Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Electronic Supporting Information

Hydrogel films prepared from plant-seed mucilage: An emerging biopolymer for therapeutic applications

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Experimental Section

1. General Information

The UV-Visible absorption spectra were archived on a Perkin-Elmer Lamda-750 UV-Vis spectrophotometer using 10 mm path length quartz cuvettes in 200-800 nm wavelengths. Baseline correction was applied for all spectra.

FT-IR Spectroscopy

The prepared hydrogels (DI and DI-L) were freeze-dried overnight in a lyophilizer (Labconco Freeze Dryer) and then finely grounded into a powder for Fourier transform infrared spectroscopy (FT-IR) analysis that was recorded at a resolution of 4 cm^{-1} in the scanning range 400–4000 cm^{-1} with a PerkinElmer (Spectrum 1) spectrophotometer.

Thin Film XRD

Rigaku Smartlab X-ray diffractometer (model TTRAX III) was employed for thin film X-ray diffraction (XRD) measurements at 50 kV, 100 mA using Cu-Kα (λ = 1.5406 Å) radiation for the analysis of hydrogel samples in the diffraction angle (2θ) range of 5–80° at a scanning rate of 0.02° s^{-1}. The originally prepared hydrogel samples (200ul) were drop cast into a coverslip and allowed to dry in a hot air oven at 40°C for 4 hours, before sample analysis.

Rheology

The rheological studies were carried out on Interfacial Rheometer (model: Physica MCR 301, make: Anton Paar (Austria)) by using a 50 mm diameter at 1° angle parallel-plate geometry at 25 °C with 0.1 mm gap. The amplitude and frequency sweep tests were performed to determine the viscoelastic nature of hydrogels. The flow behaviour of hydrogels was analyzed by the Power Law model as per literature [4] given as

$$\eta = m(\dot{\gamma})\, n^{-1}$$

where η is the apparent viscosity, $\dot{\gamma}$ is the shear rate, n is the power-law index, m is the consistency index. Amplitude sweep measurement was performed within a strain range of 0.0001 to 0.1% at a constant 10 Hz frequency and in a frequency range of 0.1 to 100 Hz, with a 0.1 % strain which was considerably below the hydrogel's deformation range. Furthermore, loss tangent (tanδ), the ratio of viscous to elastic nature of hydrogel, is given by,

$$\tan\delta = G''/G'$$

where G' is the storage modulus and G" is the loss modulus.

Surface morphology

The surface morphological characterization of synthesized hydrogels was performed by FESEM (model: Gemini SEM 300, make: Carl Zeiss). The freeze-dried hydrogel samples were deposited on the given sample stub using carbon tape, and subsequently, the stub was sputter-coated with a double layering of gold. FESEM image was recorded at 5-micron optical zoom, at a potential of 5.00 kV.

Thermogravimetric Analyses

The thermogravimetric profile of the freeze-dried hydrogel samples was performed under nitrogen atmosphere at a heating rate of 10 °C min^{-1} in a temperature region of 25-650 °C by employing a Netzsch STA-409CD thermal analyzer.

Zeta potential

The zeta potential was determined by Dynamic Light Scattering (DLS) by Zetasizer (Anton-par). The lyophilised hydrogel samples were sonicated in an ultra-sonic bath for 30 minutes, and then the sonicated and diluted samples were used for surface charge analysis.

Swelling Studies

A known weight of both the lyophilised hydrogel samples was immersed in DI water and Phosphate buffered Saline (PBS) in its original pH, allowed to swell and reach the equilibrium condition. After 12 hours, the now swollen hydrogel samples were taken out, filtered and weighed again. The swelling ratio was obtained as follows,

$$\text{Swelling Ratio \%} = \frac{Wf - W_i}{W_i} x100$$

Where W_i=Initial weight of the hydrogel and W_f=Final weight of the hydrogel.

2.Determination of total phenolic content (TPC)

Total phenolic content was evaluated using the Folin–Ciocalteu reagent (Manzoor, Zeng, et al., 2019). An Ultraviolet–Vis spectrophotometer was used to produce a calibration curve with a standard solution of gallic acid and measured at 760 nm. All of the tests were done in triplets. The concentration of hydrogels were kept at (100 mg/ml).The calibration standard was gallic acid, and the findings were stated in milligrams of equivalent gallic acid dry extract (mg GAE/g). (3)

2.1 Determination of DPPH (Radical Scavenging Assay)

Radical scavenging activity of the hydrogels were determined essentially as described by Blois (1958). The concentration of hydrogels were varied from 25-150 mg/ml). The volume was adjusted to 100 µl by adding MeOH. 5.0 ml of 0.1 mM methanolic solution of DPPH was added to these tubes and shaken vigorously. The tubes were allowed to stand at 25 °C for 30 min. The control was prepared as above without any extract and MeOH was used for the baseline correction. The changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula,

$$\text{Radical Scavenging Activity \%} = \frac{C_{O.D} - S_{O.D}}{C_{O.D}} \times 100$$

Where C is the control and S is the sample undergoing analysis.

2.2 Determination of anti-diabetic activity

The anti-diabetic assay was performed using alpha-amylase inhibition assay.(2) For this, different aliquots with sample (50-250 µL of sonicated hydrogel with concentration ranging from 50-250mg/ml) was placed in a tube and 125 µL of 0.02 M sodium phosphate buffer (pH 6.9) having alpha-amylase solution (5mg/ml) was added. 500 µL of 0.02 M sodium phosphate buffer (pH 6.9) was mixed and the solution was incubated at 25 ° C for 20 min. Then 600 µL of 2% starch solution (in 0.03M sodium phosphate buffer, pH 6.9) was mixed and incubated at 25 ° C for 15 min. 500 µL of DNSA reagent was added to terminate the reaction. The tubes were then incubated at 90° C for 10 min and cooled. 6 ml of water was added and absorbance was measured at 540 nm. The alpha-amylase inhibition activity was calculated as :-

$$\%\text{Inhibition} = \frac{\text{Abs}_{\text{Test}} - \text{Abs}_{\text{Control}}}{\text{Abs}_{\text{Test}}} \times 100$$

For control, the sample was replaced with water.

2.3a) Determination of anti-inflammatory activity

Tryptophan fluorescent spectroscopy analysis was used for protein denaturation assay. (2) For this reaction mixture was prepared having 0.5 mL 1.5% BSA,

6.0 mL of PBS (pH 6.4) with 0.5 ml hydrogel sonicated samples. After that tubes having all components were kept at 37 °C for 20 min followed by heating at 70 °C for 7 min and mixture

was immediately cool down. Two milliliters of mixture was studied by fluorescent spectroscopy analysis (excitation wavelength 280 nm) on Horiba Fluoromax Spectrophotometer.

2.3 b) Stern-Volmer Plot

The quenching behaviour was studied by Stern-Volmer equation,

$$I_0 / I = 1 + Ksv\,[Q],$$

where I_0, I are the fluorescence intensities before and after addition of the quencher, K_{sv} is the Stern- Volmer quenching rate constant and [Q] is the concentration of the quencher. [5]

2.4 Antibacterial Studies

The antibacterial performance of the hydrogels were evaluated against gram-positive (*B. subtilis* MTCC 441, *S. aureus* MTCC 96) and gram-negative (*E.coli* DH5α MTCC 433, *P. aeruginosa* MTCC 424) strains by zone inhibition and Growth curve tests.

Growth Curve Test:

From an overnight grown fresh culture, 1% inoculum of gram-negative bacteria and gram-positive bacterial culture was given in Nutrient broth medium in each of 6 flasks. One was kept as control, with only the respective bacterial cultures, another flask had only Fenu hydrogel (100 mg/ml), another flask had Flax hydrogel (100 mg/ml). The flasks were incubated at 37 ° C 180 rpm, and periodic samples were withdrawn for O.D measurement at 600 nm in a UV-Spectrophotometer.

Evaluation of Zone of Inhibition by Well-Diffusion Method

The antibacterial property of the synthesized ligand was further ascertained by the determination of zone of inhibition by the well-diffusion method. Firstly, the respective bacterial lawn was prepared in sterile nutrient agar media plates using sterile cotton swab sticks. 10^6 CFU/ml cultures of freshly overnight-grown bacterial suspensions of representative gram-positive and gram-negative bacterial strains were used to prepare the lawn culture on the plates. Following this, wells of approximately 5mm in diameter were made, and 100 mg of hydrogel samples werw added to the wells. The plates were then kept for incubation at 37°C, overnight. [2]

2.5 Biodegradation Analysis:

The biodegradability of the hydrogel films carried using the method adopted by [4] in soil as well as in simulated body fluid. The films were cut into a square shape (2 mm × 2 mm) and were dried in a hot air oven at 105 °C for 24 h and weighed as initial weight (Wi). The samples were buried 10 cm deep in a paper tea cup (150 mm × 60 mm × 1.5 mm) containing compost soil, and in 2 ml eppendorf tubes containing freshly prepared SBF. (2ml) The film sample pieces were recollected from the soil, and the SBF media at regular intervals and cleaned by wiping tenderly with tissue paper. Further, the samples were dried in hot air oven at 105 °C for 4 h and weighed (Wt) to determine the % of Weight Loss using the following equation.

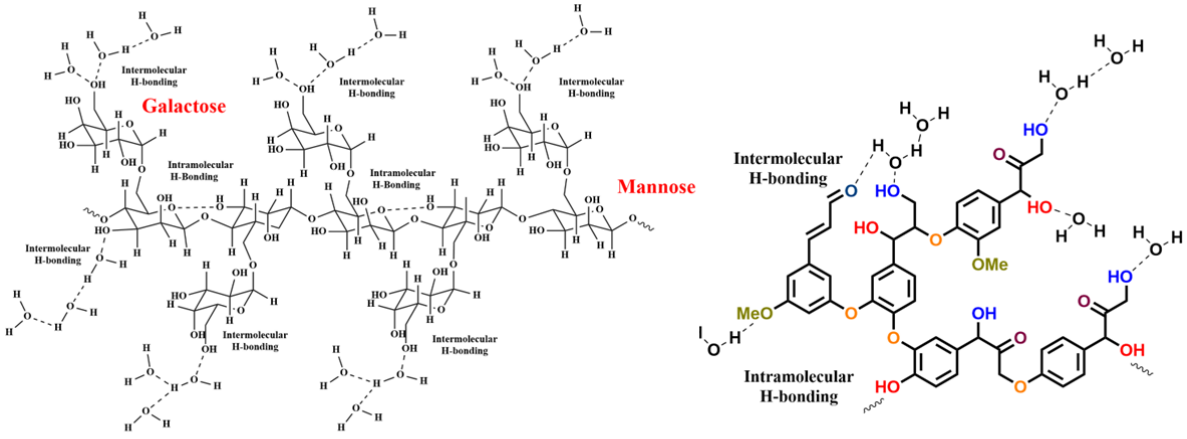
$$\text{Weight loss } \% = \frac{W_i - W_f}{W_i} \text{x} 100$$

Table S1. CHNS Analysis of Seed Extracts

SAMPLE	C %	H%	N%	S%	C/N RATIO
FENUGREEK SEED EXTRACT	0.69	9.36	0.10	3.80	6.77
FLAX SEED EXTRACT	0.35	9.70	0.12	3.69	3.08

Table S2. Physiochemical Analysis of Seed Extracts

PHYTOCHEMICAL TEST	FENUGREEK SEED EXTRACT	FLAX SEED EXTRACT
CARBOHYRATES	+	+
PROTEINS	+	+
AMINO ACIDS	+	+
REDUCING SUGARS	+	+
ALKALOIDS	+	+
FLAVONOIDS	+	+
POLYPHENOLS	+	+



Fenugreek Hydrogel Film **Flax Hydrogel Film**

Fig. S1a. Probable mechanism of Gelation in Fenugreek and Flax Hydrogel Films

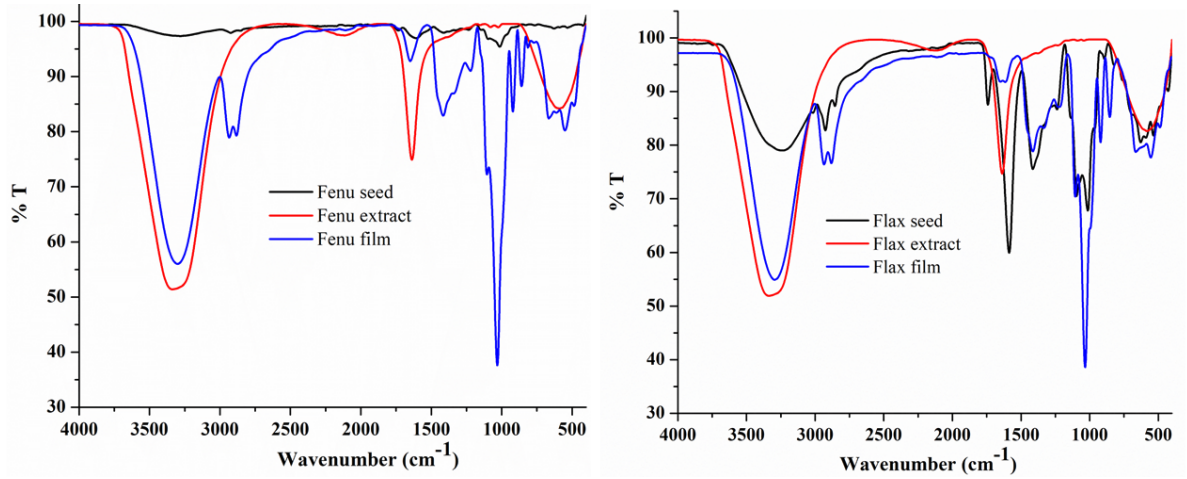


Fig. S1b. FTIR spectra of Fenugreek and Flax Seed, Extract and Hydrogel Films

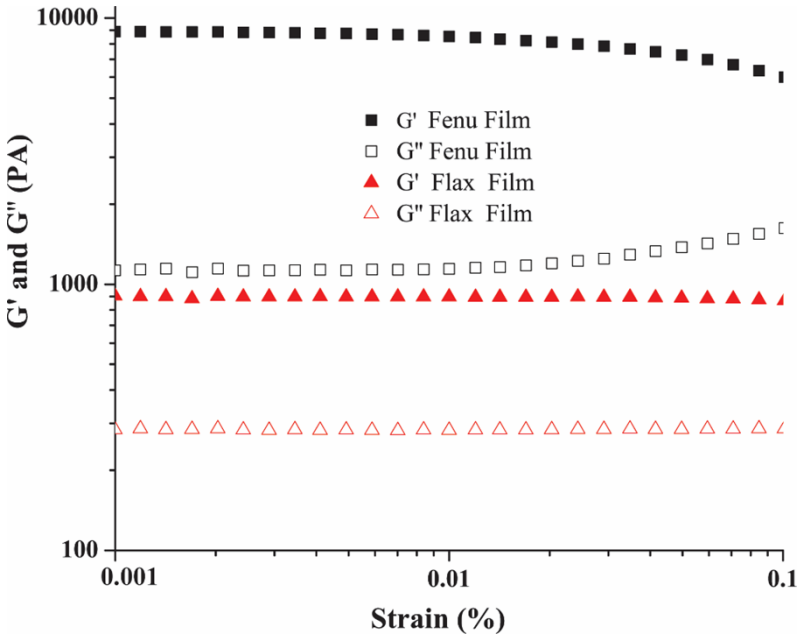


Fig. S2. Amplitude sweep curve of Fenu and Flax Hydrogels

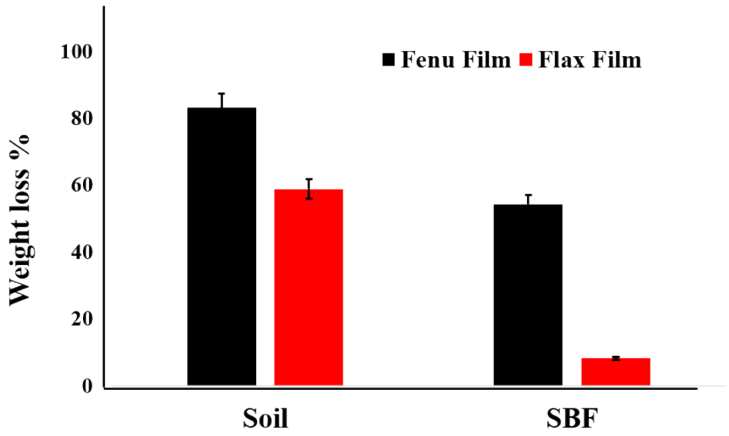


Fig. S3. Biodegradability study of Fenu and Flax hydrogels in Soil and SBF.

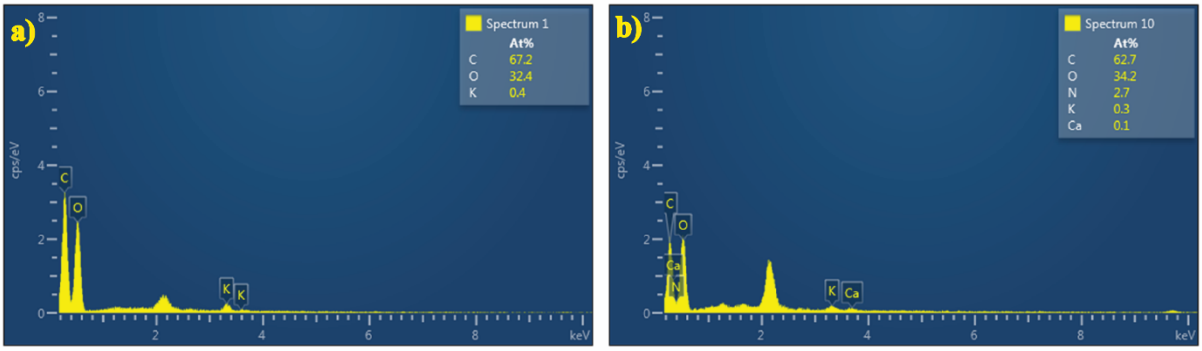


Fig. S4. EDX Spectra of Fenu and Flax Hydrogels.

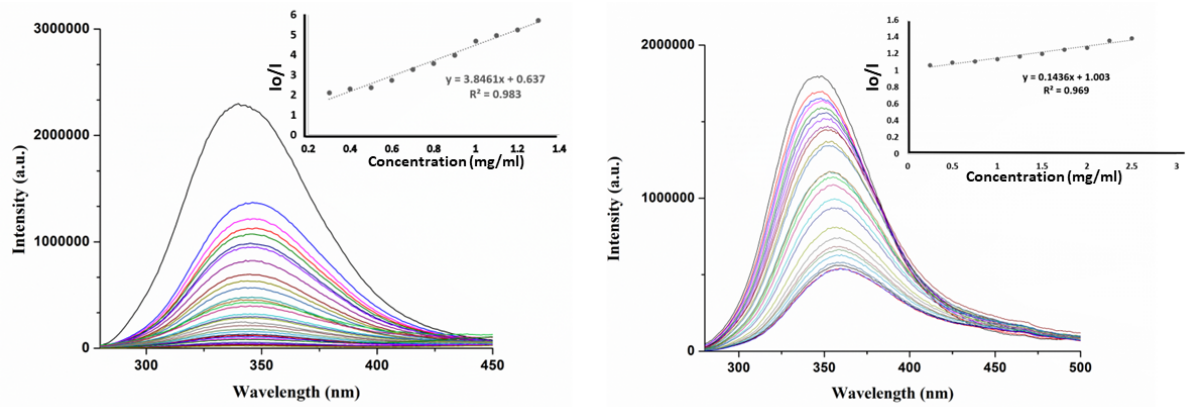


Fig. S5. Fluorescence spectra of BSA quenching using Fenu hydrogel and Flax Hydrogel and determination of Stern Volmer constant (inset).

Table S3. Statistical Data of Flax and Fenu hydrogel

Flax hydrogel

Regression Statistics	
Multiple R	0.984356312
R Square	0.968957349
Adjusted R Square	0.965077018
Standard Error	0.020627799
Observations	10

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.1062531	0.106253	249.7099503	2.57133E-07
Residual	8	0.003404049	0.000426		
Total	9	0.109657148			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	1.003018153	0.014091469	71.1791	1.69017E-12
X Variable 1	0.143550207	0.009084183	15.80221	2.57133E-07

Fenu Hydrogel

Regression Statistics	
Multiple R	0.991486
R Square	0.983045
Adjusted R Square	0.981162
Standard Error	0.176583
Observations	11

ANOVA

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	16.27148	16.27148	521.83169	2.80285E-09
Residual	9	0.280633	0.031181		
Total	10	16.55212			

	Coefficients	*Standard Error*	*t Stat*	*P-value*
Intercept	0.636979	0.144833	4.398019	0.0017253
X Variable 1	3.846071	0.168365	22.84364	2.803E-09

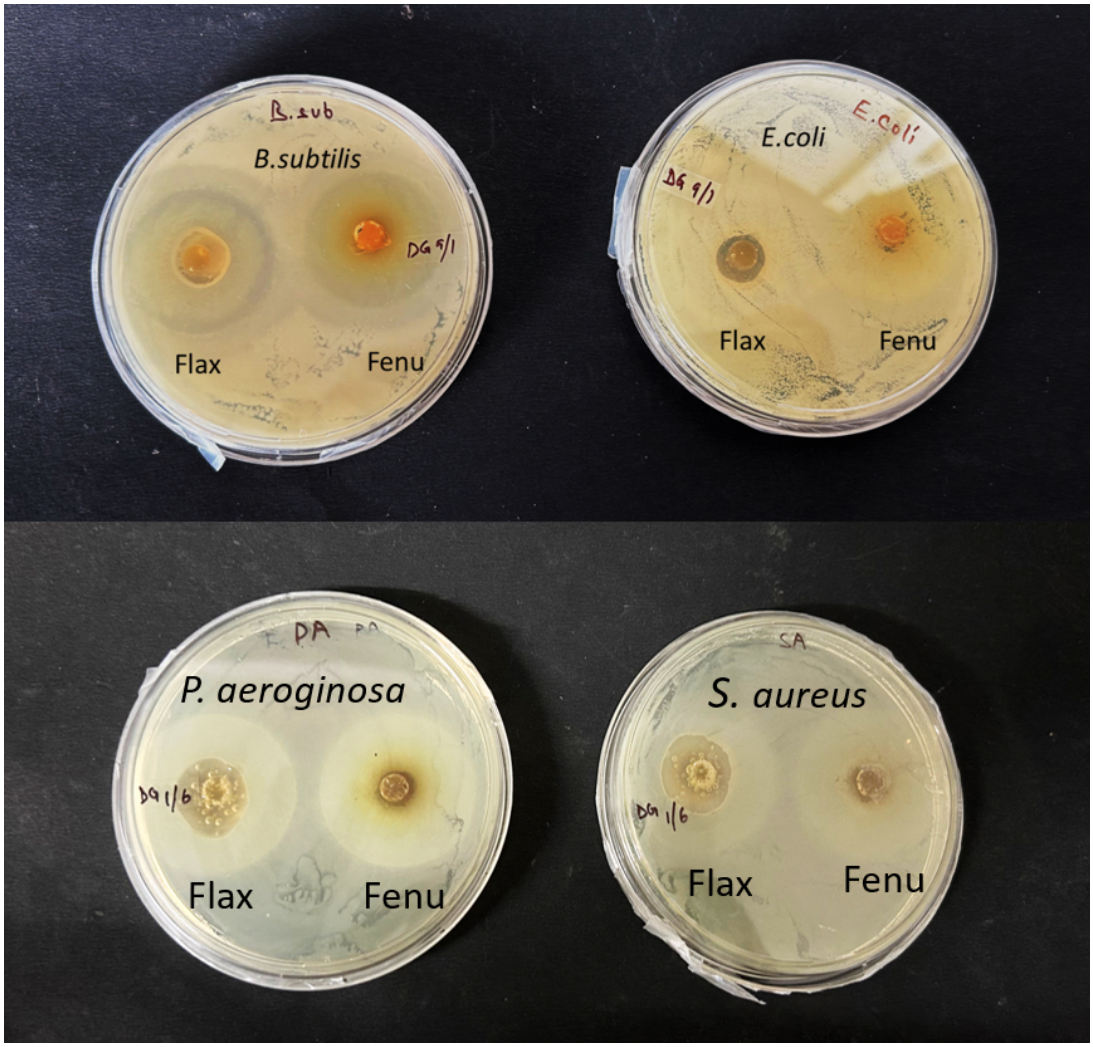


Fig. S6. Zone inhibition studies of Fenu and Flax Hydrogels *in B.subtilis* and *E.coli* strains and *P.aeroginosa* and *S.aureus* strains.

Sl. No	Mucoadhesive source	Cross-linker/ Copolymer	Gelation	Mechanical Strength	Swelling Property	Biodegradability	Antibacterial Property or Biocompatibility	Applications	References
1.	Carboxymethyl Tamarind Gum	Citric acid	Esterification	Not studied	High	Not studied	Yes	Drug delivery of moxifloxacin	[6]
2.	Okra mucilage	Sodium alginate	H bonding and electrostatic interactions	Not studied	High	High	Yes	Biological applications	[7]
3.	Basil seed mucilage	PVA and Glutaraldehyde	Chemical cross-linking via acetal groups	High	High	Not studied	Yes	Drug delivery of tetracycline/ wound dressing	[8]
4.	Elephant apple	Synthetic amphiphilic polyphenol	H bonding	Moderate	High	Not studied	Yes	Antioxidant studies and Fe(III) adsorption	[9]
5.	Fenugreek galactomannan	Borax	Borate/di-diol complexation	High	High	Not studied	Not studied	Water retaining agent	[10]
6.	Fenugreek seed mucilage	Poly-methylacrylate	Free radical polymerization	Not studied	High	Not studied	Yes	Drug delivery of methotrexate	[11]
7.	Flax seed mucilage	Polyacrylamide	Microwave-assisted grafting and cross-linking	Not studied	Moderate	Not studied	Yes	Preparation of polymeric scaffold for tissue proliferation	[12]
8.	Flax seed gum	Sodium Alginate, Epichlorohydrin,	Chemical cross-linking	High	Very high	Not studied	Not studied	Forward osmosis desalination	[13]

		Poly ethylene glycol							
9.	Fenu Film	NA	Intra and Intermolecular H bonding	High	High	Yes	Yes	Biological applications	**This work**
10.	Flax Film	NA	Intra and Inter molecular H bonding	Moderate	High	Yes	Yes	Biological applications	**This work**

Table S4. Comparative properties of prepared hydrogel films with other reported natural hydrogels from literature

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