopolymer films

Bodily fluids	Fenugreek polymer	Fenugreek glycerol	Fenugreek agar- agar
Saliva fluid	90%	85%	96%
Blood plasma	80-85%	80%	80%
Gastric fluid	50%	95%	85-90%
Intestinal fluid	90%	90%	80-85%

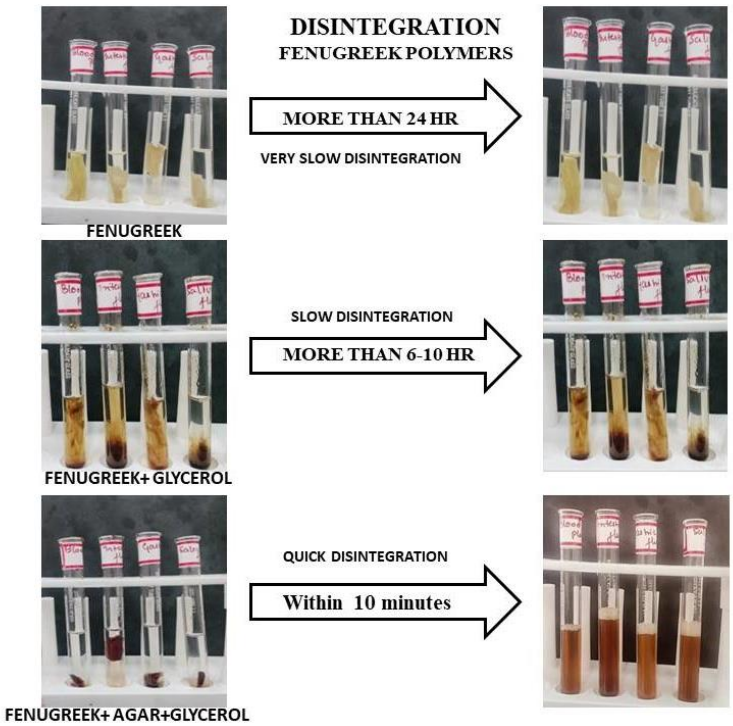

Figure 5: Disintegration of fenugreek polymers in saliva fluid

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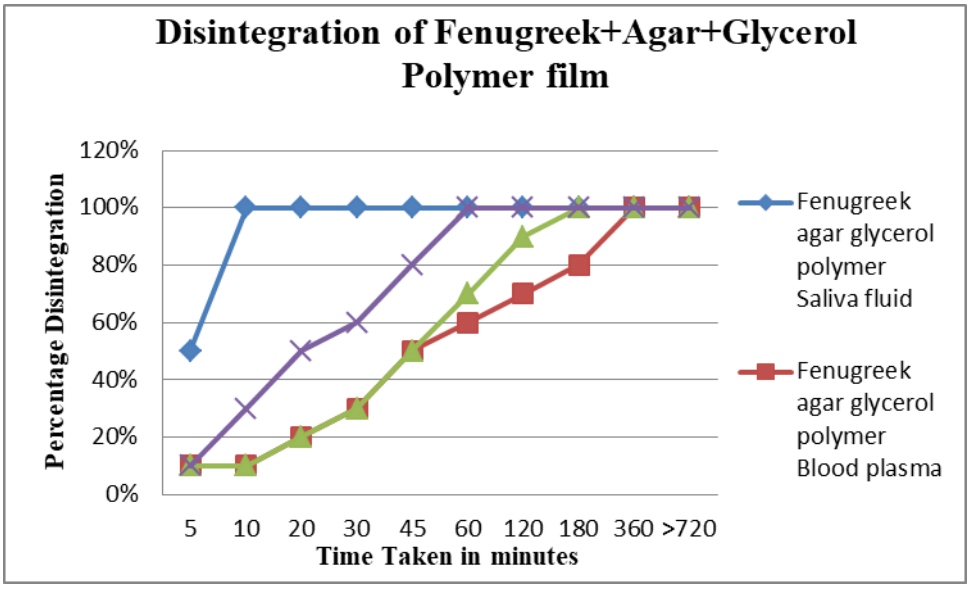

Figure 6: Graph representing the time taken for disintegration of Fenugreek Agar Glycerol polymer

3.3.FTIR characterization of biopolymer films

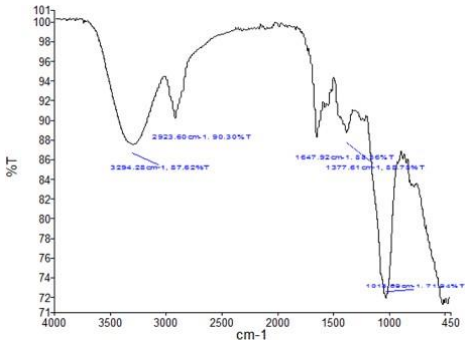

Figure 7: FTIR Spectrum for Fenugreek Polymer (FP)

The FTIR Spectrum of the Fenugreek-based polymer shows different peaks corresponding to various functional groups. A broad and intense peak at 3294.28 cm^{-} corresponds to O-H strectching vibrations, representing the presence of alcohol groups. The absorption at 2923.6 cm^{-} is due to C-H strectching, characteristic of alkane chains. A prominent band at 1647.92 cm^{-} is attributed to C=O stretching, vibartions, suggesting the existence of amide or alkene groups. The peak observed at 1377.61 cm^{-} represents C-H bending vibrations from methyl (CH_3) groups. Finally, the band at 1018.69 cm^{-} is related with C-O stretching vibrations, pointing to alcohols and possibly siloxane linkages. These spectral features confirm the complex polymeric nature and functional diversity of the fenugreek polymer.

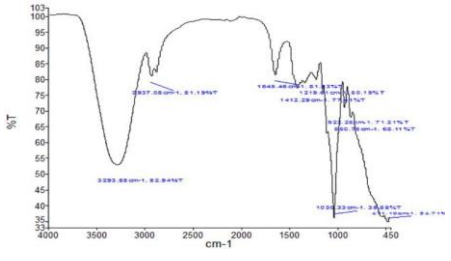

Figure 8: FTIR Spectrum for Fenugreek Glycerol Polymer (FGP)

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The FTIR Spectrum shows the existence of various functional groups charactersitsic of polysaccharides and glycerol. A broad peak at 3293.55 cm^{-1} corresponds to O-H stretching, confirming strong hydrogen bonding typical of polysaccharides and glycerol. The 2937.08 cm^{-1} peak arises from C-H stretching vibrations due to glycerol. A significant band at 1645.46 cm^{-1} is attributed to C=O and N-H stretching, suggesting the presence of amide groups or proteins. The absorption at 1412.29 cm^{-1} indicates CH_2 bending, further confirming glycerol content. A band at 12129.61 $_2$ represnets C-O and C-C-O stretching, characteristic of the glycerol backobone. Peaks at 1030.33 cm^{-1} indicate C–O–C linkages and C–O stretches found in glycerol and galactomannans. The 922.28 cm^{-1} peak is associated with C–O–H bending from the glycerol ring. Lastly, the small absorption at 868.11 cm^{-1} relates to C–H stretching in polysaccharide structures, supporting the presence of carbohydrate components.

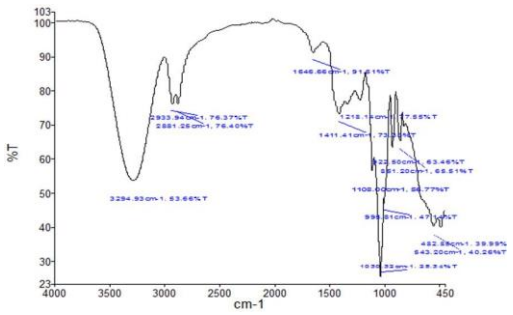

Figure 9: FTIR Spectrum for Fenugreek agar-agar Polymer (FAP)

The FTIR spectrum of the polymer blend comprising agar, glycerol, and polysaccharides shows distinct peaks indicative of key functional groups. A broad and strong peak at 3294.93 cm^{-1} is due to O–H stretching, confirming the presence of hydroxyl groups from polysaccharides, agar, and glycerol. Peaks at 2933.94 cm^{-1} and 2881.25 cm^{-1} correspond to CH_2 stretching vibrations, typically arising from the glycerol component. A band at 1646.66 cm^{-1} is associated with C=O and N–H stretching, indicating proteins, amides, or water molecules.The abosrption at 1411.41 cm^{-1} is due to CH_2 bending from glycerol. Peaks at 1218.14 cm^{-1}, 1108 cm^{-1}, and 1030.32 cm^{-1} reflect C–O and C–C–O stretching vibrations related to the glycerol backbone and agarose units. Characteristic peaks at 995.81, 922.5, and 851.2 cm^{-1} confirm the presence of 3,6- anhydrogalactose, a key marker for agar, supporting the integrity of the polysaccharide structure in the blend.

3.4 Measurement of thickness polymer films

The thickness of various polymer films was measured to evaluate the influence of different polymers and plasticizers on film formation. Results showed significant variation in thickness across formulations, indicating the impact of both polymer type and additives used. Amongst the samples analysed, the chia polymer film exhibited a thickness of 25µm, while its counterpart formulated with glycerol showed a reduced thickness of 12.5 µm, suggesting that glycerol, a plasticizer, enhances film flexibility and reduces thickness.

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Table 3: Thickness of biopolymer films using micrometry

Sl no	Polymers	Thickness
1	Fenugreek Polymer	18.75 µm
2	Fenugreek glycerol	37.5 µ m
3	Fenugreek agar-agar	31.25 µm

The Fenugreek Polymer film had a thickness of 18.75 µm, thinner than the native chia polymer, indicating different film-forming behavior. When glycerol was added to Fenugreek, the thickness increased significantly to 37.5 µm, unlike the trend observed with chia, implying that glycerol interacts differently with fenugreek mucilage, possibly promoting swelling. The Fenugreek agar-agar film showed a moderate thickness of 31.25 µm, slighly lower than the chia agar-agar film, emphasing the role of base polymer in determining final film structure. Overall, the study shows that the type of polymer (Fenugreek) and the type of additive (glycerol or agar-agar) considerably influence the thickness of the resulting film. These variations are likely due to differences in molecular interactions, water retention, and gelation behaviour of the components. Understanding these factors is critical in tailoring polymer films for specific applicatons such as biodegradable packaging or edible coatings.

Value of 1 division of ocular micrometer = 100/8 = 12.5 µm
Fenugreek polymer = 1.5 x 12.5 = 18.75 µm
Fenugreek glycerol = 3 x 12.5 = 37.5 µm
Fenugreek agar-agar = 2.5 x 12.5 = 31.25 µm



Figure 10: Measurement of thickness polymer films using micrometry

Conclusion

The present study efficaciously formulated and evaluated *Tridax*-loaded biopolymer films using mucilage extracted from *Trigonella foenum-graecum* (fenugreek). The films exhibited outstanding uniformity, transparency, and structural integrity, emphasizing the compatibility of the natural mucilage components with alginate. Incorporation of glycerol as a plasticizer considerably enhanced the flexibility and handling properties of the films, while also increasing *Tridax extract* encapsulation and continuous release profiles. Physicochemical characterization shown optimal mechanical strength, water vapour permeability, and thermal stability representing potential for both functional and packaging applications. The *Tridax extract* release studies confirmed a controlled and sustained release behavior, influenced by the nature of the biopolymer blend and glycerol concentration.

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Biodegradability evaluations exhibited fast degradation under natural conditions, confirming the environmental sustainability of the films. FTIR analyses confirmed the successful incorporation of mucilage and *Tridax extract* within the alginate matrix, with homogenous morphology and no significant phase separation. These results display the potential of these biopolymer-based films as eco-friendly carriers for natural *Tridax extract*s in pharmaceutical, cosmetic, and food-related applications. Overall, the study offers a promising approach for the development of biodegradable, sustainable, and functional *Tridax extract* delivery systems using plant-derived mucilage.

Conflict of Interest

The authors have no conflict of interest

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