



Evaluation Of Wound Healing Of Gauze Fabric Embedded With Tridax Procumbens Coated Film For Wound Healing Efficacy

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Abstract

The tridax procumbens extracts loaded sodium alginate and incorporated with gauze film was fabricated through solution casting techniques. After conducting a phytochemical test on the TP extracts, various chemicals including alkaloids, anthraquinone, carbohydrates, saponins, and cardiac glycosides were discovered to be present. Following an FTIR examination, the functional groups detected in tridax, alginate, gauze, and film were identified. Using EDX and FESEM, the film's surface shape and elemental composition were investigated. HPLC was used to confirm that the TPE contained quercetin. The film was then tested for in vitro cytotoxicity using the L929 cell line, yielding a maximum of 81% cell viability and 19% toxicity at varying dosages. The E. Coli, Bacillus, and Aeromonas bacteria were the targets of the in vitro antibacterial activity. To assess the antibacterial activity, the zone of inhibition was identified. Elastic modulus and tensile strength without 7. 52 MPa, 8. 83 MPa, 1. 67 GPa, and 2. 45 GPa were discovered with gauze and with gauze sheet, correspondingly. When the film's extracts were given the chance to interact with the L929 cell line, 86% of the cells migrated, proving the film's ability to promote wound healing. After examining the drug release, kinetics, and swelling of the film, the drug release profile and mechanism were discovered. For the purpose of healing wounds, tridax procumbens extracts filled alginate with gauze film.

Keywords: Tridax procumbens, Alginate, Film

1. Introduction

Plants play a major role in primary healthcare as therapeutic remedies in developing countries. Herbal remedies have long been the cornerstone of traditional Indian medical practices like Ayurveda, Unani, and Sidha, providing relief from a wide range of ailments. Medicinal herbs have an amazing role in the healing of wounds.

Recent research has spurred the creation of new wound dressings that incorporate active chemicals or extracts from medicinal plants as potential substitutes for conventional wound dressings. Individual herbs are not enough to produce the intended therapeutic effect, according to Ayurveda. When the composition of numerous herbs is tuned in a specific ratio, it can yield a more beneficial and less hazardous medicinal impact. Previous research indicates that Tridax procumbens (TP) has a good antibacterial activity and may be able to treat skin infections. TP has anticoagulant, wound healing, hair tonic, insect repellent, antifungal, and antidiarrheal properties.

Based on the literature study, there are no research has been tried on gauze with herbal extract on direct application on wound. Whereas research done on Tridax Procumbens herbal medicine as powder and tablets form up to 2023 Using crude extracts of this plant, a comprehensive biological screening was conducted with gram-positive and gram-negative bacteria, yeasts, and fungi. The flowers' n-hexane extract exhibited anti-Escherichia coli properties. Salmonella group C, Salmonella paratyphi, Escherichia coli, and Mycobacterium smegmatis were all susceptible to the identical extract of the whole aerial portions [1]. An entirely novel flavonoid, 2"-glucosyl-8-C-glucosyl-4'-O-methylapigenin (1), was isolated from the aerial portions of Chrysanthemum viscidifolium [2]. According to the current research, T. procumbens leaves may be utilized to cure illnesses brought on by the examined pathogens. [3] When compared to silver nitrate and tridax procumbens leaf extracts, the produced silver nanoparticles from the plant demonstrated improved wound healing activity in fish as well as improved the epithelialization and look of the lesion. Analysis of the nanofibrous mat's antimicrobial activity against Escherichia coli and Staphylococcus aureus bacteria revealed that it had superior resistance power and an excellent zone of inhibition against both gram positive and gram negative bacteria [5].

The T. procumbens leaf methanol extract shown much higher antioxidant activity. Compared to breast cancer cell lines, the studied plant leaf extract shown higher efficacy against human lung cancer cells [6]. Folk medicine has long utilized T. procumbens to treat wounds, skin conditions, and to prevent blood clotting [7]. Findings demonstrated the curcumin-MPEG-chitosan film's efficacy in the treatment of wound healing [8]. Numerous pharmacological activities have been identified by it, including hepatoprotective activity, anti-inflammatory, wound healing, antidiabetic activity, hypotensive effect, immunomodulation property, bronchial catarrh, dysentery, and diarrhea, as well as antimicrobial activity against both gram-positive and gram-negative bacteria and the prevention of hair loss and hair growth [9].

Tanning and flavonoids were responsible for the healing of wounds. The results validated tridax procumbens (L.) ethanomedicinal claim in wound healing in both non-diabetic and diabetic circumstances [10]. On the other hand, little is known about the biological functions of its leaves. Diffuse transmittance was used in this investigation to examine the phenolic contents, possible anti-aging action in vitro, and toxicity of the ethanol extract from the leaves [11].

The plant is known for its various qualities, including those that are astringent, carminative, stomachic, diuretic, antidiabetic, anti-inflammatory, radio-protective, gastroprotective, antioxidant, ant allergy, anti-cancer, anti-bacterial, and cardioprotective [12,13].

The purpose of this work is to create a film with 10% weight percentage of TP fresh extracts combined with 10% weight percentage of sodium alginate. It was decided to combine TP fresh extracts with gauze film and load them with sodium alginate. The goals are to assess the chemicals present in the fresh extracts from TP using phytochemical screening, FTIR for functional groups, FESEM for surface morphology and elemental compositions, and EDX for elemental compositions. to assess the film's ability to cure wounds using both in-vitro and in-vivo characterisation. to assess the film's in vitro cytotoxicity and antibacterial properties.

To assess the tensile strength, kinetics, in-vitro drug release, contact angle, and swelling behavior

2. Materials and Methods

TP fresh leaves was collected from in and around the campus Hindustan Institute of Technology & Science Deemed University in India, Padur, Chennai. The sodium alginate (density) and gauze 150-200 GSM was procured from sithalapatty Coimbatore. TP fresh extracts loaded sodium alginate and incorporated with single layer gauze film was fabricated through solution casting method.

2a. Development of coated gauze fabric

TP fresh extracts loaded sodium alginate and incorporated with single layer gauze film was fabricated through solution casting method is shown in Figure.1.

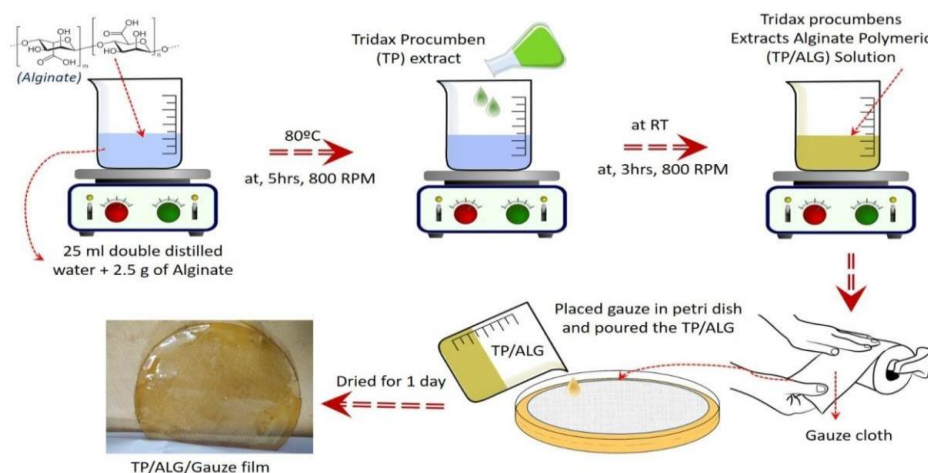


Figure. 1 wt. 10 % of TP Fresh Extracts Loaded with wt. 10% of Sodium Alginate and Incorporated with Gauze Film

To develop the gauze with herbal film coated to cure the wound directly from the skin. 25 ml double distilled water and dissolved 2.5 g of alginate and stirred for 5 hours at 800 rpm with 80 degrees Celsius. After obtaining polymeric solution cooled for 1 hour then add tridax procumbens extracts in polymeric solution for 3 hours in 800 rpm without heat. Then placed gauze in petri dish and poured the tridax procumbens extracts loaded alginate polymeric. Then homogeneous polymeric solution was poured in the petri dish after placing single layer gauze on the bottom of Petra dish and allowed to dry. Then after 24 hours the film was revealed from the dish and further characterization were carried out. The film was incorporated gauze in first time. The novelty is incorporation of gauze in the film to enhance the stability of the film in terms of strength. Also expecting the slow release of drug through the gauze.

2b. Characterizations

Phytochemical Screening

The phytochemical screening (Table 1) of the developed TP was carried out at Avinashi lingam Institute for Home Science and Higher Education for Women, deemed to be University, Coimbatore, Tamil Nadu, India.

HPLC

HPLC analysis was carried out to determine quercetin in TP at Kayunga University, Coimbatore, Tamil Nadu, India.

FTIR

FTIR spectroscopy (BRUCKER, ALPHA) analysis was carried out to determine functional groups present in TP (3 ml), Alginate (2 g), and film (3 mm x 3 mm x 0.5 mm) at Coimbatore Institute of Technology, Coimbatore, Tamil Nadu, India-641014.

XRD

The XRD analysis was carried out to determine crystalline peaks average crystallite size present in TP at Avinashi lingam Institute for Home Science and Higher Education for Women, Deemed to be University, Coimbatore, Tamil Nadu, India.

FESEM

The surface morphology and elemental composition of the TPELAWGF (5 mm x 5 mm x 0.5 mm) film were examined through FESEM (CARL ZEISS (USA), MODEL: SIGMA), and EDX was carried out at Avinashi lingam Institute for Home Science and Higher Education for Women, Deemed to be University, Coimbatore-641 043, Tamil Nadu, India.

EDX

The elemental composition of the TPELAWGF (5 mm x 5 mm x 0.5 mm) film were examined through FESEM (CARL ZEISS (USA), MODEL: SIGMA), and EDX was carried out at Avinashi lingam Institute for Home Science and Higher Education for Women, Deemed to be University, Coimbatore-641 043, Tamil Nadu, India.

In Vitro Cell Viability Assay

The in-vitro cell viability assay was performed using an L929 cell line with different concentrations (20, 50, 75, and 100 µg/µl) of TP/ALG for different durations (0, 4, 18, and 24 h) was carried out at Centre of Excellence for Medical

Textiles, The South India Textile Research Association, Coimbatore-641 014, Tamil Nadu, India

In Vitro Antibacterial

The in-vitro antibacterial activities were done against Escherichia Coli, Staphylococcus aureus, streptococcus A, and Vibrio cholera at The South India Textile Research Association, Tamil Nadu, India-641014. The zone of inhibition was determined to evaluate the antimicrobial activity.

Tensile Strength

The tensile strength of the TPELAWGF (1" X 6") was carried out at Coimbatore Institute of Technology, Coimbatore, Tamil Nadu, India-641014 through Universal Tensile Tester (Deepak Poly Plast 30 kN) as per ASTM D882 at the speed of 2 mm/min

Swelling Behaviour

The swelling behaviour of the nanofiber film was assessed. The phosphate buffer (PBS) solution with a pH of 7.4 was poured over the nanofiber film, and they were then left to soak for 18 days at 37 °C. The nanofiber film were removed from the solution each day, all surface water was removed, and the swelling behaviour of the film was determined using the equation below. = $W_t - W_i / W_i$ swelling capacity (%) $100 \times (W_t - W_i) / W_i$ (2) where W_i is the starting weight of nanofiber mats and W_t is the weight of swollen nanofiber mats that were drip-dried by filter paper.

Optical Contact Angle

Optical contact angle analysis was carried out to determine contact angle of in TP at Karunya University, Coimbatore, Tamil Nadu, India.

Drug Release and Kinetics

Drug release and kinetics analysis was carried out to determine Drug release and kinetics of in TPELAWGF at Coimbatore Institute of Technology, Coimbatore, Tamil Nadu, India-641014.

3. Results and Discussions

3.1 Phytochemical Screening

The phytochemical screening was carried out for Tridax Procumbens extracts and found the following compounds presence and confirmed through test is shown in Figure. 2. The presence compounds are aids for the wound healing and enhanced the tissue regeneration on the wound. Also, the plant extracts which is

having antibacterial properties were play vital role in the cells growth on the wound. The obtained results were compared with previous work on this plant and confirmed the same effects on the same. Tridax Procumbens extracts were loaded in Carboxymethylcellulose was revealed the better wound healing on mice within 11 days [16, 17].

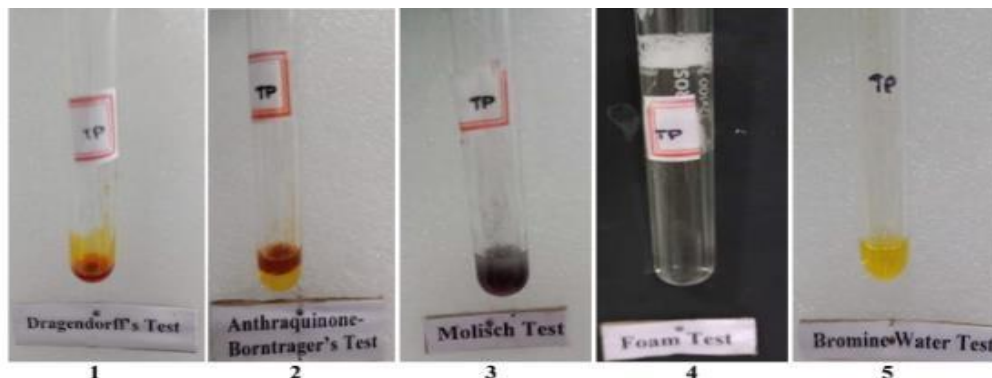


Figure. 2 Phytochemical Screening of Tridax Procumbens Extracts

The phytochemical screening of TP was carried out and found the presence of 1. Alkaloids, 2. Anthraquinone (Borntrager's test), 3. Carbohydrates, 4. Saponins, 5. Cardiac glycosides which were confirmed through the Dragendorff's Reagent, FeCl_3 + Conc. HCl + diethyl ether + Ammonia, Molisch's test, shaken with water, and Bromine water test, and identified based on the reactions as shown in Figure. 2 The phytochemical screening of tridax procumbens extracts were given in table.

Table. 1 Phytochemical Screening of Tridax Procumbens Extracts

Sl. No	Phytochemical Screening	Reactions	Compounds
1	Dragendorff's Reagent FeCl_3 + Conc. HCl + Diethyl Ether + Ammonia	Presence of Reddish Brown Precipitate	Alkaloids
2	Molisch's Test Shaken With Water	Presence of Reddish Orange Color	Anthraquinone (Borntrager's test)
3	Bromine Water Test	Presence of Violet Ring	Carbohydrates
4		Presence of Foam	Saponins
5		Presence of Yellow Precipitate	Cardiac Glycosides

3.2 HPLC

The film was carried out the HPLC analysis confirmed the presence of quercetin is shown in Figure.3. The Q value of 8.205 which nearly matches with Q value of pure quercetin. The Q value was confirmed with previous work with standard quercetin [23]. The quercetin plays significant role in wound healing. The quercetin reduces free radicals because of its antibacterial properties. Also which reduce the wound inflammation and with anti-cancerous properties. The quercetins even control the blood pressure and helps for controlling the allergies. Which are find in many fruits and food consuming daily as well as its controls cholesterol level in the body. The drug release from the film would enhance the wound healing and its contractions. Then it does improve tissue growth and its regeneration in the wound on the skin [18, 19].

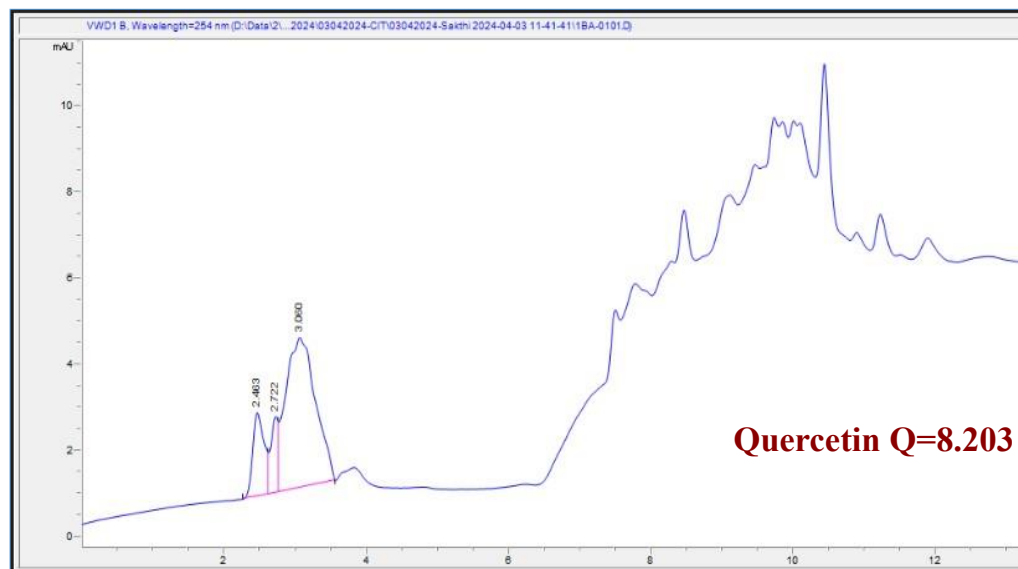


Figure. 3 HPLC Spectrum of Tridax Procumbens Extracts

3.3 FTIR

The major peaks of Alg/TP/Gauze wound dressing was shown at wavenumber, 3332 cm^{-1} , 2978 cm^{-1} , 1589 cm^{-1} , 1388 cm^{-1} , 1257 cm^{-1} and 1056 cm^{-1} . The peak at 3332 cm^{-1} is specifically due to the vibration of OH groups present in both sodium alginate and Tridax procumbens (TP) leaf extract. In the FTIR pattern of pure TP extract, a strong intense peak is observed at 3356 cm^{-1} is due to the predominant alcoholic OH groups, which are usually present in plant extracts. The peak due to the OH group present in pure alginate is found at a wavenumber 3657 cm^{-1} . The peaks at wavenumber, 2978 cm^{-1} , 1589 cm^{-1} , 1388 cm^{-1} and 1056 cm^{-1} is due to the C-H stretching, asymmetric stretching of COO^- , symmetric stretching of COO^- and C-O-C groups of alginate polymer. The shift in peaks compared to pure sodium alginate polymer and the pure TP extract compared to the Alg/TP/Gauze, wound dressing composite indicates the successful incorporation of TP into sodium alginate polymer [14, 15] is shown in Figure.4. Figure.5. Figure.6. Figure.7.

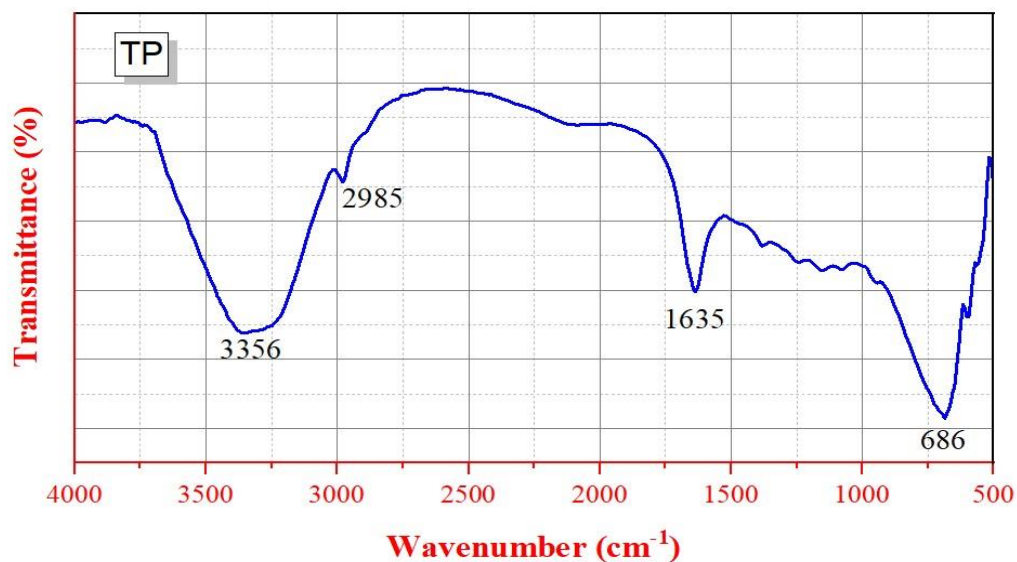


Figure. 4 FTIR Spectrum of Tridax Procumbens Extracts

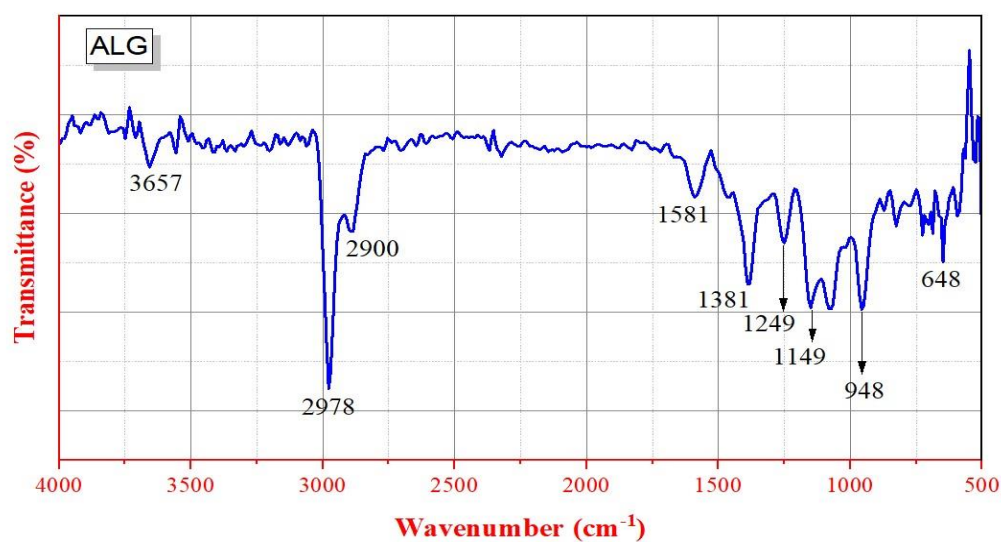


Figure. 5 FTIR Spectrum of Alginate

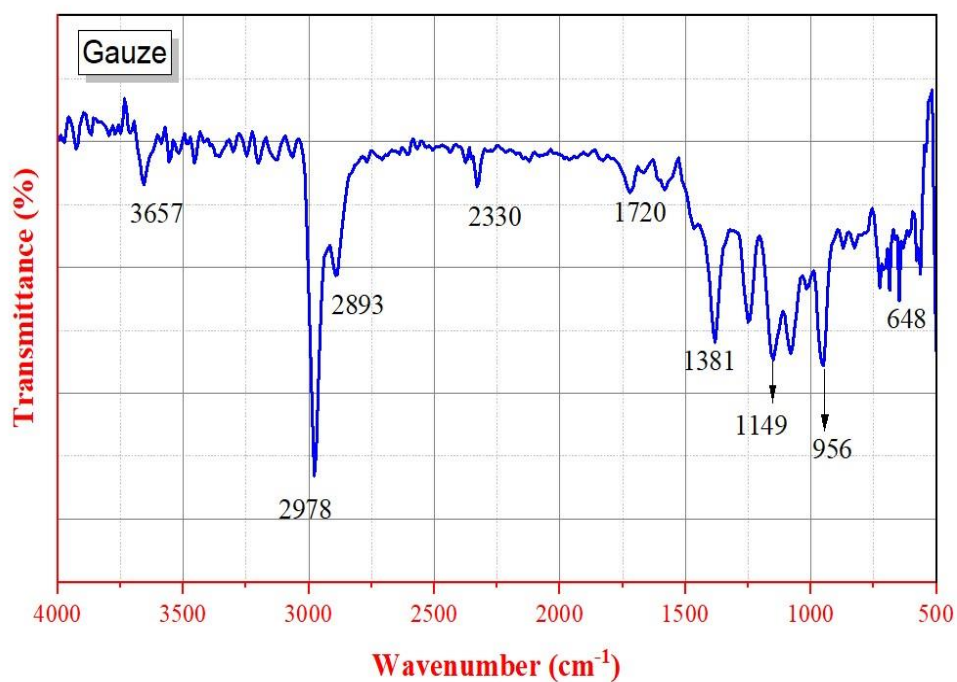


Figure. 6 FTIR Spectrum of Gauze

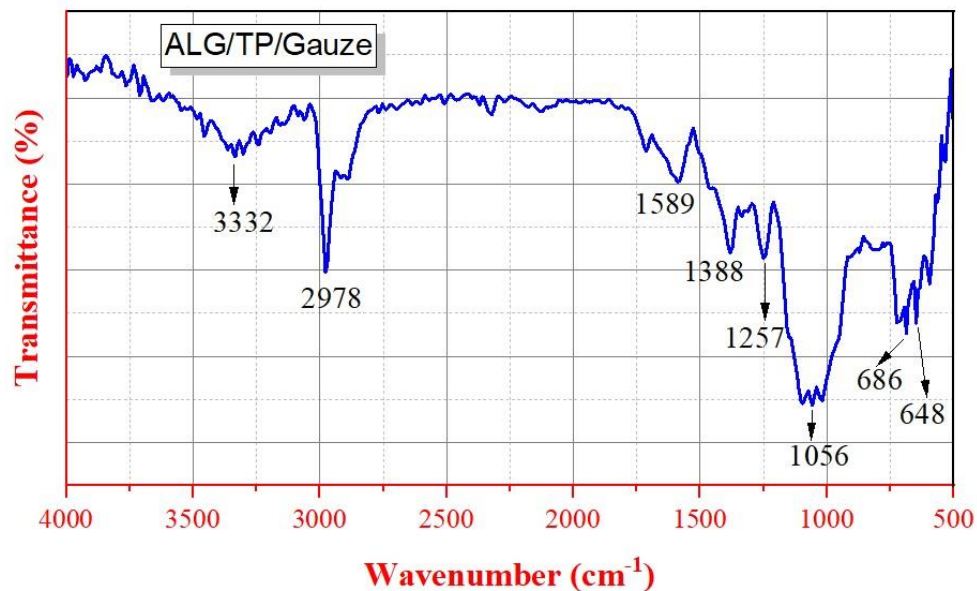


Figure. 7 FTIR Tridax Procumbens Extracts Loaded Alginate with Gauze Film

3.4 XRD

The XRD analysis of tridax procumbens extracts loaded alginate with gauze film shows the crystalline nature of the fabricated gauze dressing Figure.8.

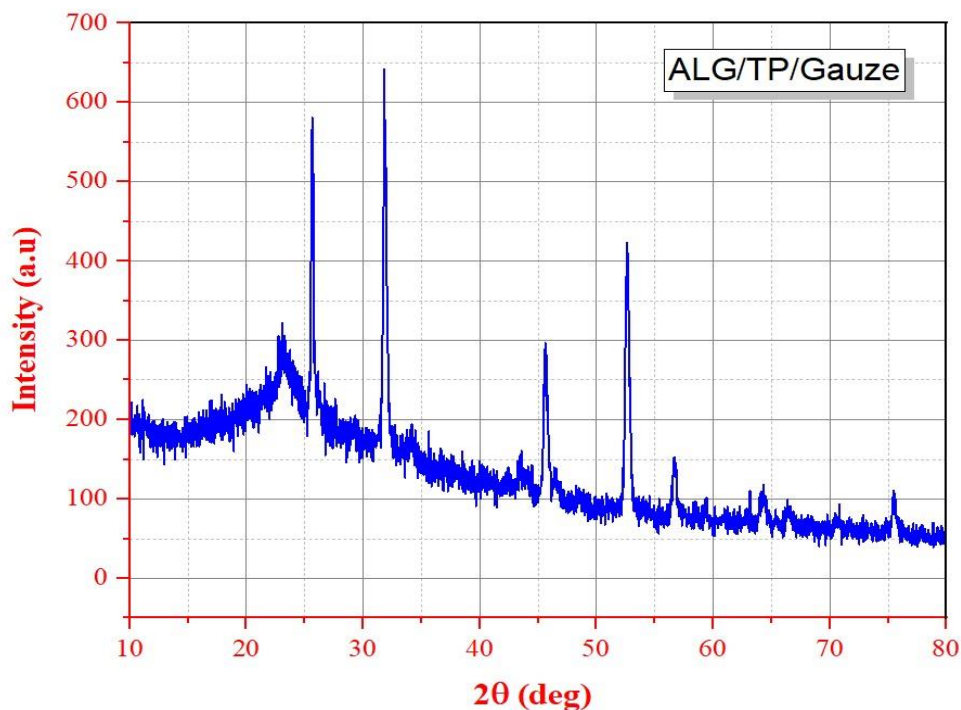


Figure. 8 XRD of Tridax Procumbens Extracts Loaded Alginate with Gauze Film

The high intense crystalline peaks are observed at 2θ values 23.1664, 25.64, 31.82, 43.72, 52.64, 56.71, 45.66, 64.24, 66.24, 66.44 and 75.49 degrees.

The peaks at 2θ value 23.11664 degree occurs due to the presence of alginate polymer which is usually amorphous in nature. The other intense peaks are due to the added TP extract. It is observed that the crystallinity of the tridax procumbens extracts loaded alginate with gauze film shows appreciably increased

on addition of TP extracts. The average crystallite size of the tridax procumbens extracts loaded alginate with gauze film is calculated as 38.426 nm using debye scherrre formula [9, 10, 11].

3.5 FESEM

The surface morphology and different compositions of elements were present in the tridax procumbens extracts loaded alginate with gauze film was carried out through FESEM and EDX analysis Figure.9 and Figure. 10. The dispersion of tridax procumbens extracts were present in the film was found. The fillers were settled by own its location in the matrix. The tridax procumbens extracts were dried and, in the form, small fillers were revealed in FESEM images. The fillers were form tiny groups and settled in matrix by its own gravity. After stirring the tridax procumbens extracts in alginate and obtained polymeric solution was poured in petri dish.

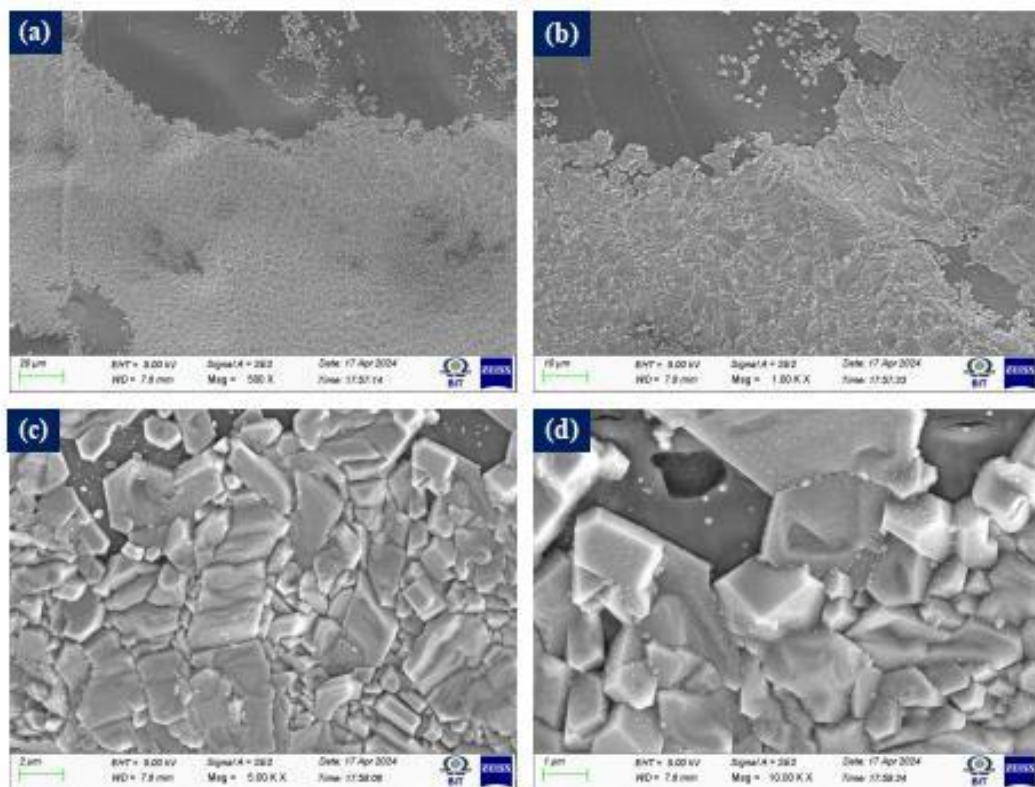


Figure. 9 Tridax Procumbens Extracts Loaded Alginate with Gauze Film

The tridax procumbens extracts were stirred continuously in polymeric solution. Hence the fillers were dispersed and by its gravity and stooped its rotations and settled down on the matrix. The FESEM images show that the addition of plant extract as fillers produce an irregular structure on the alginate matrix surface. More over the use of cotton gauze showed an irregular solid line embedded tridax procumbens leaf extract particulates. The FESEM analysis of the tridax procumbens extracts loaded alginate with gauze film shows that the particles of TP extract are of particle size of almost 1 micron [12, 13].

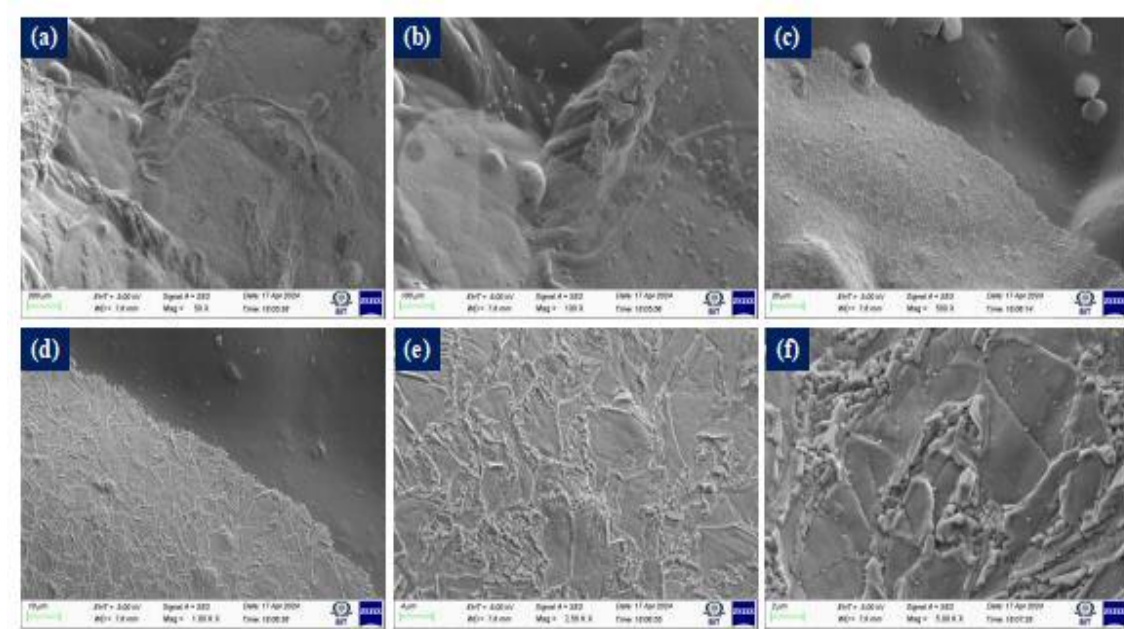


Figure.10 Tridax Procumbens Extracts Loaded Alginate with Gauze Film

3. 6 EDX

The 10 wt. % tridax procumbens extracts loaded alginate with gauze film was carried EDX analysis and found the different elemental compositions Figure.11 .

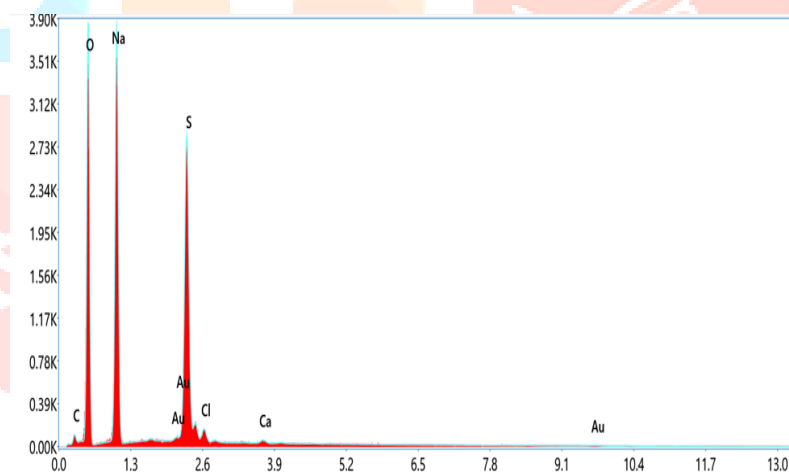


Figure. 11 EDX Analysis of Tridax Procumbens Extracts Loaded Alginate with Gauze Film

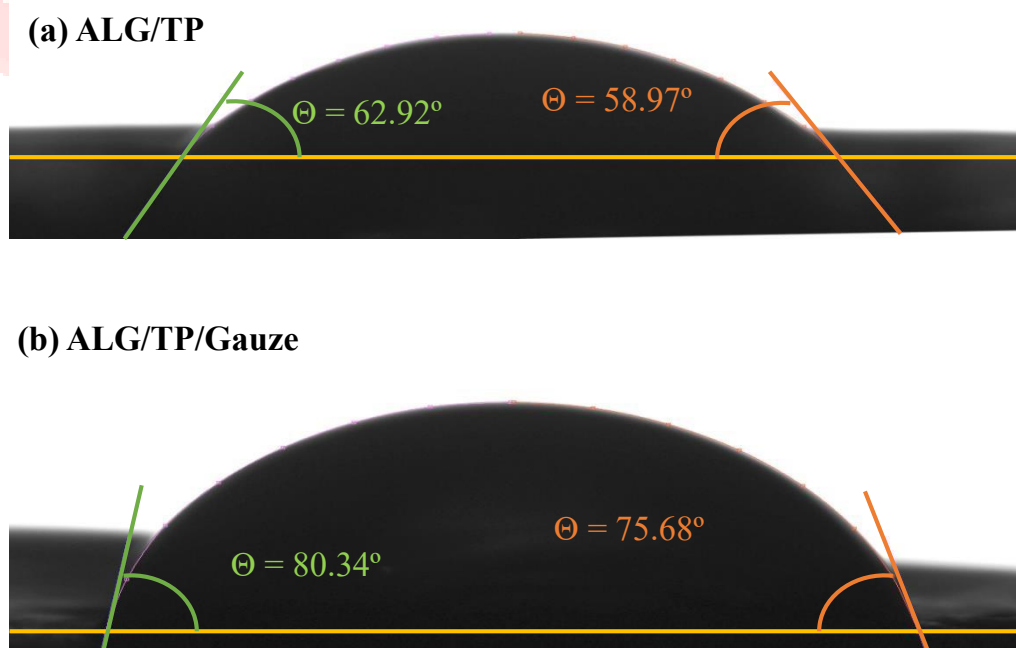
The film was cut in to 5 mm x 5 mm and gold sputtered before the EDX analysis. The film was cut in to 5 mm x 5 mm and gold sputtered before the EDX analysis. The results revealed that the following elements were present in the film such as C, O, Na, Cl, Ca, and Au. The atomic wt. of each element was found as 11.76, 54.54, 23.36, 8.70, 0.43, 0.13, and 0.09. The elements were plays significant role in wound healing. The 10 wt. % tridax procumbens extracts loaded alginate with gauze film was carried EDX analysis and found the different elemental compositions.

Table. 2 EDX Analysis of Tridax Procumbens Extracts Loaded Alginate with Gauze Film

S.	Elements	Weight. %	Atomic. %	No
1	C	7.47		11.76
2	O	46.13		54.54
3	Na	29.61		24.36
4	S	14.74		8.70
5	Cl	0.80		0.43
6	Ca	0.27		0.13
7	Au	0.98		0.09

3.7 Optical Contact Angle

The wettability of tridax procumbens extracts loaded alginate without and with gauze film was studied using optical contact angle studies. A drop of water was placed on top of the gauze film and the angle between the droplet of water and the bottom of the gauze film was measured using a contact angle meter. It was observed that the optical angle on addition of tridax procumbens extract and the use of gauze decreased is shown in Figure. 12 (a, b). This indicated the hydrophilic nature of the film which indicated the nature of film to absorb moisture and help faster healing of wound. This also helps in better absorption of wound exudates helping in faster healing of wounds [20, 21].

**Figure. 12 Optical Contact Angle a) ALG/TP b) ALG/TP/Gauze**

3.8 Antimicrobial studies

The culture media utilized in this experiment was either potato dextrose agar or nutrient agar. In pre-sterilized petri dishes (25 ml), the molten nutrition agar or potato dextrose agar was dispensed and allowed to cool. The test bacterium was first suspended in distilled water and then uniformly cultured on these agar plates. The plates were left to become firm. Using a flamed cork borer, 6 mm diameter holes or wells were punched into the agar after solidification. For every plate, three wells are made. A 100% concentration of plant extract was used to fill one well, while a 50% concentration was used in another. Solvent was used to fill the last hole as a control. For twenty-four hours, the Petri plates were incubated at 37 °C to grow bacteria. Following the incubation phase, measured the created around each entire (well/cup), recording the data as they are displayed in Figure 13. For every experiment, a well was made by adding only solvent as a negative control. For analysis, the value produced here is subtracted from the experimental values.

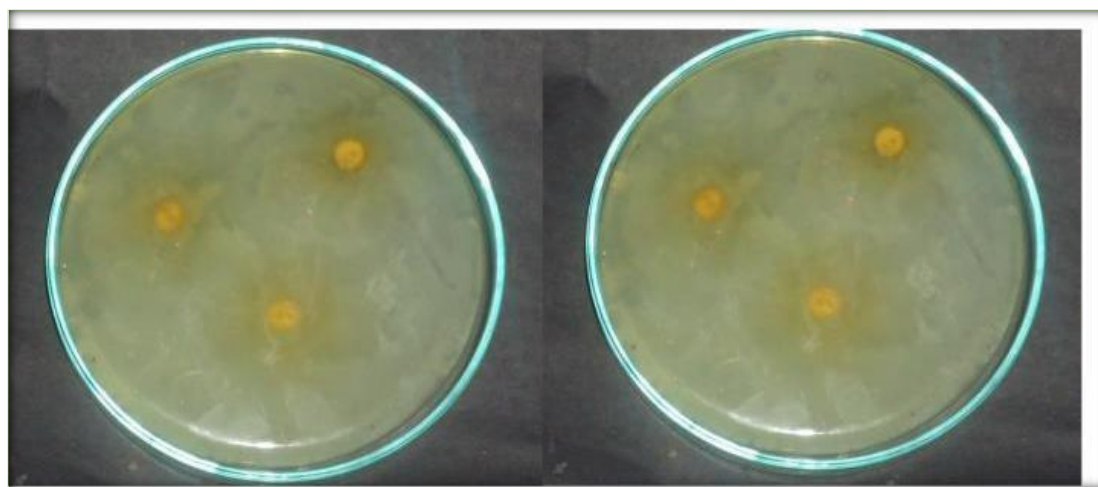


Figure. 13 Antimicrobial studies

The wound dressing was tested for its antibacterial activity, against microbes such as E.coli and Bacillus, P.aureginosa. The zone of inhibition in case of each microbe was measured. The antibacterial activity of Alg/TP/gauze wound dressing against E.coli, Bacillus, P. aeruginosa and was having a zone of inhibition upto 12.3 mm, 10.3 mm and 12 mm

Table. 3 Antimicrobial Studies

S. No	Organisms	Zone of I nhibition in (mm)		
		Control (Gentamycin)	Without Gauze	With Gauze
1	E.coli	15.0	10.0	12.3
2	Bacillus	12.0	09.0	10.3
3	P. aeruginosa	16.6	10.6	12.0

3.9 In-Vitro Cytotoxicity

The 10 wt. % tridax procumbens extracts loaded 10 wt. % of alginate with gauze film was carried out in-vitro cytotoxicity analysis through L929 cell line with different concentrations of 5, 25, 50, 75, and

100 μ l. The cell viability and toxicity was found through qualitatively through the cells images is shown in Figure.14. Figure. Cells morphology for different concentrations (5 μ l, 25 μ l, 50 μ l, 75 μ l, 100 μ l) tp fresh extracts loaded with 10 wt. % of sodium alginate gauze film interacted with L929 cell line. The living and non-living cells were found for different concentrations of extracts were interacted with L929 cell line. The growth of fiber blast cells were found in the cells images after interaction with cell line. This revealed cells proliferation and cells morphology. The morphology of cells was found through spectrometer and founds its % of cells viability and % of toxicity. The maximum cell viability and toxicity was found 81 % and 19 %. The cells were seen in spectrometer and found its surface morphology is shown in Figure.15. By increasing the concentrations the slight decrease in cell viability slight increases in toxicity which is given in table.

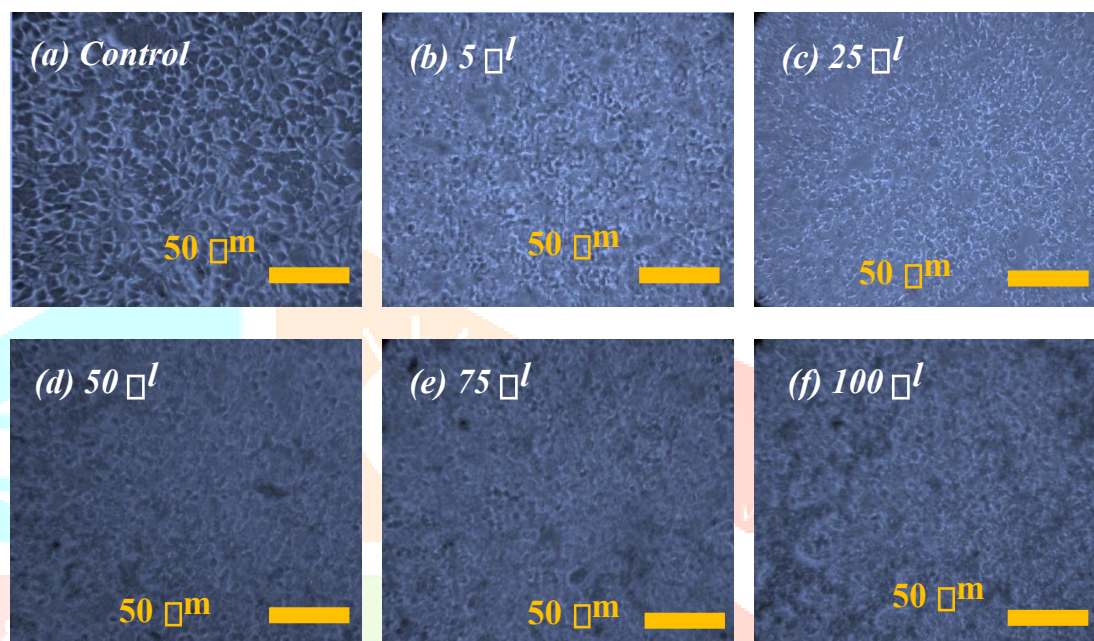


Figure. 14 In-Vitro Cells Morphology of Tridax Procumbens Extracts Loaded Alginate with Gauze Film

Table. 4 Comparisons of Cell Viability and Cytotoxicity

Sl. No	Concentrations	Cytotoxicity %	Cell viability %	Reactivity Level
1	5	19	81	Slight
2	25	23	77	Mild
3	50	27	73	Mild
4	75	32	68	Mild
5	100	38	62	Mild

The results were compared with previous research work and found it better.

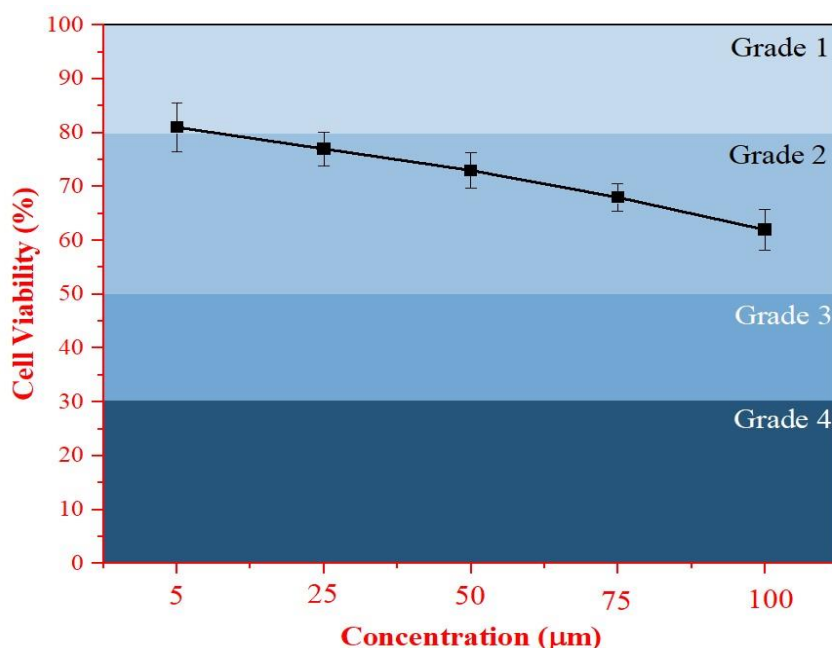


Figure.15 In-vitro Cell viability of 10 wt. % of TP Fresh Extracts Loaded with 10 wt. % of Sodium Alginate with Gauze Film

Also the results were standard reference table. The new combination of film would be used for wound healing applications [26, 27]. The maximum cell viability and toxicity was found 81 % and 19 %. The cells were seen in spectrometer and found its surface morphology is shown in Figure.16.

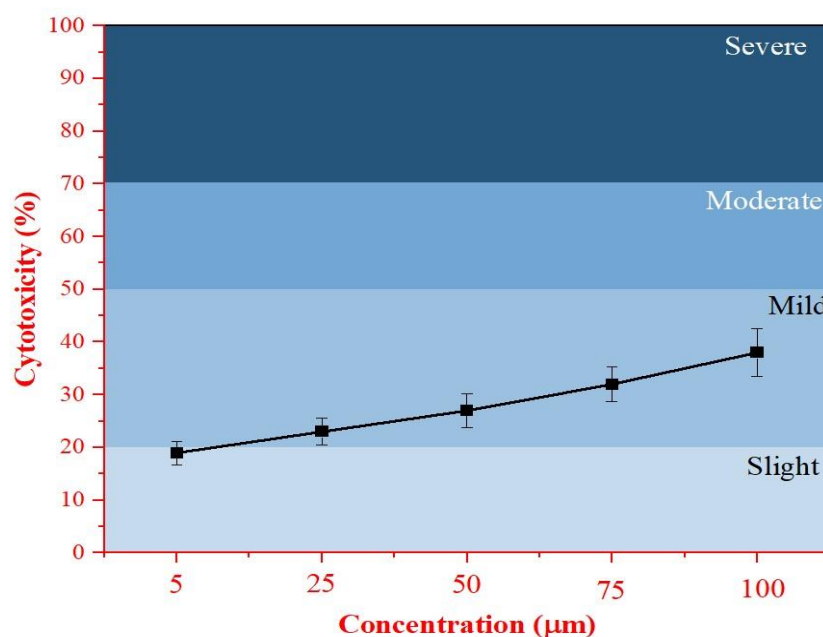


Figure.16 In-vitro Cytotoxicity of 10 wt. % of TP Fresh Extracts Loaded with 10 wt. % of Sodium Alginate with Gauze Film

3.10 In-vitro Wound Scratch

The 10 wt. % tridax plant extracts loaded alginate with gauze film was carried out in-vitro wound scratch analysis through L929 cell line with different concentrations 25 µl, 50 µl, 75 µl, and 100 µl. The different concentrations of extracts 25 µl, 50 µl, 75 µl, and 100 µl were allowed to interact with cell line for different durations (0 hours, 4 hours, 18 hours, and 24 hours).

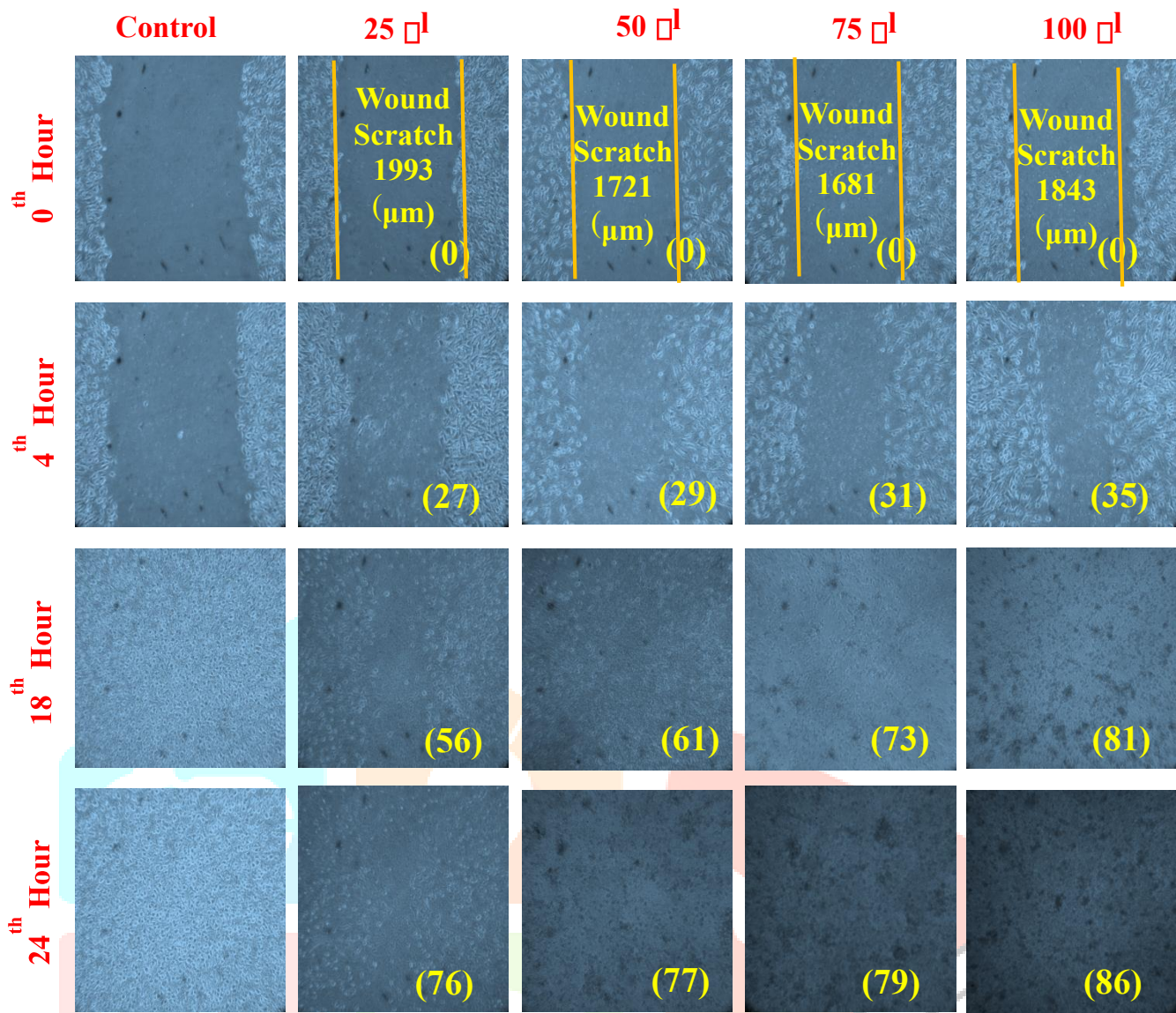


Figure. 17 In-Vitro wound scratch of 10 wt. % of TP Fresh Extracts Loaded with 10 wt. % of Sodium Alginate with Gauze Film

Then the cells migrations and cells proliferations were found through its cells morphology. Then cells migrations were found and given in Table. The maximum % of cells migrations were found as 76, 77, 79, and 86 % for 24 hours of interaction of L929 cell line with different concentrations of 25, 50, 75, and 100 ml. The quantitative results revealed that within 24 hours almost all concentrations were attained the stable migrations of cells through in-vitro wound scratch study. Also maximum cells migrations were attained 86 % for 100 μ l was interacted the cell line with 24 hours is shown in Figure.17.

By incorporating more concentrations of extracts in alginate film would enhance the cells migrations further with short durations. The results were compared with previous research and found the significant improvement in these new combinations of film. The qualitative results revealed the wound scratch and cells migrations for each concentrations at different hours of interaction.

Table. 5 Comparisons of Cell Migrations (%)

Sl. No	Concentrations μg/μl	Wound Area (μm)	Duration (Hours)			
			0	4	18	24
			(Hours)	(Hours)	(Hours)	(Hours)
			Percentage of Cell Migrations (%)			
1	25	1993	0	27	56	76
2	50	1721	0	29	61	77
3	75	1681	0	31	73	79
4	100	1843	0	35	81	86

The cells migrations were confirmed that different compounds present in the Tridax Procumbens Extracts were enhanced the cells migrations and proliferations. Which further enhance the wound healing and regenerate new tissues on the wound compared and confirmed. Also, the quercetin present in tridax procumbens extract was induced the cell migrations and wound healing activities [28, 29].

3.11 Tensile Strength

The tensile strength was carried through UTM and found the tensile stress and modulus of elasticity for i) 10wt.% of tridax procumbens extracts loaded 10 wt.% Alginatej without gauze and ii) 10 wt.% of Tridax Procumbens Extracts loaded 10 wt.% of Alginate with gauze. The film was prepared the size of 1 mm x 5 mm x 80 mm. The film was holded the both ends in UTM and started the test using inbuilt software in UTM machine. The travel speed was fixed 2 mm /minutes. After completing the load deflection curve of tridax procumbens extract loaded sodium alginate film without gauze and with gauze curve was obtained is shown in Figure.18 and Figure.20. After completing the test stress strain curve of tridax procumbens extract loaded sodium alginate film without gauze and with gauze curve was obtained is shown in Figure.19 and Figure.21.

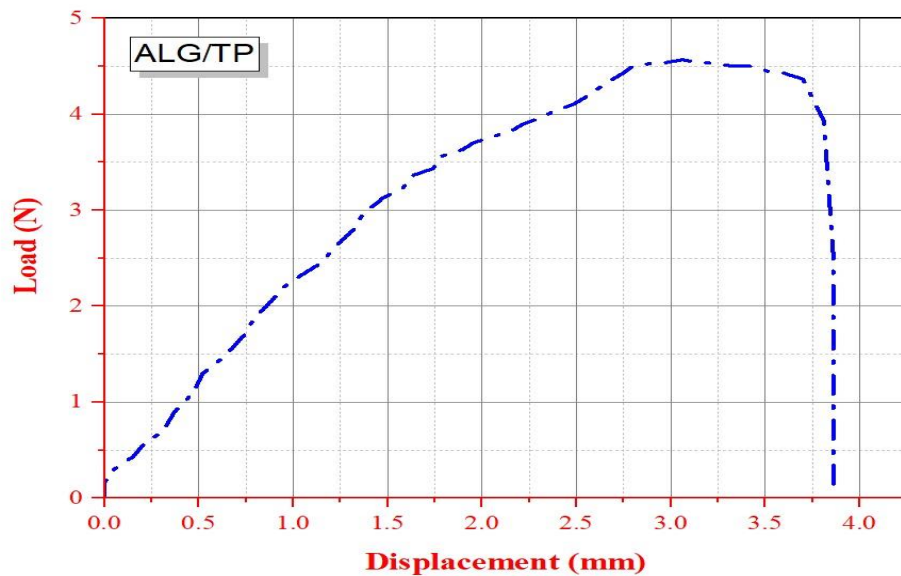


Figure. 18 Load Deflection Curve of Tridax Procumbens Extract Loaded Sodium Alginate Film without Gauze

Then found the tensile strength and modulus of elasticity. i) 10wt.% of Tridax Procumbens Extracts loaded 10 wt.% Alginatej without gauze film revealed the tensile strength if 7.52 MPa and modulus of elasticity 1.67 GPA. ii) 10wt.% of Tridax Procumbens Extracts loaded 10 wt.% Alginatej with gauze film revealed the tensile strength if 8.83 MPa and modulus of elasticity 2.44 GPA. The tensile was improved 9.6 % after incorporating the gauze on the film. Also, modulus elasticity was increased 31.55 %. The % of elingation was increased from 6.43 % to 7.05 %. Also breaking force increased from 117.5 N to 141.6 N. Since the gauze incorporated on the film enhanced the strength for better stability [24, 25].

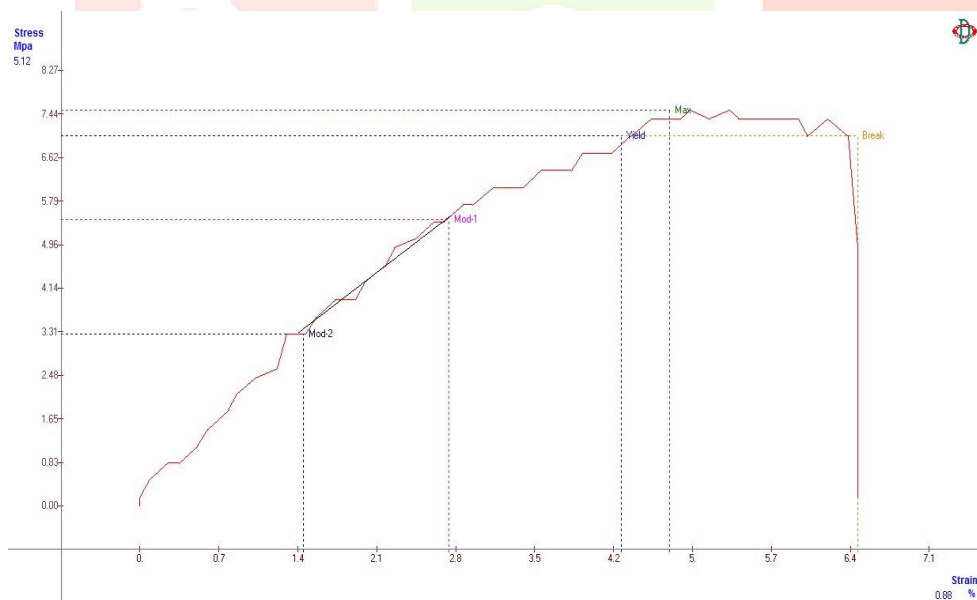


Figure. 19 Stress Strain Curve of Tridax Procumbens Extract Loaded Sodium Alginate Film without Gauze

The tensile strength was carried through UTM and found the tensile stress and modulus of elasticity for i) 10wt.% of tridax procumbens extracts loaded 10 wt.% alginate without gauze and ii) 10 wt.% of tridax procumbens extracts loaded 10 wt.% of Alginate with gauze. The film was prepared the size of 1 mm x 5 mm x 80 mm. The film was holded the both ends in UTM and started the test using inbuilt software in UTM

machine. The travel speed was fixed 2 mm /minutes. After completing the test stress strain curve was obtained.

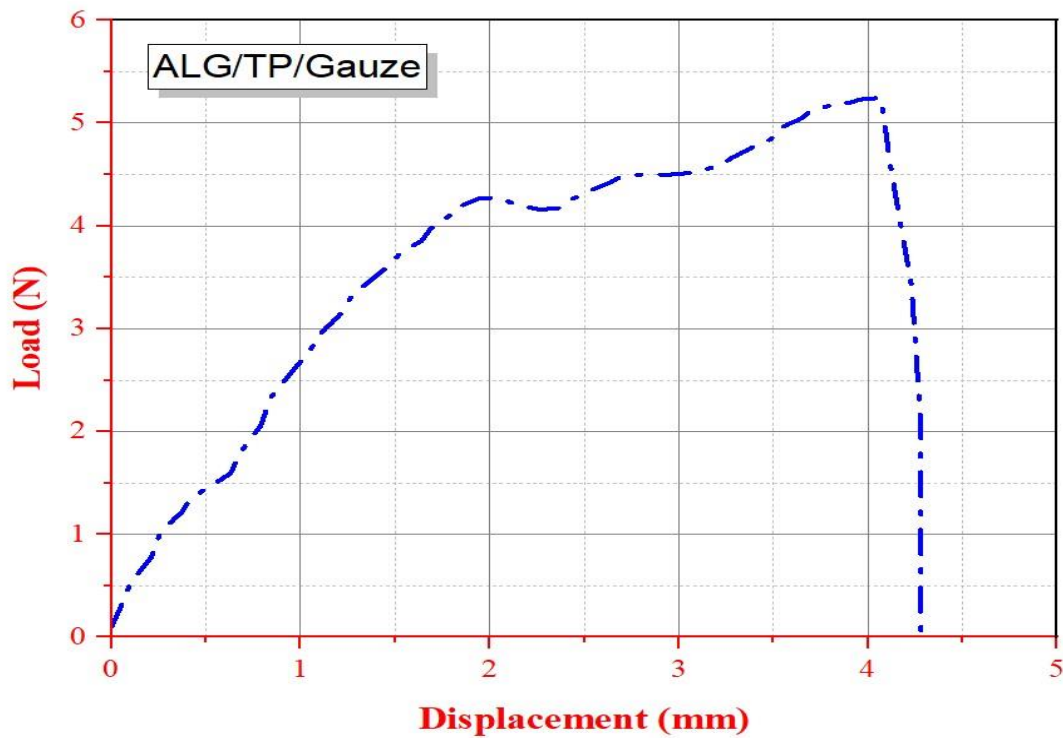


Figure. 20 Load Deflection Curve of Tridax Procumbens Extract Loaded Sodium Alginate Film without Gauze

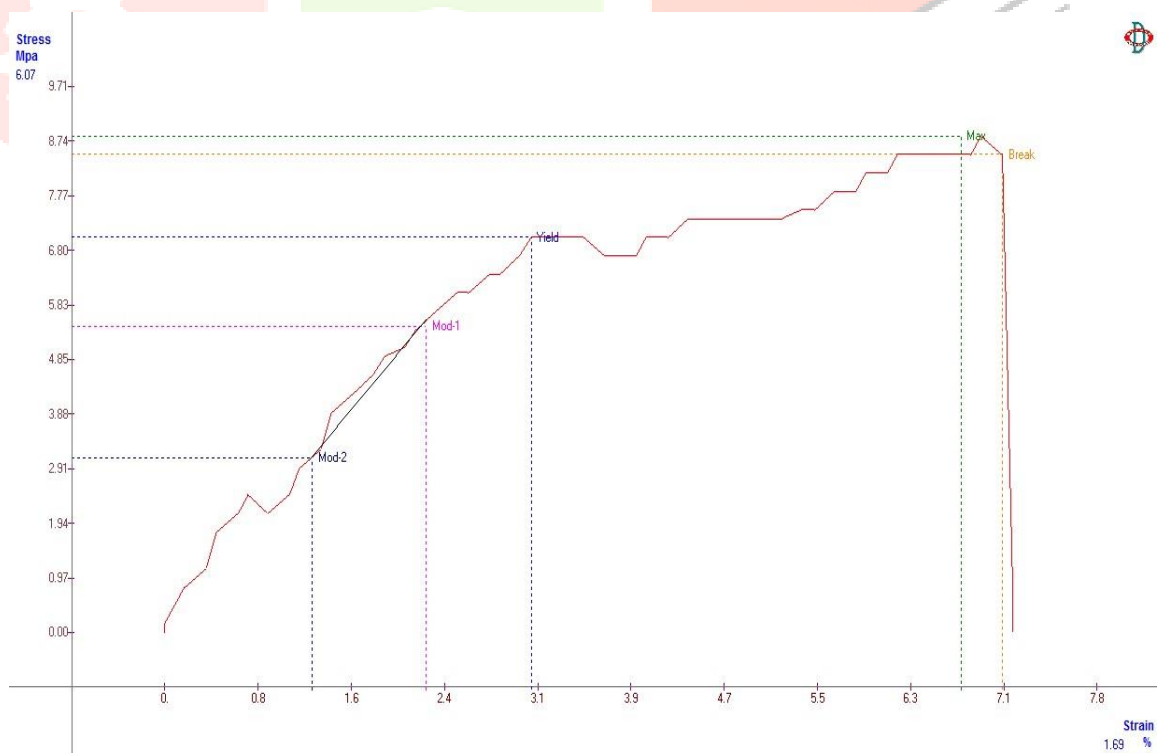


Figure. 21 Stress Strain Curve of Tridax Procumbens Extract Loaded Sodium Alginate Film with Gauze

Then found the tensile strength and modulus of elasticity. i) 10wt.% of tridax procumbens extracts loaded 10 wt.% Alginate without gauze film revealed the tensile strength if 7.52 MPa and modulus of elasticity 1.67 GPa. ii) 10 wt. % of tridax procumbens extracts loaded 10 wt.% alginate with gauze film revealed the tensile strength if 8.83 MPa and modulus of elasticity 2.44 GPa.

Table. 6 Comparison of Tensile Strength of Tridax Procumbens Extract Loaded Sodium Alginate Film with and without Gauze

Tensile Strength of Tridax

Procumbens Extract Loaded

Sl.	Sodium Alginate Film No	Results	Units	
			without Gauze	with Gauze
			Value	Value
2	Tensile Stress at Yield	MPa	7.03	7.03
3	% Strain at yield	%	4.32	3.08
4	Tensile Stress at Break	MPa	7.0	8.5
5	% Strain at Break	%	6.43	7.05
6	Yield Force	N	42.17	42.17
7	Break Force	N	117.13	141.65
8	% Elongation	%	6.43	7.05
9	Tensile Stress at Max	MPa	7.52	8.83
10	% Strain at Max	%	6.43	7.05
11	Modulus of Elasticity		1.67 GPa	2.45 GPa

1 Area cm²
0.06 0.06

The tensile was improved 9.6 % after incorporating the gauze on the film. Also, modulus elasticity was increased 31.55 %. The % of elongation was increased from 6.43 % to 7.05 %. Also breaking force increased from 117.5 N to 141.6 N. Since the gauze incorporated on the film enhanced the strength for better stability. The incorporation of gauze in the film the stability of film was enhanced and found that the increases in strength and modulus of elasticity

3.12. Drug release profile:

The Alg-TP-Gauze, film was immersed in a pH 7.4 PBS solution at 37 °C and continuously stirred at 100 rpm to assess the quercetin release profile. Fig. 22 depicted the quercetin release pattern from the film. The Alg-TP-Gauze film dissolved completely in 20 minutes when it was submerged in PBS solution, according to the observations. The film's dissolution shows that all of the quercetin that was contained in the film matrix was released into the surrounding solution. The full release during the first 20 minutes was confirmed by a decrease in the percentage of quercetin after 25 minutes. It was determined that 0.0039 g/g of quercetin had been released from the picture overall.

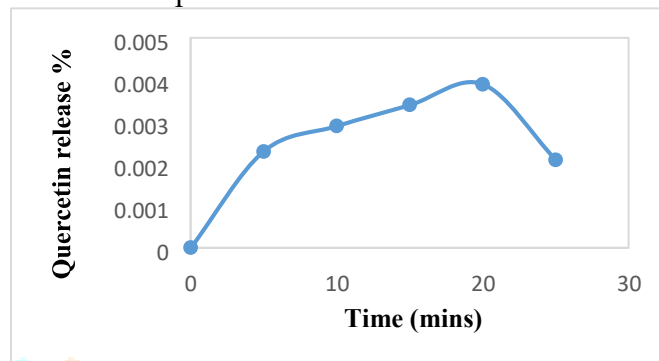


Figure 22. Drug release profile of Alg-TP-gauze wound dressing

3.13. Drug release kinetics:

Several pharmacokinetic models, such as the zero order, first order, Korsmeyer-Peppas, and Higuchi models, were used to investigate the kinetics and mechanism of quercetin release from the Alg-TP-Gauze film. These models' outcomes are shown in **Table and Fig. 23**. A controlled drug release mechanism where the release rate is constant and unaffected by drug concentration is described by zero-order kinetics. The zero-order formula is as follows:

$Q_t = Q_0 + K_0 t$, where Q_0 is the starting dose of the medication in the system, K_0 is the zeroorder release constant, t is the time, and Q_t is the amount released at time t . The drug's release profile is described by the first-order release kinetic model, which holds that the amount of drug left in the system determines how quickly the drug releases from it. The following is the first-order release kinetic equation:

$\log Q_t = \log Q_0 + K_1 t / 2.303$ where, Q_0 is the starting dose of the medication, K_1 is the first-order release constant, and Q_t is the amount released at time t . Drugs that are water soluble or low soluble are studied for their release behavior from nanofiber mats using the Higuchi model. The following is the mathematical relationship: $Q_t = Q_0 + K_H t / 2$, where Q_t is the total amount of medicine released at time t ; K_H is the Higuchi rate constant; and Q_0 is the initial amount of medication that was encapsulated. Where Q_t is the amount of medication released at time t , Q_0 is the initial drug amount, and K_H is the Higuchi rate constant, we get $Q = Q_0 + K_H t^{1/2}$. An empirical equation was developed by the Korsmeyer-Peppas model to investigate the Fickian and non-Fickian release of pharmaceuticals from swelling and nonswelling drug delivery devices. $\log(Q_t / Q_0) = \log K + n \log t$ where Q_t / Q_0 is the amount of drug released at time t , K is the rate of drug release constant, and n is the drug release exponent. The drug release in this model is governed by the Fickian diffusion mechanism if $n \leq 0.5$, the zeroorder case II transport if $n < 1$, and the anomalous diffusion or super case II transport mechanism if $n > 1$. With an R^2 value of 0.2263, the Alg-TP-Gauze film did not fit well with first-order kinetics. This might be explained by the water repellent character of the film, which caused the controlled release of drug. Since there was no consistent release rate in this instance, zero-order kinetics cannot be used. The zero-order kinetics model, on the other hand, suited the data well, with an R^2 value of 0.9548. This implies that the drug release and quick swelling behaviour of the film are consistent with the firstorder kinetics assumption. According to first-order kinetics, the rate of release is directly related to the amount of medication left in the film.

In this case, the Higuchi and Korsmeyer-Peppas models—which are frequently employed to explain drug release from polymeric systems—did not make sense. This is explained by the film's increased swelling and the drug's unpredictable release within a few minutes, which goes against the presumptions of these models.

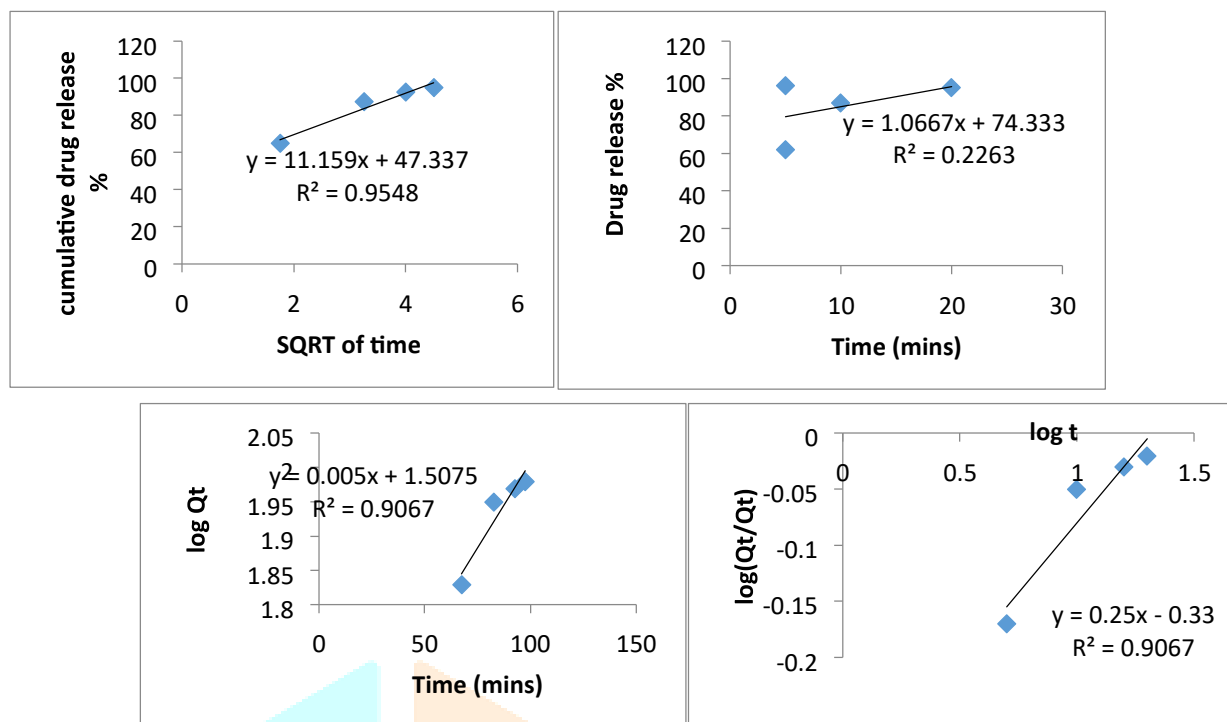


Figure 23. Drug release kinetics of Alg-TP-gauze wound dressing

3.14. Swelling studies:

Analysis of swelling properties in wound healing applications, the way that Alg-TP-Gauze film swells is a crucial factor in determining how the loaded medicine releases. In this investigation, the film's percentage swelling was calculated at 37 °C in PBS solutions with pH value of 7.4. **Fig. 24** shows the variations in 7.4 pH. However, prolonged exposure led to the film's degradation. In a pH 7.4 PBS solution, the maximal swelling of 256 percent was seen in 25 minutes. The Alg-TP-Gauze film exhibited a higher swelling capacity and a faster rate of degradation in the pH 7.4 PBS solution, as per the findings. The pH difference might have affected the loaded drug's release, and it might have also affected the film's kinetics of disintegration and swelling behaviour.

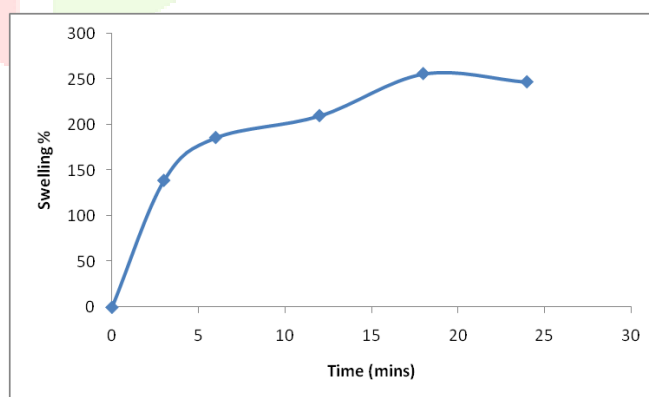


Figure 24. Swelling % of Alg-TP-gauze wound patch

6 Conclusion

The tridax procumbens extracts loaded alginate with gauze film has been developed through solution casting techniques. The TP extracts were carried out phytochemical screening and found the presence of different compounds such as Alkaloids, Anthraquinone, Carbohydrates, Saponins, Cardiac Glycosides. The in-vitro antibacterial activity was done against E.coli, Bacillus, and aeruginosa. The zone of inhibition was

determined to evaluate the antimicrobial activity. The surface morphology and elemental composition (C, O, Na, Cl, Ca, and Au) of the film was examined through FESEM and EDX. The quercetin present in the TPE was confirmed through HPLC. The tensile strength of film and modulus of elasticity without gauze and with gauze was found 7.52 MPa, 8.83 MPa, 1.67 GPa and 2.45 GPa respectively. The incorporation of gauze in the film the stability of film was enhanced and found that the increases in strength and modulus of elasticity. The extracts from the film was allowed to interact with L929 cell line and found the 86 % cell migrations which confirm the wound healing efficacy of the film. Then the film was carried out in-vitro cytotoxicity and found maximum 81% cell viability and 19 % toxicity through L929 cell line with different concentrations. The incorporations of different concentration of TP in the alginate the wound efficacy may increases and duration wound healing would reduce further. The drug release, kinetics and swelling of the film was carried out and found the drug release profile and it's order of mechanism. The *Tridax procumbens* extracts loaded alginate with gauze film would be used for wound healing applications.

References

1. Mahmood H., Khan I. U., Asif M., Khan R. U., Asghar S., Khalid I., et al. (2021). In vitro and in vivo evaluation of gellan gum hydrogel films: Assessing the co impact of therapeutic oils and ofloxacin on wound healing. *Int. J. Biol. Macromol.* 166, 483–495.
10.1016/j.ijbiomac.2020.10.206
2. Tenorová K., Masteiková R., Pavloková S., Kostelanská K., Bernatoniene J., Vetchý D. (2022). Formulation and evaluation of novel film wound dressing based on collagen/microfibrillated carboxymethylcellulose blend. *Pharmaceutics* 14 (4), 782.
10.3390/pharmaceutics14040782
3. Santos L. G., Silva G. F. A., Gomes B. M., Martins V. G. (2021). A novel sodium alginate active films functionalized with purple onion peel extract (*Allium cepa*). *Biocatal. Agric. Biotechnol.* 35, 102096.
10.1016/j.bcab.2021.102096
4. Marangoni Júnior L., Jamróz E., Gonçalves S. d. Á., da Silva R. G., Alves R. M. V., Vieira R. P. (2022). Preparation and characterization of sodium alginate films with propolis extract and nano-SiO₂. *Food Hydrocoll. Health* 2, 100094. 10.1016/j.fhfh.2022.100094
5. Mutlu B., Erci F., Çakir Koç R. (2022). Production of alginate films containing *Hypericum perforatum* extract as an antibacterial and antioxidant wound dressing material. *J. Bioact. Compatible Polym.* 37 (2), 134–148. 10.1177/08839115211073155
6. Aydın G., Zorlu E. B. (2022). Characterisation and antibacterial properties of novel biodegradable films based on alginate and roselle (*Hibiscus sabdariffa* L.) extract. *Waste Biomass Valorization* 13 (6), 2991–3002. 10.1007/s12649-022-01710-3
7. Bushra Ishfaq; Ikram Ullah Khan; Syed Haroon Khalid; Asghar, S.
Design and Evaluation of Sodium Alginate-Based Hydrogel Dressings Containing *Betula Utilis* Extract for Cutaneous Wound Healing. *Frontiers in Bioengineering and Biotechnology* 2023, 11.
<https://doi.org/10.3389/fbioe.2023.1042077>.

8. Qamar, M.; Akhtar, S.; Ismail, T.; Wahid, M.; Abbas, M. W.; Mubarak, M. S.; Yuan, Y.; Barnard, R. T.; Ziora, Z. M.; Esatbeyoglu, T. Phytochemical Profile, Biological Properties, and Food Applications of the Medicinal Plant *Syzygium Cumini*. *Foods* 2022, 11 (3), 378. <https://doi.org/10.3390/foods11030378>.
9. Asma Rhimi; Khira Zlaoui; Horchani_Naifer, K.; Dorra Jellouli Ennigrou. Characterization and Extraction of Sodium Alginate from Tunisian Algae: Synthesizing a Cross-Linked Ultrafiltration Membrane. *Iranian Polymer Journal* 2022, 31 (3), 367–382. <https://doi.org/10.1007/s13726-021-01005->
10. Fatima, F.; Aldawsari, M. F.; Ahmed, M. M.; Anwer, M. K.; Naz, M.; Ansari, M. J.; Hamad, A. M.; Zafar, A.; Jafar, M. Green Synthesized Silver Nanoparticles Using *Tridax Procumbens* for Topical Application: Excision Wound Model and Histopathological Studies. *Pharmaceutics* 2021, 13 (11), 1754. <https://doi.org/10.3390/pharmaceutics13111754>.
11. Bhagyaraj, S.; Krupa, I. Alginate-Mediated Synthesis of HeteroShaped Silver Nanoparticles and Their Hydrogen Peroxide Sensing Ability. *Molecules* 2020, 25 (3), 435. <https://doi.org/10.3390/molecules25030435>.
12. Rehan, M.; Ahmed-Farid, O. A.; Ibrahim, S. R.; Hassan, A. A.; Abdelrazek, A. M.; Khafaga, N. I. M.; Khattab, T. A. Green and Sustainable Encapsulation of Guava Leaf Extracts (*Psidium Guajava* L.) into Alginate/Starch Microcapsules for Multifunctional Finish over Cotton Gauze. *ACS Sustainable Chemistry & Engineering* 2019, 7 (22), 18612–18623. <https://doi.org/10.1021/acssuschemeng.9b04952>.
12. Sangeetha, R.; Niranjana, P.; Dhanalakshmi, N. Characterization of Silver Nanoparticles Synthesized Using the Extract of the Leaves of *Tridax Procumbens*. *Research Journal of Medicinal Plants* 2016, 10 (2), 159–166. <https://doi.org/10.17311/rjmp.2016.159.166>.
13. Asma Rhimi; Khira Zlaoui; Horchani_Naifer, K.; Dorra Jellouli Ennigrou. Characterization and Extraction of Sodium Alginate from Tunisian Algae: Synthesizing a Cross-Linked Ultrafiltration Membrane. *Iranian Polymer Journal* 2022, 31 (3), 367–382. <https://doi.org/10.1007/s13726-021-01005-9>.
15. Diana, M. I.; Selvin, P. C.; Selvasekarapandian, S.; Krishna, M. V. Investigations on Na-Ion Conducting Electrolyte Based on Sodium Alginate Biopolymer for All-Solid-State Sodium-Ion Batteries. *Journal of Solid State Electrochemistry* 2021, 25 (7), 2009–2020. <https://doi.org/10.1007/s10008-021-04985-z>.

14. International journal of pharmaceutical sciences and research 2019, 10 (5).
[https://doi.org/10.13040/ijpsr.09758232.10\(5\).2492-96](https://doi.org/10.13040/ijpsr.09758232.10(5).2492-96).
15. Tejaswini, K., B V Pradeep, K. Rudrama Devi, S. Shylaja and K. Jyothsna. "PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF PLANT EXTRACT OF TRIDAX PROCUMBENS." (2011).
16. Shah, R. et al. "Validated HPLC fingerprint analysis for Simultaneous Determination of Quercetin and Kaempferol in Methanolic Extract of Tridax procumbens." (2012).
17. Sanghavi, Nikita B. et al. "RP-HPLC method development and validation of Quercetin isolated from the plant Tridax procumbens L." Journal of Scientific and Innovative Research (2014): n. pag.
18. Huang, W.-H.; Hung, C.-Y.; Chiang, P.-C.; Lee, H.; Lin, I-Ting.; Lai, P.-C.; Chan, Y.-H.; Sheng Wei Feng. Physicochemical Characterization, Biocompatibility, and Antibacterial Properties of CMC/PVA/Calendula Officinalis Films for Biomedical Applications. 2023, 15 (6), 1454–1454.
<https://doi.org/10.3390/polym15061454>.
19. Huang, W.-H.; Hung, C.-Y.; Chiang, P.-C.; Lee, H.; Lin, I-Ting.; Lai, P.-C.; Chan, Y.-H.; Sheng Wei Feng. Physicochemical Characterization, Biocompatibility, and Antibacterial Properties of CMC/PVA/Calendula Officinalis Films for Biomedical Applications. 2023, 15 (6), 1454–1454.
<https://doi.org/10.3390/polym15061454>.
20. Syed, A.; Benit, N.; Alyousef, A. A.; Abdulaziz Alqasim; Arshad, M. In-Vitro Antibacterial, Antioxidant Potentials and Cytotoxic Activity of the Leaves of Tridax Procumbens. Saudi Journal of Biological Sciences 2020, 27 (2), 757–761. <https://doi.org/10.1016/j.sjbs.2019.12.031>.
21. Andriana, Y.; Xuan, T.; Quy, T.; Minh, T.; Van, T.; Viet, T. Antihyperuricemia, Antioxidant, and Antibacterial Activities of Tridax Procumbens L. Foods 2019, 8 (1), 21. <https://doi.org/10.3390/foods8010021>.
22. Choi, I.-Y.; Chang, Y.; So Lim Shin; Joo, E.; Hyun Kyu Song; Eom, H.; Han, J. Development of Biopolymer Composite Films Using a Microfluidization Technique for Carboxymethylcellulose and Apple Skin Particles. 2017, 18 (6), 1278–1278. <https://doi.org/10.3390/ijms18061278>.
23. Balaji Ayyanar Chinnappan; M. Krishnaveni; Bal, T.; Aditya Dev Rajora. In Vitro-in Vivo Wound Healing Efficacy of Tridax Procumbens

Extract Loaded Carboxymethylcellulose Film. International Journal of Biological Macromolecules 2023, 253, 126695–126695.
<https://doi.org/10.1016/j.ijbiomac.2023.126695>.

24. Syed, A.; Benit, N.; Alyousef, A. A.; Abdulaziz Alqasim; Arshad, M. In-Vitro Antibacterial, Antioxidant Potentials and Cytotoxic Activity of the Leaves of Tridax Procumbens. Saudi Journal of Biological Sciences 2020, 27 (2), 757–761. <https://doi.org/10.1016/j.sjbs.2019.12.031>.
25. Evaluation of tridax procumbens leaf extract loaded pva film for wound healing application | international journal of pharmaceutical sciences and research. <https://ijpsr.com/bft-article/evaluation-of-tridaxprocumbens-leaf-extract-loaded-pva-film-for-wound-healingapplication/> (accessed 2024-05-31).
26. B Yaduvanshi; Mathur, R.; Mathur, S. B.; Thirumurthy Velpandian. Evaluation of Wound Healing Potential of Topical Formulation of Leaf Juice of Tridax Procumbens L. In Mice. PubMed 2011. <https://doi.org/10.4103/0250-474x.93523>.

